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Haemoglobin polymorphism in Nigerian breeds of sheep and goats

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Target Audience: Animal Breeders, Geneticists and Molecular Scientists

Abstract

This study described the genetic structure of Nigerian breeds of sheep and goats at haemoglobin (Hb) locus level. Animals used for the study comprised 100 each of Red Sokoto and West African Dwarf (WAD) goats, and 400 sheep comprising 100 per breed of Balami, Yankasa, Uda and WAD with 50 animals per sex within breed. Five milliliters of blood was obtained from each animal by jugular venipuncture and dispensed into sample bottles containing Ethylene Diamine Tetra Acetic Acid and analyzed using cellulose acetate paper electrophoresis. Genotypic frequencies were then estimated. Goats showed Hb AA, AB and BB genotypic frequencies of 0.485, 0.430 and 0.085, respectively while sheep had only the Hb AA genotype. Red Sokoto goats had Hb AA, AB, BB frequencies of 0.490, 0.430 and 0.080, respectively while the WAD goats had corresponding values of 0.480, 0.430 and 0.090. Genotypic frequencies were similar in both sexes of sheep and goats indicating that there was no effect of sex on Hb frequency. It is concluded that Hb polymorphism in goats is defined by the expression of three genotypes (2 homozygotes, Hb AA, Hb BB, and 1 heterozygote Hb AB), while in sheep there was no polymorphism at the Hb locus; only the Hb AA was exhibited.

Key words: Sheep, goats, Nigeria, Haemoglobin, genotype frequency, gene frequency.

Description of Problem

Although goat and sheep breeding is a very ancient tradition, no breeding and improvement programme was ever organized in Nigeria. Also, nowadays, the research in the field of Nigerian small ruminant is less reported compared to non-Nigerian ones and other livestock and poultry species. More so, researches on biochemical genetics in goats and sheep are scarce worldwide (1). Nigerian goats and sheep breeds have been variously evaluated for genetic variation based on morphological production, reproduction and behavioural features (2,3). However, those characters underestimate the levels of genetic variations. Therefore, polymorphism variation of different proteins, enzymes, mineral elements or blood group factors represents accuracy procedures for a better measurement of genetic variation in caprine species (4, 5) and by extension ovine species. One of the important blood proteins is haemoglobin which has attracted attention because of its biochemical, biophysical and physiological properties, having relevance to selection phenomenon of animals (6).

Haemoglobins are historically important for their part in the demonstration of relationship between genetic information and protein structure. Haemoglobin have been previously reported to have variants and these variants have been reported to be associated

with environmental adaptability as well as being chemically important as causes of a variety of genetic disorders of blood. Consequently, the genetic background of haemoglobin merits examination in some detail (7).

Blood, protein polymorphisms have been used by several researchers as markers to study evolutionary relationships in mammals. For example, evolution relationship between different sheep breeds, deer species, goat breed and chicken genotypes have been examined (8,9,10). It has been reported by several researchers including (11) as well as (12), that for an animal that has a gene for a specific substance, the substance can be detected in the blood by appropriate procedures, such as electrophoresis and the presence or absence of a specific substance is directly related to the genotype. This study aimed to use electrophoresis to describe the genetic structure of Nigerian sheep and goats from the level of determinant locus of haemoglobin. This is the first step to use in the biochemical and molecular polymorphism, incorporated in systematic breeding technologies as an important and objective tool for improving Nigerian sheep and goats.

Materials and Method

The breeds of sheep and goats used for this study were those kept by small holder farmers and institutional farms within Jos Plateau. The area is a pear-shape upland located in the middle of Nigeria within latitudes 8^0 N and 10^0N and longitude 7^0 and 11^0E; experiences equable climate with average monthly temperature ranging between 21^0Cand 25^0C, average humidity of 60% and rainfall of 1400m; mostly covered by extensive grassland and few trees; and with cattle, sheep, goats, pigs and poultry which form the major sources of dietary protein playing important roles in the socio-economic lives of the people. The animals were managed under the traditional free-range in the dry season and semi-intensive and intensive system in the wet season. However, in some cases where flock size was small, animals were tethered.

The studied animals comprised 100 each of Red Sokoto and West African Dwarf (WAD) goats, and 400 sheep also comprising 100 per breed of Balami, Yankasa, Uda and WAD sheep with 50 animals per sex per breed in goats and sheep, respectively. 5ml of blood was drawn from each animal by jugular venipuncture using needle and syringe and put into sample bottles containing Ethylene Diamine Tetra Acetic Acid (ETDA) as anticoagulant, properly labeled with respect to species, sex and breed and preserved in a cooler containing ice blocks from whence it was transported to the of laboratories of National Veterinary Research Institution, Vom and Na Allah, Nakowa Hospital, Mangu, Plateau State for electrophoresis. About 2ml of blood sample was placed into a clean test tube, 5ml of cold saline was added and the diluents obtained centrifuged at 4000 rpm for 10 – 15 minutes. The supernatant was discarded and the sample was washed again three times. The supernatant was discarded and the sediment was re-suspended using about 2ml of cold distilled water. When the lysate was well separated after standing, it was stored in refrigeration temperature until electrophoresis.

