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ASSESSMENT OF HAEMOGLOBIN POLYMORPHISM AS A POTENTIAL PROTEIN MARKER IN SELECTION FOR GENETIC IMPROVEMENT OF THE WEST AFRICAN DWARF GOAT POPULATION IN NIGERIA

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ABSTRACT

This study was conducted to ascertain the level of genetic diversity at the haemoglobin (Hb) locus and to assess its potential as a protein marker-assisted selection for genetic improvement of Nigerian West African Dwarf goat populations. Haemoglobin genotyping was performed on forty (40) West African Dwarf goat obtained from Okpokuu local government area of Benue state, Nigeria, using cellulose acetate electrophoresis. Data on Haemoglobin genotypes were also subjected to chi-square test. Two co-dominant haemoglobin alleles Hb^A and Hb^B observed controlled three haemoglobin genotypes (Hb^{AA}, Hb^{AB} and Hb^{BB}). The genotypic frequencies were Hb ^{AA} 0.42, Hb^{AB} 0.50 and Hb^{BB} 0.08 for does populations, while that in the bucks population were Hb^{AA} 0.38, Hb^{AB} 0.44 and Hb^{BB} 0.19 respectively. The most frequent haemoglobin genotype observed in the study was Hb^{AB}. The heamoglobin genes (allelic) frequencies observed in this study were 0.64 and 0.36 for Hb^B and Hb^A respectively. The result of chi-square test was not significant ($P>0.05$), critical value was 3.84 at 1degree of freedom. Which implies that the oberseved and the expected genotypic frequencies at the heamoglobin locus in west African dwarf goat populations in okpokwu local government area were in handy-Weinberg proportion. Since protein molecules are easily accesseble through electrophoresis, heamoglobin blood protein polymorphism can be used as a potential biochemical marker in selection for genetic improvement of the West African Dwarf goat populations among rural farming communities in Nigeria.

Contribution/Originality: The study indicated that two co-dominant haemoglobin alleles Hb^A and Hb^B controlled three haemoglobin genotypes. Genotypic frequencies at the heamoglobin locus in West African dwarf goat populations in the area were in handy-Weinberg proportion. Heamoglobin blood protein polymorphism through electrophoresis can be use as a potential biochemical marker in selection for genetic improvement of the West African Dwarf goat populations among rural farming comunities in Nigeria.

1. INTRODUCTION

Goat breeding is a very old tradition among indigenous farmers, no purposeful breeding programme has been arranged for the species in Nigeria (Adedeji *et al.*, 2011). Nigeria is a country with a heavy human population of about 168.8 million (USCB, 2012) this population is continuously on the increase. This trend has led to the high demand for the available animal and animal products in all parts of the country to meet up with the minimum animal protein requirement per individual per day (FAOSTAT, 2010). The small ruminants of Nigeria have been variously evaluated for genetic variation based on morphological and productive characters (Adu and Ngere, 1979).

Goat is the most prolific ruminant among all domesticated ruminant animals under tropical and the subtropical conditions. Goat forms the largest group of small ruminant livestock in Nigeria totaling about 53.8 million and also constituting 6.2% of the world's goat population (FAOSTAT, 2011). Survey has shown that up to 85% of rural households, poor farmer and small-time business people of all age groups and sexes reared goat (FDLPCS, 2007). The ability of goat to tolerate harsh climate, the presence of the trypanotolerance in some breed (Salako, 2004) suitability to traditional systems on account of small size, short generation interval (Aziz, 2010) and ability to thrive on poor quality diets provided by scarce grazing on marginal lands (Hoste *et al*., 1988; Ozoje, 1998) all combine to make goat production strategic to increasing livestock productivity in rural agricultural systems (Fitzugh *et al*., 1992).

