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ASSESSMENT OF GENETIC DIVERSITY AT THE HEAMOGLOBIN LOCUS IN SELECTED WEST AFRICAN DWARF GOAT POPULATIONS IN NIGERIA

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ABSTRACT

Blood samples were collected from 40 (forty) goats in Kwande Local Government Area, of Benue State, Nigeria. Blood samples collected were subjected to electrophoresis to study haemoglobin polymorphism and its distribution in West African Dwarf goat populations in Kwande. Data on Haemoglobin genotypes were subjected to sample variance (S^2) population genetics simulation software for the analysis. Three heamoglobin genotypes (Hb^{AA}, Hb^{AB} and Hb^{BB}) were observed in the West African dwarf goat populations. The haemoglobin gynotypes (Hb^{AA}, Hb^{AB} and Hb^{BB}) were controlled by two co-dominant haemoglobin alleles Hb^A and Hb^B. The genotypic frequencies were 0.24, 0.57 and 0.19 for Hb^{AA}, Hb^{AB} and Hb^{BB} respectively. Genotypic frequencies at the haemoglobin locus in West African Dwarf goat were in hardy-weinberg equilibrium. The gene frequencies of Hb^A and Hb^B observed were 0.52 and 0.48 respectively with Hb^A being the most frequent. The low allelic variation may have occur when populations are separated but only for a short time, that the processes of genetic drift and mutation that leads to differentiations of allelic frequencies at selected loci are yet to occur. As the amount of time in which two isolated populations increases, the differences in allelic frequencies will also increase until each population is completely fixed for a number of alleles. These factors may have accounted for the low variation in the heamoglobin allelic frequencies observed in this study. There was also gene-controlled diversity at the haemoglobin locus in the WAD goat populations with heterozygosity (He) value of 0.49, which indicated a moderate level of genetic diversity at the haemoglobin locus in WAD goat populations in Kwande Local Government Area of Benue State, Nigeria.

Contribution/Originality: The study indicated that the populations of the West African dwarf goat were separated for a short time, that the processes of genetic drift and mutation that leads to differentiation of allelic frequencies at selected loci are yet to occur and that no population of the West African dwarf goat is fixed in heamoglobin allelic frequencies.

1. INTRODUCTION

The domestic goat (*Capra hircus*) often is described as the "poor man's cow" (Ayoade, 2010) for its ability to thrive on meager fodder and cope with harsh environments. However, this shows the economic and archeological importance of the species. From an agricultural standpoint, the world's 700 million goats provide reliable access to meat, milk, skins and fibre for small farmers particularly in developing countries like Nigeria (Aziz, 2010). Assessment of genetic variability in domestic animals is an important issue to preserve genetic resource for future

breeding options in order to satisfy the demands of changeable climate and markets. Animal breeding practices have emphasized productivity utilizing few genes and from a favoured small number of breeds (Devendra, 1997). As a result, locally adapted native breeds to a large extent has been neglected, abandoned and/or replaced by more efficient and higher productive modern goat breeds. Goats are highly adaptable to different environmental conditions and are being raised the world over for milk, meat and fibre production. Although they present reasonable reproductive and productive performance, it is necessary to improve their production efficiency to become more competitive with other livestock industries (Davendra and Burns, 1983). In this regard, genetic selection plays a very important role and substantial genetic gain has been achieved using traditional breeding methods (Meuwissen *et al*., 2001).

Blood components are undoubtedly essential biological characteristics and warrant consideration for the study of a breed. Studying the haematological picture is helpful for clinical diagnostics (Obi and Anosa, 2001) but is also essential to reflect the particular evolution of a breed or a population; the fact that some blood factors are related to the suitability of the breed under particular environmental conditions has been noted (Tibbo *et al*., 2008). Haemoglobin (Hb) has been one of the most studied polymorphisms invertebrate species since the infancy of both population and evolutionary genetics. The close relationship between structure and function of Hb complex protein remains important especially its molecular, genetic and adaptive features and has been recently defined as "an evergreen red protein" (Bettati *et al*., 2009). Goats exhibit a very complex Hb polymorphism due to the presence of a number of allelic and non-allelic chains both in the alpha and beta globin systems (Pieragostini *et al*., 2005). The Hb^{BB} locus is highly polymorphic, while only four different alpha globulin variants have been found so far: Hb^{A1A} and $Hb^{A2T,}$ the most frequent genes (more than 0.98 and almost 0.80 respectively, followed by Hb^{A2A} (about 0.20) and Hb^{A1B} very rare (Pieragostini *et al*., 2005).