At electrophoresis, the red cell lysate was impregnated on a cellulose acetate paper with a control placed on the electrophoresis tank using forceps and subjected to electrophoresis according to standard procedure described (13). The tank was powered with the lead closed and sampled allowed to separate for about 10 – 15 minutes. By electrophoresis, the haemoglobin fractions were separated and the identification of the haemoglobin types was achieved in accordance with the migration speed on the electrophoretical substratum detected from the start line towards the cathodal zone. The haemoglobin

polymorphism was pointed out by detection of three migration zones; a single faster band designated as AA homozygote a single slower band designated as BB homozygote and both bands designated as AB heterozygote. After separation, the cellulose acetate paper was blotted dry using filter paper and the result was taken.

Because only two alleles (A and B) were detected, the haemoglobin genotype and gene frequencies were estimated thus; Genotype frequency AA=number of AA/total number x 100; Genotype frequency AB=number of AB/total number x 100; Genotype frequency BB=number of BB/total number x 100; based on species, breed and sex.

For the estimation of gene frequency, the equation adopted was as follows:
$P = (2N_{AA} + N_{AB})/2N$ and $Q = (2N_{BB} + N_{AB})/2N$; where: P = gene frequency for allele A, Q = gene frequency for allele B, N = total number of individuals, N_{AA} = observed genotype number for AA, N_{AB} = observed genotype number for AB, N_{BB} = observed genotype number for BB. Note that Hardy Weinberg equilibrium is $P + Q = 1$.

Results and Discussion

Gene and genotype frequencies according to species and breeds are presented on Table 1. Goats showed haemoglobin genotype distribution (number) Hb AA, Hb AB and Hb BB to be 98, 86, and 17 representing genotype frequencies of 0.485, 0.430 and 0.085, respectively. The Red Sokoto goat has Hb AA, Hb AB and Hb AA genotype frequencies to be 0.4890, 0.430 and 0.080, respectively, and allele frequencies of 0.705 and 0.295 for A and B, respectively. The West African Dwarf goat had the respective genotype frequencies to be 0.480, 0.430 and 0.090 with allele frequency of A and B to be 0.695 and 0.305, respectively. In this study, three haemoglobin genotypes were therefore detected for goats, two homozygotes (AA and BB) and one heterozygote (AB). This corresponds to the gene observation of A and B alleles with their corresponding genotypes AA, AB, and BB in different species (14,15,16, 17).

The observation of the haemoglobin Hb BB types in goats and their breeds agrees with the report of (21) and (16); although (22) did not find this phenotype in Red Sokoto breed of goats. (23) further stated that it is not readily apparent why the BB phenotype is not widely distributed. Moreover, (22) advanced that frequency of certain phenotype might be strong enough evidence of differential mortality. (16) reported from the Niger Delta of Nigeria 1.0 gene and genotype frequency each in WAD goat and 0.70; 0.20 and 0.10 for AA, AB and BB, respectively for A and B in Red Sokoto goats, while the Sahel goats had AA, AB and BB to 0.50, 0.450 and 0.00, respectively with frequencies of allele A and B to be 0.77 and 0.23, respectively. The authors (16) also reported AA, AB and BB having respective frequencies of 0.7500, 0.2167 and 0.033, while the frequencies were 0.86 and 0.14 in goats generally. (3) however found three alleles, A, B, and C which gave rise to two homozygotes (AA and BB) and two heterozygotes AB and AC) in goats.

The sheep and its four (Balami, Uda, Yankasa and WAD) breeds had only the HB AA genotype and A allele at the haemoglobin locus each with frequency of 1.0. Only HbAA genotype was therefore observed in sheep used for this study. This is in agreement with the report of (24) who also found only HbAA genotype in sheep in Port Harcourt, Nigeria. However, these findings contradict that of (3) who showed that HbBB predominates followed by HbAA and HbAB in both male and female sheep. The existence of only HbAA in this study could be an adaptive survivability feature. Different genotypes have been reported to exhibit selective advantages in different geographical regions (18). This

might have led to the fixation of HbAA in sheep of present study. (19) reported that carriers of HbAA demonstrated significant resistance against helminth infections. According to (20), this could be due to better functional properties such as greater affinity to oxygen, higher Hb concentration and packed cell volume. Conversely, (25) found HbBB alleles fixed in Kwale, Makueni and Siaya sheep breeds of Kenya. (16) stated that there is indication that vegetation or climate changes affect Hb type. With homozygosity at the Hb locus, selection for improvement cannot be based on sheep genotype.