Genetic diversity is shaped by the past population and environmental challenges for the future. The production practices, choice of values and the breeding systems are other avenues that modify genetic diversity of farm animals. WAD goats are reared exclusively on free-range utilizing pasture mating. There is thus uncontrol breeding which causes high genotypic variations among individual animals leading to different productivities, enviromental tolerance and adaptation capacities to common enviromental challenges. Thus, maintenance of genetic diversity is key to the long-term survival of most species (Hall and Bradley, 1995). Farm animal genetic diversity is required to meet current production needs in various environments to allow sustained genetic improvement and to facilitate rapid adaptation to changing breeding objective and environmental challenges (Kumar *et al*., 2006). If genetic diversity is very low, none of the individuals in a population may have the potentials needed to cope with the new environmental conditions or challenges, Such populations faces the danger of elimination due to increased vulnerability to common environmental threat and sudden catastrophic event such as disease outbreaks and be wiped out due to loss of diversity. Also, low genetic diversity may indicate a high level of inbreeding with its associated problems of expression of deleterious alleles or loss of over-dominance. Change in the distribution of the pattern of genetic diversity can destroy local adaptation and break up co-adapted gene-complexes. These problems combined, may lead to an increasing poorer 'match' of the population to its habitat and eventually lead to the breed loss of adaptive features and eventual extinction (Mahmoudi *et al*., 2010).

Estimations of genetic variations increasingly are being based upon information at the DNA level by various molecular marker such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP) (Rincón *et al*., 2000) Simple Sequence Repeat (SSR) or microsatellite (Dalvit *et al*., 2008).

Range of innovations in molecular genetics to study variation and evolution of populations using DNA marker genotypic variation plays an important role in developing national breeding strategies for economical animal species (Maudet *et al*., 2002). These DNA based techniques are yet to be exploited in developing countries like Nigeria due to infrastructural, financial and technical deficiencies. Analysis of genetic markers based on protein variants detected by electrophoretic method has been a tool for studying genetic differentiation among populations (Maudet *et al*., 2002). It application is less complex and with minimal cost, that it can be applied to evalute animal genetic resource diversity. Animals belonging to one breed may be reared under different populations and under different environmental conditions, phenotypic differences observed may simply reflect genotype–environment interaction. In such cases, polymorphic biochemical markers can help to compare genetic variability within and between populations (Dalvit *et al*., 2008). Molecular biochemical markers can be used as important tools to tag loci underlying the expression of traits which have breeding importance.

Gene diversity is an appropriate measure of genetic variability within a population. Population showing higher intra-breed similarity and lower portion of polymorphic loci are likely to have less heterozygosity. Genetic diversity of goat populations in Nigeria need a thorough investigation so as to identify the undisclosed genetic potentials associated with these animals. This limited performance may not only be explained by the breeds genetic potentials but also traditional management practices. WAD goat breed are well adapted to rather difficult conditions over

long periods. However, resistance and adaptation capabilities might have declined due to indiscriminate mating, cross-breeding, sub-structuring and consequent genetic drift in these populations over time. Furthermore, as the demand for certified products such as meat and milk is increasing, there is a need to consider the local indigenous WAD goat breeds. Indigenous populations will be at a higher risk of genetic erosion due to selection pressure, indiscriminate mating and lack of characterization, A detail genetic study of local goat populations are important to assess the structure of genetic diversity to optimize conservation and utilization strategy. The investigation of genetic diversity and similarity between and within breeds are important to give useful genetic information necessary for developing effective management plans for the conservation and improvement of the genetic resource.

Goats, particularly Nigerian breeds are genetically diverse, so it is important to analyze their genetic diversity in order to identify populations and individuals of particular merit. Therefore, the genetic study of the goat populations in Nigeria is crucial, to integrate the result into the livestock sector data base and identify characteristics of superior genotypes at the molecular level on genetic structure and diversity of Nigerian WAD goat breeds.

The general objectives of this study were to evaluate heamoglobin genotypes and polymorphism as a potential protein marker in selection for genetic improvement of the WAD goat in Nigeria.

2. MATERIALS AND METHOD

2.1. Study Area

This study was carried out in Okpokwu Local Government Area in Benue State. Okpokwu Local Governmnet Area is located between latitude 6^{o}' 30^{o}N to 8^{o}' 30^{o}'N and longitude 70^{o}' 30^{o}'E to 10^{o}' E. it has an area of 731km^{2} and a population of 176, 647 according to the 2006 census. The local government is located about one hundred and seventy (170km) South West of Makurdi, the state capital Benue State. It shares land borders with OhiminiLoca Government Area on the North, Ogbadibo Local Government Area on its Western end, Ado and Otukpo Local Government Area on the East, Isiuzo local government area of Enugu State on the South and Olamaboro local government area of Kogi state to the North West.

