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HAEMOGLOBIN POLYMORPHISM IN NIGERIAN INDIGENOUS GOATS IN THE NIGER DELTA REGION, NIGERIA

Agaviezor, B. O., Ajayi, F. O. & Benneth, H. N.
Department of Animal Science and Fisheries, Faculty of Agriculture, University of Port Harcourt, P.M.B. 5323, Choba, Port Harcourt, Nigeria

ABSTRACT
This study was conducted to evaluate the haemoglobin (Hb) genotypes and their frequencies as well as their effect on some phenotypic characteristics in the indigenous goat breeds of Nigeria in the Niger Delta area of the country. Blood samples were collected from 60 goats of the various indigenous breeds, 20 each including; the West African Dwarf (WAD), the Red Sokoto and the long legged Sahelian goat breeds. The samples were from live animals in the big markets / abattoir and small holder livestock farms in the humid rainforest region of southern Nigeria. Blood samples were analyzed for haemoglobin types determined by cellulose acetate electrophoresis. The haemoglobin polymorphism was detected by identification in the electrophoretical field of three migration zones: the fast haemoglobin named HbA type, the haemoglobin with intermediate migration labeled HbAB type and the slow haemoglobin designed HbB type. These phenotypes are determined by two co-dominant alleles, HbA and HbB. The resultant data were subjected to descriptive statistical analysis to determine the effect of Hb types on the phenotypic characteristics of the various breeds. The results showed that haemoglobin genotypes varied between and within breeds but there was not enough evidence to ascertain if this disparity is also due to sex. However, the haemoglobin genotype for the WAD breed (AA) was uniform having a genotype frequency of 100% with allelic frequency of 1.00. Various other Hb types including (AA, AB and BB) were observed in the Red Sokoto goats as well as the Sahel breeds and these were in various proportions having the HbAA type predominating in all the breeds sampled. The goat population indigenous to Nigeria was however found to be in Hardy Weinberg's equilibrium.

KEY WORDS: Indigenous goats, Haemoglobin, Electrophoresis, Genotypes.

INTRODUCTION

Goats play an important role in food production in developing countries. Their great popularity can be explained by their good adaptation to many different climates (ecological adaptation) and the many uses for which they can be kept. Indigenous goats have been classified into two main groups, the long-eared and short-eared. This is not a particularly useful system and a more appropriate one ascribes goats to large, small and dwarf types. Indigenous goats in Nigeria belong to three distinct breeds. The long-legged Sahel found in the arid and Sahel regions, the relatively small body-sized Red Sokoto found in the savanna zone and the hardy, short-legged West African Dwarf (WAD) restricted to high altitude areas and humid forest of the south (Adu *et al.,* 1976 and Osinowo, 1992). Haemoglobins are historically important for their part in the demonstration of the 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 clinically important as causes of a variety of genetic disorders of blood. Consequently, the genetic background of the haemoglobins merits examination in some detail (Peters *et al*., 2004). Polymorphism is a genetic variant that appears in at least 1% of a population in a herd. It is a discontinuous genetic variation where two or more forms, stages, or types exist in the same species within the same population. It can apply to biochemical, morphological, and behavioral characteristics, but must be discontinuous. 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 (Guney *et al*., 2003; Malan *et al*., 2003; Yang and Jiang, 2005). It has been reported by several researchers including Legates and Warwick (1990) as well as Adebambo (2004), 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 specific substance is directly related to the genotype. Different haemoglobin types may be selective advantages in different geographical regions (Ndamukong, 1995). For example, FAO (1988) reported that carriers of hemoglobin A have both demonstrated significant resistance against helminth infections. Di Stasio (1997) suggests 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).

MATERIALS & METHODS

Research animals

The animals used for this study were obtained from several small holder flocks especially for the breeds

predominant in the area; as well as big markets/abattoirs especially for the other breeds predominant to the dry climates of the north. A total of sixty animals (20 animals per breed – Red Sokoto, Sahel and West African Dwarf (WAD)) were used. Sampled animals were these three indigenous Nigerian goat breeds comprising of both female and male adults within breeding age group.

Blood collection

5ml of blood was collected from each of the sampled animals by jugular venipuncture using needle and syringe and into sample bottles containing Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant, properly labeled with respect to the different breeds and sex and preserved in a cooler containing ice blocks from whence it is transported to the laboratory where the samples were analyzed.