Emphasis has being directed on molecular polymorphism of blood Hb to which selection can be applied for breeding strategies Alpan and Ertugrul (1991); Atroshi and Sandholm (1982) and Aygun and Mert (2007) had reported the potential of blood heamoglobin as a candidate for selection. Recently, attention has been focused on functional genetics, an area that is currently included amongst animal breeding research priorities. The functional effect of the Hb phenotype on haematological patterns has been demonstrated in humans as well as in farm animals (Evans and Phillipson, 1957; Krishnamurthy and Rathnasathy, 1978; Marian *et al*., 1983; Gonzales *et al*., 1984). In sheep, individuals carrying extra alpha-globin genes exhibit an overall blood picture mimicking a thalassemia-like syndrome, while positively charged variants have been found to be somehow related to a decreased mean corpuscular volume and hematoctrit (Pieragostini *et al*., 2006). One relevant parameter to the implementation of genomic selection in a breeding program is the extent to which linkage disequilibrium (LD) persist across the genome and how it varies between populations. LD is define as a non-random association of alleles at two or more loci and is influence by population history, breeding system and the pattern of geographic subdivision. In addition to linkage disequilibrium, the accuracy of genomic selection also depends on the number of records available to estimate marker effects (training population).

The primary unit in animal genetic resources is a breed, strain or geographically defined groups, the numbers of which share particular morphological characteristics that distinguished them from other groups. Several techniques have been developed to estimate the genetic variation or polymorphisms in population and hence, the genetic relationship amongst populations.

Allele frequencies can be used to estimate all genotype frequencies (including null heterozygotes) for the analyses of genetic variation in each population, differentiation among populations and genetic distance. Although there have been some substantial studies on haemoglobin genotyping, alleles frequencies in sheep and cattle, very few studies have been done on goats in Nigeria. In addition, studies carried out are more in the temperate regions like United State of America, Canada among others; this study was limited to the Nigerian tropical environment in the upper highland areas of Benue state. The main aim of this study was to provide information on goat

haemoglobin genotypes, allele frequency and distribution in the upland region of Kwande Local Government Area of Benue State, Nigeria.

2. MATERIALS AND METHOD

2.1. Experimental Location

This research work was carried out in three council wards (Turanjato-Aka, Injorov and Mbadura) in Kwande local government area of Benue State, Nigeria. Jato-Aka, Injorov Mbakideerinjorsha, Mbawegh, Ijever, Tuum, Mbadura, Turan and Tyogbeda were locations selected for the study. These locations were rural communities in Benue state of Nigeria.The community is located at latitude 8^0 $42^{0'}$ N and longitude 9^0 $18^{0'}$ E, with an attitude of $216m^2$. The temperature range was 25 to 33^0C and the relative humidity range of 55% to 75%, while precipitation ranged from 35% to 40%. The environment was generally variable with soil pH (5.5-8.5), there was natural vegetation of tree cover making up 40%. The areas have grassland-based environment. The production practices were the mixed farming system (crop and livestock) while the goat was managed under the free-range (extensive) system.

2.2. Experimental Animals and their Management.

The animals used for this research were West African Dwarf goats comprising of both sexes. The total number of animals used for the study were 40 (forty), comprising of 20 (twenty) females (doe) and twenty (20) buck. Out of the total number of male goats that were used for the study, four were castrated male goats, 16 were breeding bucks. While the twenty female goats that were used for the study, six were kids, fourteen were breeding the doe. The animals were managed under free range with no supplementary feeding and there was no evidence of veterinary care for the animals.

2.3. Blood Sample Collection

4ml of blood samples were collected from each of the forty goats in four locations. Part of Kwande local government area in Mbadura council ward are namely: Mbakideerinjorsha, Tuuminjorsh, Ijever and Tyogbeda locations, all in Turan, Kwande local government area of Benue State. The blood samples were collected from the jugular vein using needle and syringe into an ethylene diaminetetraacetic acid (EDTA) sample bottle containing boric acid as an anticoagulant to prevent the conversion of fibrinogen to fibril or chelate calcium. The blood samples were placed in an ice flask covered with cotton wool and thereafter transported to Tosema Specialist Diagnostic Laboratory in Makurdi for the genotype test.

2.4. Materials Used for the Experiment

The materials used for Heamoglobin Genotyping were Electrophoresis machine, Acetate paper, Buffer, alkaline, Filler paper, Goat blood samples, EDTA (ethylene diaminetetraacetic acid), sample bottles, Buffer alkaline PH of 8.4, Test tubes and Hand gloves.