2.2. Animals Management

The goats were managed under a semi-intensive system of management. The animals were taken out to graze sometime in the morning from 8:00 am to 5:00pm but not daily with the help of children and were confined. Shade and water trough were provided and supplemented with crop byproducts feeds like groundnut haulms, dry grasses, fresh cassava leaves and kitchen waste.

2.3. Blood Collection

Two (2) ml of blood samples were collected from 40 goats in four locations namel: location A (Omusu), Location B (Igama), location C (Opialu) and location D (Okpolikpo) in Ojigo ward. The blood samples were collected from jugular vein using needle and syringe into a test tube containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant and sample were properly labeled. On the 6th November, 2017, I collected sample in Location A and proceeded to location B.On the 14th November, 2017 and lastly, I collected sample in location C and D and were taken to laboratory on each occasion. Two lyse drops of the blood sample with 4ml of distilled water properly mixed in a test tube in the tris buffer soak cellulose acetate paper remove cellulose paper from buffer using forceps and lightly blot between two sheets of filter paper to remove excess moisture. Place the cellulose paper across the bridges of the electrophoresis tank and secure. Apply the heamolysate (blood sample) near the cathode (+) bridges using a fine applicator. Repeat heamolysate using known AA, AB, BB and CC controls. The lid was replaced on top of the tank to prevent evaporation while power supply was connected, electrophoresis was carried out at 225-230 volts for 15-20 minutes.

2.4. Data Analysis

Genotype frequencies of the haemoglobin genotypes alleles frequencies of the Hb alleles were estimated. Genotype frequencies were calculated as follows:

$$\text{Genotype frequency of } Hb^{AA} = \frac{No\ of\ individuals\ with\ HbAA}{No\ of\ individual\ sampled}$$

$$\text{Genotype frequency of } Hb^{BB} = \frac{No\ of\ individuals\ with\ HbBB}{No\ of\ individual\ sampled}$$

$$\text{Allele frequency of } Hb^{A}\ (P) = \frac{2nAA + nAB}{2N}$$

$$\text{Allele frequency of } Hb^{B}\ (Q) = \frac{2nBB + nAB}{2N}$$

Where:

nAA = Number of individuals with Hb^{AA} genotype

nAB = Number of individuals with Hb^{AB} genotype

nBB = Number of individuals with Hb^{BB} genotype

N = Total number of individuals sampled

Data on genotype frequencies were subjected to chi-square analysis to test for goodness- of-fit for observed and expected frequencies under Hardy-Weinberg equilibrium (HWE).

Estimates of heterozygosity were calculated as shown by the expression below:

Heterozygosity (He) = 1 - $\sum_{i=1}^{n} X^2$

Where:

n = Number of loci

Xi = Frequencies of the alleles

3. RESULTS

The genotype and allele frequencies of the heamoglobin locus in West African Dwarf goat in selected populations of Okpokwu local Government Area are shown in Table 1.

Tablel-1. Genotype and alleles frequencies at the heamoglobin locus in WAD goats in selected populations of Okpokwu.

Sex	Genotype number			Genotype frequencies			Allele frequencies	
	Hb^{AA}	Hb^{AB}	Hb^{BB}	Hb^{AA}	Hb^{AB}	Hb^{BB}	Hb^{A}	Hb^{B}
Male	6	7	3	0.38	0.44	0.19	0.59	0.41
Female	10	12	2	0.42	0.50	0.08	0.67	0.33
Total	16	19	5	0.40	0.48	0.13	0.64	0.36

Hb: Heaoblobin, AA; Homozugous Genotype, BB; Homozugous Genotype, AB; Hetrozygous Genotype, A; Alleles, B; Alleles.

Table 2 shows the observed and expected number of haemoglobin genotype in West African Dwarf goat Bucks in Okpokwu local government area.

Table-2. Observed and Expected number of heamoglobin genotypes in the WAD Bucks in OkpokwuL.G.A.