Blood Preparation

About 2ml of blood sample from each sample was placed into a clean test tube, 5ml of cold saline was added and the obtained diluents centrifuged at 4000 rpm for 10-15 minutes. The supernatant was discarded and the sample rewashed 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 at refrigeration temperature pending electrophoresis.

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 the standard procedure described by Smithies (1955) that was later modified by Boyer and Hiner (1963). The tank was powered with the lead closed and samples allowed to separate for about 10 – 15 minutes. By electrophoresis, the haemoglobin fractions were separated. The identification of the haemoglobin types in the different breeds of sampled goats was achieved in accordance with the migration speed of the light spots 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 the AA homozygote.
- The presence of a single slower band designated as BB homozygote
- The presence of both bands designated AB heterozygote

After separation, the cellulose acetate paper was blotted dry using filter paper and the result was taken.

Statistical analysis

Since only two alleles (A and B) were detected; then, hemoglobin genotype and gene frequencies were estimated as follows:

Genotype frequency AA = No of AA/ No sampled x 100/1
Genotype frequency AB = No of AB/ No sampled x 100/1
Genotype frequency BB = No of BB/ No sampled x 100/1.

Genotype frequency was estimated with respect to breeds and sex sampled. For the estimation of Gene frequency the equation was adopted as thus;

$P = (2N_{AA} + N_{AB}) / 2N$ 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 sampled
N_{AA}= observed genotype number for AA
N_{AB}= observed genotype number for AB
N_{BB}= observed genotype number for BB.
N/B: Hardy Weinberg equilibrium P + Q = 1.

RESULTS

Data obtained from this study were analyzed using descriptive statistical tools, 20 samples of each breed showed the haemoglobin distribution HbAA, HbAB and HbBB for Red Sokoto breed to be 10 , 3, 1 and 4 , 1, 1; Sahel breeds being 9, 6, 0 and 2, 3, 0; while WAD breeds were 17, 0, 0 and 3, 0, 0 respectively for the sampled female and male goats (Table 1).

TABLE 1: Effect of breed and sex on the haemoglobin types of Nigerian indigenous goats

BREED	n	SEX	n	AA	AB	BB
Red Sokoto	20	Female	14	10	3	1
		Male	6	4	1	1
		Total	20	14	4	2
Sahel	20	Female	15	9	6	0
		Male	5	2	3	0
		Total	20	11	9	0
WAD	20	Female	17	17	0	0
		Male	3	3	0	0
		Total	20	20	0	0

The genotype frequency (Hb %) of the sampled animals according to breed were 70, 20 and 10% for the Red Sokoto breeds; 55, 45 and 0% for the Sahel and 100, 0 and 0% for the WAD breeds; 75, 21.67 and 3.33% when samples were polled (all together) and 78.26, 19.57, 2.17% and 64.29, 28.57 7.14% for the females and males respectively irrespective of the breed (Table 2).

TABLE 2: Genotype frequency of three indigenous Nigerian goats with respect to breeds and sex

BREED	n	AA%	AB%	BB%	TOTAL%
Red sokoto	20	70.00	20.00	10.00	100.00
Sahel	20	55.00	45.00	0.00	100.00
WAD	20	100.00	0.00	0.00	100.00
Polled (All)	60	75.00	21.67	3.33	100.00
SEX					
Female	46	78.26	19.57	2.17	100.00
Male	14	64.29	28.57	7.14	100.00

Gene frequencies for the two alleles expressed *i.e.* A and B obeyed Hardy Weinberg's Equilibrium were (P + Q = 1) for breeds and sex. Values obtained for gene frequency for both breeds and sex include; 0.80 and 0.20, 0.77 and 0.23, and 1.00 and 0.00 respectively for Red Sokoto, Sahel and WAD breeds; 0.86 and 0.14 respectively when polled(all together); as well, 0.88, 0.12 and 0.79, 0.21 for female and male animals respectively as shown in Table 3 below.