2.5. Experimental Procedure

Hemoglobin Genotyping was determined by cellulose acetate electrophoresis as described by Aikhuomobhogbe and Orheruata (2006). Two lyse drop of the blood samples with 4ml of distilled water was properly mixed in a test tube with the trisbuffer. The soaked cellulose acetate paper was removed from the buffer using forceps and lightly blot between two sheets of filter paper to remove excess moisture. The cellulose paper was place across the bridge of the electrophoresis tank and secured properly. The haemolysate (blood sample) was applied near the positive electrode bridge using a fine applicator. The haemolysate was repeated using known AA, AB, BB, and CC control. The electrophoresis was switched on at 225-230 volts and read after 30-40 minutes.

2.6. Data Collection and Statistic Analysis

The data were collected through stratified sampling and was subjected to simple calculation of statistic method.Genotype frequencies of the haemoglobin genotype and allele frequencies of the Hb alleles were estimated. The frequency genotypes AA, BB, and AB were calculated using the formula below.

$$\text{Genotype frequency of } Hb^{AA} = \frac{\text{numbers of an individual with } Hb^{AA}}{\text{Total numbers of individual sampled}}$$

$$\text{Genotype frequency of } Hb^{AB} = \frac{\text{number of individual with } Hb^{AB}}{\text{Total numbers of individual sampled}}$$

$$\text{Genotype frequency of } Hb^{BB} = \frac{\text{number of individual with } Hb^{BB}}{\text{Total numbers of individual sampled}}$$

$$\text{Allele frequency of } Hb^{A}(p) = \frac{2n^{AA} + Nab}{2N}$$

$$\text{Allele frequency of } Hb^{B}(q) = \frac{2n^{BB} + N^{AB}}{2N}$$

Where N^{AA} =numbers of an individual with Hb^{AA} genotype.

N^{BB} = Numbers of an individual with Hb^{BB} genotype

N^{AB} = Numbers of an individual with Hb^{AB} genotype

N = total number of goats sample.

The genotypic and the gene frequencies were calculated using the Hardiweinberg relationship as shown below.

Hardiweinberg relationship $P^2 + 2pq + q^2 = 1$

Where P^2 = Homozygous dominant

$2pq$ = Heterozygous individuals

q^2 = Homozygous recessive

Data on genotypic frequencies were subjected to chi-square analysis to test for goodness-of –fit of the observed genotype to their expected values.

2.7. Haemoglobin Genotype Analysis

The Gene S^2 population genetics simulation software was used in the analysis.

3. RESULTS

The genotype and allele frequencies at the haemoglobin locus of the west African dwarf goats in kwande are shown in Table 1. The gene (allele) frequencies of Hb^A and Hb^B observed in the study were 0.52 and 0.48 respectively. The genotypic frequencies of the blood haemoglobin observed in this study were 0.24 (Hb^{AA}), 0.57 (Hb^{AB}) and 0.19 (Hb^{BB}) for West African dwarf buck populations. While 0.32 Hb^{AA}, 0.58 Hb^{AB} and 0.11 Hb^{BB} genotypic frequencies were observed in the West African dwarf doe populations.

Table-1. Genotype and allele frequencies at the haemoglobin locus in the WAD goatkwande local government in Benue State.

SEX	No.of samples	Genotype Number			Genotype frequencies			Allele frequencies	
		Hb^{AA}	Hb^{AB}	Hb^{BB}	Hb^{AA}	Hb^{AB}	Hb^{BB}	Hb^{A}	Hb^{B}
Male (buck)	21	5	12	4	0.24	0.57	0.19	0.52	0.48
Female (Doe)	19	6	11	2	0.32	0.58	0.11	0.61	0.39
Total no.	40	11	23	6	0.28	0.58	0.15	0.56	0.44

Hb^{AA} = Homozygous genotype AA, Hb^{BB} Homozygous genotype BB, Hb^{AB} Heterozygous genotype, A ,B = Alleles, B.

The test of a variation of the Hb genotypes between the observed and the expected numbers of haemoglobin genotypes in West African dwarf bucks populations is presented in Table 2. The test for the variation of the Hb genotypes between the observed and the expected numbers of haemoglobin genotypes in west African dwarf doe

populations is shown in Table 3. Table 4 shows the test of the variation between the observed and the expected numbers of haemoglobin genotypes in the pooled data (Buck and Doe) populations of the West African Dwarf goat. Three distinct haemoglobin genotypes Hb^{AA}, Hb^{AB} and Hb^{BB} were observed in the study populations. The three Hb genotypes were controlled by two co-dominant alleles Hb^{A} and Hb^{B}.