Genotype	Observed	Expected	$X^2df = 1$
Hb^{AA}	6	5.64	
Hb^{AB}	7	7.72	
Hb^{BB}	3	2.64	0.139 ^{ns}

ns =Not significant (P > 0.05) ,Critical Value =3.84 at 1 df , Hb; Heamoglobin, Hb^{AA}; Homozygous genotype, Hb^{BB}; Homozygous genotype, X^2; Chi- square, df; Degree of Freedom.

Table 3 shows the observed an expected number of haemoglobin genotypes in West African Dwarf goat Does in Okpokwu local government area.

Tablel-3. Observed and Expected number of heamoglobin genotypes in the WAD Does in Okpokwu L.G.A.

Genotype	Observed	Expected	X^2df = 1
Hb^{AA}	10	10.67	
Hb^{AB}	12	10.67	
Hb^{BB}	2	2.67	0.375^{ns}

ns =Not significant (P > 0.05), critical Value =3.84 at df = 1 Hb^{AA} Heamoglobin, Hb^{BB} Homozygous genotype, Hb^{AB} Heterozygote genotype, X^2; Chi- square, df Degree of Freedom.

Table 4 shows the observed and expected number of haemoglobin genotypes in West African Dwarf goat in Okpokwu local government area.

Tablel-4. Observed and Expected number of heamoglobin genotypes in the WAD Goats in Okpokwu L.G.A.

Genotype	Observed	Expected	X^2df = 1
Hb^{AA}	16	16.26	
Hb^{AB}	19	18.49	
Hb^{BB}	5	5.26	0.031^{ns}

Ns = not significant (P>0.05), critical Value=3.84 at df = 1, Hb, Heamoglobin, (Hb^{BB} Hb^{AA}) Homozygousgenotypes,X^2Chi-square.

Table 5 shows heterozygosities at the heamoglobin locus in West African Dwarf goat populations in Okpokwu local government area of Benue state, Nigeria.

Table-5. Heterozygosities at the Hb locus in populations of the WAD Goat in Okpokwu.

Population	Heterozygosity
Buck	0.48
Does	0.44
Entire Population	0.46

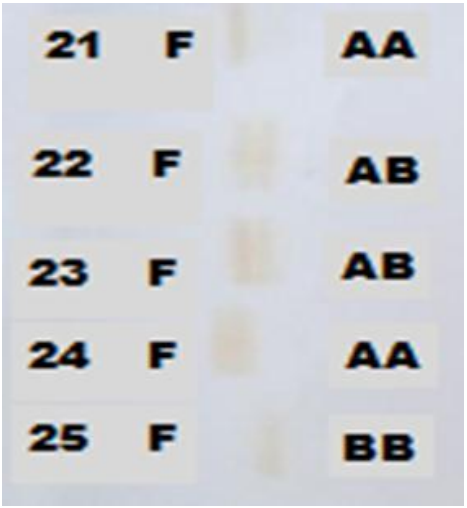

Plate-1. Electrophoresis film pictures.

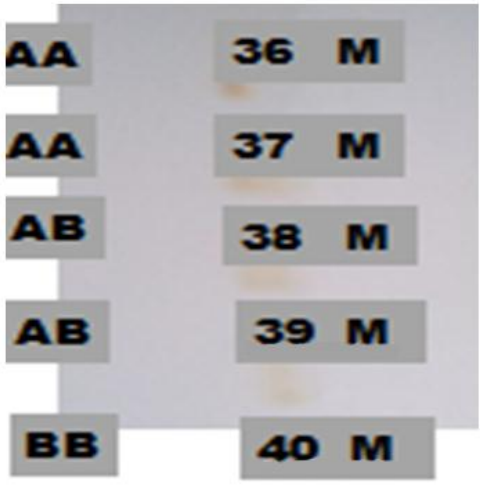

Plate-2. Electrophoresis film pictures.

