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Effects of sex and haemoglobin types on gene and allelic frequencies in Red Sokoto goats in semi-arid region of Nigeria

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Target Audience: *Animal Scientists, Breeders, Goat Farmers, Government.*

Abstract

The experiment was conducted to determine the haemoglobin types and their gene and Genotypic frequencies in Red Sokoto goats. A total of 321 (34 bucks and 287 does) Red Sokoto goats were sampled for the study and 213 of them were sampled for haemoglobin type. The populations of the Red Sokoto goat were studied using Hardy-Weinberg equilibrium procedures. Four haemoglobin types were discovered, namely; HbAA, HbAB, HbBB and HbAC. Only two (2) animals sampled had the rare pre-adult haemoglobin type (HbAC). Haemoglobin AC, AA and BB were identified as the fastest single bands retrogressively with AB being a double band. The gene (allelic) frequency for haemoglobin A and B were 0.6 and 0.4 representing a 'high-low' pattern. The genotypic frequencies for HbAA, AB and BB were 0.36, 0.48 and 0.16 representing a-low-high pattern and a greater preponderance for the HbAB. The expected ratio of Hb variants (HbAA, HbAB and HbBB) in this population of goats was 2:2:1 which was different ($P<0.01$) from the observed ratio of 3:6:1. The observed ratio of 3:5:1 for the does did not differ ($P>0.05$) from the expected ratio of 2:3:1. However, it differed for the bucks which had observed ratio of 1:4:0. The HbAC typed goats were the least in performance compared to HbBB, HbAA and HbAB variants in Red Sokoto goats. In essence, the haemoglobin genotype HbAB in Red Sokoto goats seems to be favoured by natural selection with preponderance for the HbA allele which suits the northern highlands of Nigeria, though goats with HbBB genotype showed greater promise for improved production.

Key words: *Goat, Red Sokoto, Haemoglobin types, Genotypic frequency, Semi-arid.*

Description of Problem

Small ruminants are increasingly becoming a major source of animal protein in Nigeria; they are almost as ubiquitous as poultry, though not as numerous. They have a total estimate of 56.6 million throughout the country, with goats outnumbering sheep by three to two (1). The Red Sokoto goat (RSG) or Maradi is the most predominant goat and accounts for about 70% of Nigeria's total goat population which has been estimated at 34.45 million (2). The breed is predominantly reddish brown in colour and is found in the Savanna zone of Nigeria (8°N-11°N) where it constitutes more than 90% of the goat population in that area. The breed weighs about 1.5-2.0kg at birth and reaches about 12.0kg when weaned at 3 months under good management. Weights of adult does and bucks are 20-35kg and 25-40kg, respectively (3).

Genetic variations among the small ruminant varieties that are indigenous to

Nigeria have been a point of concern to geneticists. This is because the amount of genetic variation detectable in an animal population is related to the magnitude of genetic improvement achievable within the species. Thus genetic variation has become the objective tool traditionally used for improving animal species (4). The small ruminants of Nigeria have been variously evaluated for genetic variation based on morphological and productive characters/data. However, morphological variations have been documented to underestimate true levels of genetic variations. In the recent decades, advances in the field of biotechnology have opened up a completely new area at molecular levels with the introduction of techniques such as routine electrophoresis employed for the detection of polymorphism at protein and enzyme loci as well as other serological and immunogenetic procedures for the measurement of variation (4).

Data obtained from this type of study could be useful as genetic markers for important economic characters and diseases and could aid significantly in selection of superior animals for breeding purposes. Reports and documentations on these animals from the molecular standpoint are scarce. One of the important erythrocyte proteins that have attracted attention because of its relevance to the selection process is the hemoglobin, an alpha and beta containing polypeptide chain. The existence of two types of Haemoglobin (Hb), types A and B, has been well documented in goats (5). They are expressed as homozygous (HbAA and HbBB) and heterozygous (HbAB and HbAC); phenotypes with HbAC being a pre-adult form of Hb (5). The understanding of biochemical polymorphism has contributed immensely towards unraveling some of the mysteries behind certain biological phenomena. In so doing, gene codes for proteins like haemoglobins and transferrins (6; 7) have found use as genetic markers. Knowledge of the association between these haemoglobin types and genotypic frequency of Red Sokoto goat will go a long way to improving the genetic background of our indigenous herd. Therefore, the objective of this study was to determine the haemoglobin types and gene frequencies in Red Sokoto goat in semi-arid region of Nigeria.

Materials and Methods

Experimental Location

The field research was conducted in the semi-arid zone of Nigeria. The semi-arid zone of Nigeria starts from about 11ºN latitude and ends at the Nigeria-Niger frontier. It encompasses the Sudan and Sahel Savanna and part of the Northern Guinea Savanna. The mean annual temperature runs between 26 and 28ºC. There is a single rainy season from May to October, with mean annual rainfall ranging from 1016mm in the wettest parts to less than 508mm in the driest parts. The length of growing period is about 100-150 days which makes it possible to cultivate a wide variety of crops (8). The semi-arid zone has a land mass of 113,530km2 and a population of over 35 million people (9). The major inhabitants of this area are Hausa and Fulani who are predominantly mixed crop-livestock farmers and livestock herders respectively.