TABLE 3: Gene frequencies of three Nigerian indigenous goats with respect to breeds and sex

BREED	n	A	B	Total
Red Sokoto	20	0.80	0.20	1
Sahel	20	0.77	0.23	1
WAD	20	1.00	0.00	1
Polled (All)	60	0.86	0.14	1
SEX				
Female	46	0.88	0.12	1
Male	14	0.79	0.21	1

DISCUSSION

In this study, three haemoglobin genotype were detected, two homozygote (AA and BB) and one heterozygous(AB) which corresponds to the general observation of A and B alleles with their corresponding genotypes AA, AB and BB in different species (Evans *et al*., 1956; Maxwell and Baker, 1980; Zaragoza *et al*., 1987; Tunon *et al*., 1989). The observation of the homologous HbBB types in the Red sokoto breed agrees with the report of Huisman *et al.* (1969), although Buvanendran *et al.* (1981) did not find this phenotype in the Red Sokoto breeds of goat. Johnson *et al.* (2002) reported that it is not readily apparent while the BB phenotype is not widely distributed but Buvanendran *et al.* (1981) considered that the disparity between the observed and expected frequency of certain phenotypes might be strong enough evidence of differential mortality. The predominance of HbAA types in the WAD breed of goats could be attributed to an adaptive feature for survivability of the breed in the high rainforest zone of Nigeria. Similar reports were documented by Evans and Warren (1958) that HbA has a selective advantage in sheep at higher altitudes because it constitutes the most common allele in highland breeds of English and Scottish sheep. The haemoglobin type AA was predominant in the three breeds (Red Sokoto, Sahel and WAD) as compared to other Hb types and the trend of predominance decreases from the more humid, high altitude, rain forest breeds of goats to the dryer, low altitude regions of the Sahel zone. There is an indication that vegetation or climate changes possibly affect Hb types. The frequency of HbAA was higher than that of HbAB and HbBB respectively (Table 1). This agrees with the result of Schillhorn van Veen & Folaranmi (1978) who observed that even when no deliberate selection pressure was applied at the locus, HbA genotype increases towards the forest zone where also the WAD sheep investigated in this are predominantly found. This same trend has already been reported in Yankasa sheep for the same region (Tella *et al*., 2000). HbA is reported to confer genetic resistance to helminth infection as speculated in the West African sheep, hence, the trend of helminth resistance should follow from the rain forest WAD goats having the most predominant HbAA frequency through to the Red Sokoto breeds and then to the Sahel breeds of goats although this was not tested in this study.The effects of HbAB and HbBB alleles on productive performance in sheep had been reported (Dally *et al*., 1980; Barowicz and Pacek, 1984; Arora, 1984 and Dratch *et al*., 1986). The Sokoto red breeds and the Sahel breeds are more productive than the WAD breeds with respect to milk yield, body weight gain etc, this corresponds with the frequency of the observed HbAb and HbBB in the Red Sokoto breeds and HbAb in the Sahel breeds when compared to the Wad breeds which was observed to be devoid of this Hb types. Hence, this could correspond with the report that this Hb types may have some effects on productive performance of these animals. Generally, it was found that the Alleles AA was predominant among females of the three breeds as compared to the male goats. The genotype frequency decreases from the AA through the AB and then to the BB for both sexes. The genotype frequency for the HbAA type is higher in the females of all breeds as compared to the males while that for the AB and BB respectively is higher in males than in females for the breeds. Gene frequencies for the Allele A for both sex is higher than that for Allele B and it however corresponds with Hardy Weinberg's equilibrium (P + Q = 1). There is not enough reason however, to conclude that sex has an effect on the disparity in Hb types. This assumption is partly due to the imbalance in the sample selection for both sexes with the number of sampled females being higher in value than that of the sampled males resulting in the values as evident in the gene frequency ratio of about 4:1 for females to males respectively.

CONCLUSION

Haemoglobin polymorphism is defined by expression of the three genotypes: two homozygotes, HbAA and HbBB, and one heterozygote, HbAB. The phenotypization of the haemoglobin variants is determined by two co-dominant alleles, HbA and HbB. The allele HbA registers a higher frequency than the allele HbB. Consequently, in the haemoglobin table, the homozygotes HbAA reach a high incidence, the heterozygotes HbAB have a middling frequency and the homozygotes HbBB are less present. Finally, it may be suggested that the HbAA type evident in the WAD goats of the high altitude, Southern rain forest region of Nigeria is favoured by natural selection, possibly for resistance against certain parasitic (internal and external) and some Haemo-parasitic infections which is not most obtainable with the other indigenous breeds especially when they are brought to the said region. The HbAB predominant in the savanna breeds of goats could possibly have a rather direct or indirect relationship with their various phenotypic characteristics. The result of this study is however, subjected to some limitations being that the sample size for the analysis was rather too small and this is due to financial and time constraints as well as animal resources encountered during the course of this work.

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