Gene and genotype frequencies according to sex by breed and species are presented in Table 2. Female goats had Hb AA, AB, and BB to be 0.48, 0.44 and 0.08, respectively while the males had them to be 0.49, 0.42 and 0.09, respectively. Gene frequency of A and B were 0.7 and 0.3, respectively for each of both sexes. In sheep, the genotype frequency was 1.0 for Hb AA and 1.0 for the A allele in both sexes. Red Sokoto female goats had frequencies of Hb AA, AB and BB to be 0.50, 0.44 and 0.06 while the male had 0.48, 0.42 and 0.10 respectively, with respective A and B genes frequencies 0.72 and 0.28, and 0.69 and 0.31 for female and male. Female WAD goats had Hb AA, AB, BB frequencies to be 0.46, 0.44 and 0.1 while the male had 0.50, 0.42 and 0.08, respectively while respective A and B gene frequencies of were 0.68 and 0.32, and 0.71 and 0.29 for female and male, respectively. The occurrence of only Hb AA and allele A found in sheep in the present study does not agree with the report of (3) that BB predominates followed by AA and lastly AB in both male and female sheep.

Generally, it was found in this study that the allele A was more predominant in both sexes of goats and their two breeds than allele B while both sexes of sheep and their four breeds had complete absence of allele B. The genotype frequency decreases from AA through the AB and then to BB in goats of both sexes and breeds while in sheep both AB and BB were completely absent in both sexes. This indicates that sex may have no effect on haemoglobin types. (17) found genotype frequency for the Hb AA type higher in females than males of Nigerian breeds of goats while those of AB and BB were higher in males than in females. They however stated that there is not enough reason to conclude that sex has an effect on the difference in Hb types. They further explained that it is due to imbalance in sample size selection for both sexes. In the present study, however the sample size was equal for both sexes.

The chi-square analysis used to determine the difference between the observed and expected genotype frequencies of the pooled sheep and goat population in order to test the conformity of the haemoglobin locus to Hardy-Weinberg equilibrium yielded a non-significant value $X= 0.33013$ ($p<0.05$) for 2df. Thus the sheep and goat population may be considered to be in Hardy-Weinberg equilibrium. The result is similar to the report of (15) in goats in Abuja and (17) for goats in Niger Delta Nigeria. This implies that random mating occurred in the system under study and artificial selection has not been practiced in these species by farmers in the study area.

Conclusion and Applications

1. Haemoglobin polymorphism in goats is defined by the expression of three genotypes: two homozygotes, Hb AA and Hb BB and one heterozygote, Hb AB while in sheep, no polymorphism is found at this, locus.
2. The phenotypization of the haemoglobin variants is determined by two co-dominant alleles, Hb A and Hb B in goats and only one allele, Hb A in sheep.
3. Sex and breed seem to have no effect on the disparity in haemoglobin types in goats as well as sheep. The homozygote

Hb AA had the highest incidence, followed by Hb AB while Hb BB had the lowest. It may be suggested that the HB AA type evident in sheep is favoured by nature selection.

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Table 1: Haemoglobin genotype and gene frequencies according to species and breeds of Nigerian sheep and goats

Small ruminant	N	Genotype Frequency			Gene Frequency	
		AA	AB	BB	A	B
Species						
Goats	200	97 (0.485)	86(0.430)	17(0.085)	0.70	0.3
Sheep	400	400(1.00)	0(0)	0(0)	1.00	0
Breeds						
Goats:						
Red Sokoto	100	49(0.49)	43(0.43)	8(0.08)	0.705	0.295
WAD	100	48(0.48)	43(0.43)	9(0.09)	0.695	0.305
Sheep:						
Balami	100	100(1.0)	0(0)	0(0)	1.00	0
Uda	100	100(1.0)	0(0)	0(0)	1.00	0
Yankasa	100	100(1.0)	0(0)	0(0)	1.00	0
WAD	100	100(1.0)	0(0)	0(0)	1.00	0

Table 2: Genotype and gene frequencies of Nigerian sheep and goats according to sex by species and breeds

Small ruminants	N	Genotype Frequency			Gene Frequency	
		AA	AB	BB	A	B
Species						
Goats:						
Female	100	48(0.48)	44(0.44)	08(0.08)	0.70	0.30
Male	100	49(0.49)	42(0.42)	09(0.09)	0.70	0.30
Sheep:						
Female	200	200(1.0)	0(0)	0(0)	1.00	0
Male	200	200(1.0)	0(0)	0(0)	1.00	0
Breed						
Red Sokoto Goat						
Female	50	25(0.50)	22(0.44)	3(0.06)	0.72	0.28
Male	50	24(0.48)	21(0.42)	5(0.10)	0.69	0.31
WAD Goats						
Female	50	23(0.46)	22(0.44)	5(0.10)	0.68	0.32
Male	50	25(0.50)	21(0.42)	4(0.08)	0.71	0.29
Balami Sheep						
Female	50	50(1.00)	0(0)	0(0)	1.00	0
Male	50	50(1.00)	0(0)	0(0)	1.00	0
Uda Sheep						
Female	50	50(1.00)	0(0)	0(0)	1.00	0
Male	50	50(1.00)	0(0)	0(0)	1.00	0
Yankasa Sheep						
Female	50	50(1.00)	0(0)	0(0)	1.00	0
Male	50	50(1.00)	0(0)	0(0)	1.00	0
WAD Sheep						
Female	50	50(1.00)	0(0)	0(0)	1.00	0
Male	50	50(1.00)	0(0)	0(0)	1.00	0