Table-2. Observed and expected number of hemoglobin genotype in the West African dwarf buck populations.

Genotype	Observed Hb	Expected Hb	Degree of freedom (df). X^2 df=1
Hb^{AA}	5	5.76	
Hb^{AB}	12	10.48	
Hb^{BB}	4	4.76	0.444^{ns}

ns = Not significant difference (p>0.05), Hb= Haemoglobin, df= degree of freedom, X^2= Chi-Square, Hb^{AA}= Homozygous genotype AA, Hb^{BB} Homozygous genotype BB.

Table-3. Observed and expected number of hemoglobin genotypes in the West African dwarf Doe populations.

Genotypes	Observed number	Expected number	X^2 degree of freedom 1
Hb^{AA}	6	6.96	
Hb^{AB}	11	9.08	
Hb^{BB}	2	2.96	0.851^{ns}

Hb^{AA} =Homozygous genotype AA, Hb^{BB}Homozygous genotype BB, Hb^{AB}Homozygous genotype, x^2= Chi-square, ns =Not significant (p>0.05).

Table-4. Observed and expected number of hemoglobin genotype in West African Dwarf goat for both (buck and Doe) populations.

Genotype	Observed number	Expected number	X^2df=1
Hb^{AA}	11	12.66	
Hb^{AB}	23	19.69	
Hb^{BB}	6	7.66	1.132^{ns}

ns = not significant (p>0.05), x^2= Chi-square, Df= degree of freedom.
Critical values = 3.84.

4. DISCUSSION

The low variation in the heamoglobin allelic frequencies could mean that the studied populations had only been separated for a short time when they existed as a single cohesive unit. Or these populations are substructures of populations in which there is random mating, but within which there is a reduced amount of gene flow. The low allelic variation may also occur when populations are separated but only for a short time, that the processes of genetic drift and mutation that leads to differentiation of allelic frequencies at selected loci are yet to occur. These are possible with the WAD goat populations because, rural farmer communities in most cases acquire their breeding stocks from their neighbours, initiating their own new populations at their households. Sometimes these new populations may exist as a single breeding unit, others may exist without clear demarcation boundaries or as subpopulations with only reduce the flow of alleles while others may actually be isolated due to distance. In the first and second cases, allelic differentiation will be low. In the third case, however, even though the populations were isolated, it may require a given period for allelic differentiation to occur. The time to fixation of either of the favoured allele will determine the extent of genetic divergence of the alleles at their locus. This again may be influenced by the population size. As the period of time in which two isolated populations increases, the differences in allelic frequencies will also increase until each population is completely fixed for a number of alleles. There may also not be specific boundaries or regional units such that sub-populations are not well defined. These factors may have accounted for the low variation in the heamoglobin allelic frequencies observed at the heamoglobin locus in the West African Dwarf goat populations studied.

The result of X^2 analysis revealed that the populations of WAD goat was at hardy-wein-being equilibrium at the haemoglobin locus (p>0.05). This indicated that the frequency of each diploid genotype at the heamoglobin locus equals the expected from the random union of alleles, that is the genotypes Hb^{AA} , Hb^{AB} and Hb^{BB} were at frequencies of P^2 , 2PQ and q^2. The random fluctuations in allelic frequencies at the heamoglobin locus due to chance alone were very low that, could not alter significant variation in the gene frequecies at the heamoglobin

locus. The populations of the WAD goat studied had not diverged extensively due to chance (random sampling, genetic drift, and genetic bottleneck) to levels that can alter the populations genetic structures into boundaries.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The results of this study indicate that the haemoglobin locus in the West African dwarf goat populations in kwande local government Area of Benue State, Nigeria is controlled by two co-dominant alleles Hb^A and Hb^B. The allele Hb^A was the most frequent haemoglobin allele indicating low genetic divergence at the heamoglobin locus in the population of the West African dwarf goats studied. These populations may either have been separated for only a very short time or are sub-populations without specific boundaries or population units whose boundaries are not well defined.

5.2. Recommendation

Further research should be carried out to determine the effect of the observed haemoglobin genotypes on productive performance to enable the application of selection to the haemoglobin blood protein contents for genetic improvement of the West African dwarf goats in kwande local Government Area of Benue State, Nigeria.

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