Three distinct haemoglobin genotypes Hb^{AA}, Hb^{AB} and Hb^{BB} were observed in the study. The three heamoglobin genotypes were controlled by two co-dominant alleles Hb^{A} and Hb^{B}. The haemoglobin genotypic frequencies observed in the study were and (Hb^{AA}) 0.42, (Hb^{AB}) 0.50, (Hb^{BB}) 0.08 in the West African dwarf goat does populations Plate 1, while (Hb^{AA}) 0.38, (Hb^{AB}) 0.44, (Hb^{BB}) 0.19 in West African dwarf goat bucks populations Plate 2. The most frequent heamoglobin genotype observed in this study was Hb^{AB} with the genotypic frequency of 0.48. The gene (alleles) frequencies of Hb^{A} and Hb^{B} observed in this study were 0.64 and 0.36 respectively. The

results of chi-square test in table 2-4 were not significant (P > 0.05), critical value was 3.84 at 1 degree of fredom. This implies that the observed and expected genotypic frequencies at the heamoglobin locus in West African dwarf goats in Okpokwu local government area were in Hardy-Weinberg proportion. The estimated heterozygosities at the heamoglobin locus in West African dwarf goat are shown in table 5. The estimated heterozygosity in the entire population was 0.46.

4. DISCUSSION

Among the three genotypes of the heamoglobin blood protein at the heamoglobin locus, 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 the Hb^{BB}) genotypes. Aygun and Mert (2007); Bindu and Raghavan (2010) and Das *et al.* (2004) all reported that heamoglobin genotypes played significant roles in reproduction and milk production traits in Norduz goat Yusuncu. Blood heamoglobin has a locus in the blood protein loci. Different blood heamoglobin genotypes have different potential for blood protein content between individual within and between populations. It is reasonable to infer that, the heterozygote (Hb^{AB}) genotype has higher potential for blood protein content than either of the homozygotes (Hb^{AA} and Hb^{BB}) genotypes (Eyal, 1968; Galip and Elmaci, 2001; Daramola *et al.*, 2005; Gurcan *et al.*, 2010) all noted the influence of heamoglobin genotypes on haematological and biochemical parameters of small ruminants and recommended that haemoglobin genotyping can be use for selection of superior genotypes for adaptation to enviromental challenges.

The frequencies of the Hb^{AB} genotypes were more in the population, such that even though the Hb^{A} and Hb^{B} were the codominant heamoglobin alleles determining the genotypic frequencies of the heamoglobin genotypes in the population, the recombinant at ferttilization, natural, unconcious and concious selections had favoured the heterozygote (Hb^{AB}) genotypes in the population over the homozygotes (Lush, 1970; Oduye and Adadevoh, 1976; Krishnamurthy and Rathnasathy, 1978; Petre *et al.*, 1982; Marian *et al.*, 1983; Lande and Thompson, 1990; Pieragostini *et al.*, 2006; Salako *et al.*, 2010) all observed the relationship between heamoglobin genotypes, their relationship with production and reproduction traits, heamatological parameters and other characters of small ruminants and cattles. It is reasonable to infer that, the heterozygote genotypes had superior advantage over the homozygotes in responding to tissue energy needs and output through tissue respiration, oxygen carbonic acid gradient in circulating blood and gaseous exchange between the animal and their enviroment. Holsinger and Weir (2009) also reported similar observation (Seth *et al.*, 1974; Samorineanu *et al.*, 1984; Yaman *et al.*, 1986; Soysal and Ulku, 1998; Shaharbak *et al.*, 2010) observed the association between heamoglobin genotypes and blood proteins, serum biochemistry and other production and reproduction traits of sheep and goats. The reduction in the frequencies of the Hb^{B} allele further favoured the increased frequencies of the heterozygote (Hb^{AB}) genotype and genetic divergence at the heamoglobin locus from a single cohessive unit from which the studied WAD goat populations may have been isolated or as a sub-population to the single cohessive unit. There would therefore, be a reduction in the allelic frequencies of the Hb^{B} in the offspring population and subsquent generations of the West African Dwarf goat populations compared to their parental generations due to random sampling, genetic drift and small poplation sizes.

The decline in the allelic frequency of Hb^{B} may have arose due to random sampling that favoured the distribution of the Hb^{A} allele at higher frequencies compared to that of the Hb^{B} allele. Thus individuals in the population with the homozygous genotypes (Hb^{AA} and Hb^{BB}) had lower individuals in their offspring populations bearing their genotypes, while the frequencies of the heterozygote (Hb^{AB}) in their offspring and subsquent populations increases. The increase may be due to differential fitness and adaptation due to differential blood protein content (Seth *et al.*, 1974; Petre *et al.*, 1982; Pieragostini *et al.*, 2006; Salako *et al.*, 2010; Shaharbak *et al.*, 2010) aslo reported the potentials of heamoglobin polymorphism in enhancing adaptation and resistant to

environmental challenges in several small ruminant breeds. Although there was no loss of allele at the heamoglobin locus, there was variation in the frequencies of the heamoglobin codominant alleles. In other words, forces of selection, natural, concious and unconcious had acted on the genetic structure of the West African Dwarf goat populations among rural farming communities at the heamoglobin locus. □

The distribution of the allelic frequencies in the genotypes of subsquent populations and generations produces higher frequencies of the heterozygote (Hb^{AB}) genotypes compared to those of the homozygotes (Hb^{AA} and Hb^{BB}) genotypes. This may be attributed to processes of mutation, random sampling and genetic drift and natural selection which leads to differentiation in allelic frequencies at selected loci. The increase in the frequencies of the Hb^{A} alleles is an indication that the population of the West African Dwarf goat studied had been isolated from a single cohessive unit for a short time,or it was a sub-population in which there was a random mating but within which there was a reduce amount of gene flow. While the differentiation in the frequencies of the Hb^{B} and Hb^{A} heamoglobin alleles increases in favour of the Hb^{B} allele, the increase is yet to attain the levels at which the studied West African Dwarf goat populations becomes completely fixed for the Hb^{B} allele at the heamoglobin locus.

These differential heamoglobin genotypic frequencies also translates to differential blood protein size, structure, molecular weight and contents of the blood heamoglobin. The variation in the nature and content of the blood protein of blood heamoglobin also determines differential fitness and adaptation of the West African Dwarf goat populations among rural farmers under the free-range production system. Dally *et al.* (1980) also reported the influence of heamoglobin genotypes on reproductive efficiency and lampwool in crossbreed ewes. In other words, there were variations in the heamoglobin blood protein content between individuals within and between populations of the West African Dwarf goat populations that determines the role and efficiency of cellular, tissue respiration and gaseous exchange between the animal and their enviroment which wholistically determines adaptation (Yaman *et al.*, 1986; Vicovan and Rascu, 1989; Soysal and Ulku, 1998) also onserved the role of heamoglobin genotypes on blood protein contents supporting adaptation and performance in several populations of goats and sheep. Since protein molecules are easily accesseble through electrophoresis, heamoglobin blood protein polymorphism can be use as a potential biochemical marker in selection for genetic improvement of the2 West African Dwarf goat populations among rural farming communities in Nigeria.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The study indicated that the heamoglobin locus in WAD goats in Okpokwu local government area of Benue state, Nigeria is controlled by two co-dominant alleles Hb^{A} and Hb^{B}. The allele (Hb^{B}) was the most frequent Heamoglobin gene in the populations of the WAD goats studied. The heterozygotes heamoglobin genotypes had a higher frequency than the homozygous genotypes. There existed a moderate genetic diversity at the hemoglobin locus even though the most frequent Hb allele is yet to be fixed by random genetic forces to establish genetic divergence at the heamoglobin locus in WAD goats populations in Okpokwu Local Government Area of Benue state, Nigeria. The presence of the heterozygous (Hb^{AB}) genotype confers a degree of natural selective advantage due to variation in its blood protein content. Individuals in the WAD goats populations bearing the heterozygous genotype were more in number than the homozygotes, as they were placed at an advantage in adaptation to their natural environment.

5.2. Recommendation

The blood protein content of the heterozygote heamoglobin genotype can be used as a biochemical protein marker for selection for genetic improvement in cellular metabolism, efficiency in tissue energy output, oxygen carbonic acid gradient in the blood and gaseous exchange between the WAD goats and its environment. Further

research should be carried out to determine the effect of the heterozygous heamoglobin polymorphisms on productive performance of the WAD goats populations in Benue state, Nigeria.

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