Sampling Size, Sampling Structure and their Characterization

The Red Sokoto goats were sampled using a random sampling method to determine their body morphological traits. A total of three hundred and twenty one (321) Red Sokoto goats were used for this study while two hundred and thirteen (213) of them were evaluated for haemoglobin type or polymorphism. Thirty-four (34) of the goats sampled were male while two hundred and eighty-seven (287) were female.

The age of the goats was determined using teeth count (2) in combination with the information provided by the goat owners.

Measurements/Observations of Physical Parameters

Some of the general measurements taken on the animals include:

1. **Body weight-** The body weight was taken for each goat using a scale. Weight was taken in Kilogram (Kg).
2. **Age-** The pairs of permanent incisors in the dentition of the goat was used to determine age.
3. **Sex-** The sex of the animal was also noted.

Blood Sampling and Analysis for Hb Types

Blood samples were obtained via jugular venipuncture; about 5ml of blood was drawn by using a syringe into heparinized vacutainer tubes containing ethylene diamine tetra-acetic acid (EDTA) as blood anti-coagulant. The test tubes were then labelled according to ascribed numbers given to each goat sampled for body mensuration characteristics. The blood samples were then taken to the laboratory, washed with normal saline and then haemolysed with distilled water to release the haemoglobin. The supernatant was removed after centrifuging at 3000 rpm for 5 min and the sample haemoglobin stored until ready for electrophoresis.

Human haemoglobin AA and AS were used as controls for the first 100 samples. This was to develop a control for the caprine samples. On development of caprine control, the procedure was then repeated for all the samples using caprine haemoglobin AA and BB as controls.

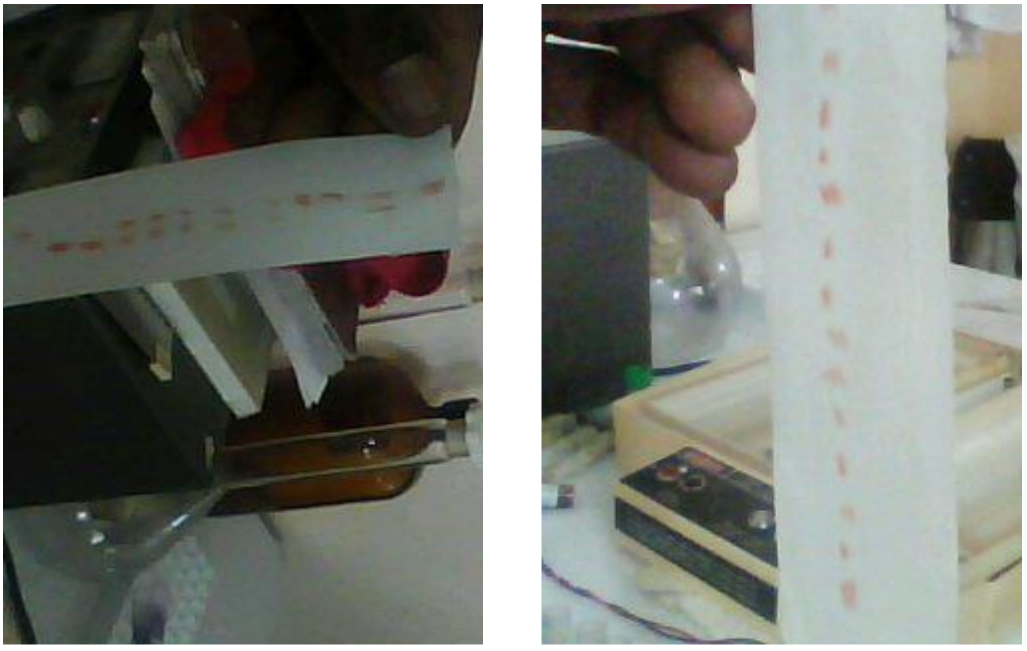

Fig 1- Showing haemoglobin bands using Electrophoresis.

Cellulose acetate paper strip electrophoresis was employed to separate the globin fractions. Electrophoresis was carried out in a Shandon electrophoresis tank on cellulose acetate strips 34.5x 150mm with 0.26MTris buffer (pH 9.1) at both the anode and cathode. The strips were run for 5 minutes at a constant voltage of 250v until a clear separation was obtained.

On separation, the strips were stained with ponceau-s stain, later washed with 5% glacial acetic acid, and dried using filter paper. Interpretations were made based on the relative mobility of the haemoglobin bands towards the anode. The genotype that migrated faster was labeled HbAA, the slow moving fraction was identified as HbBB while the heterozygote (consisting of both slow and fast bands) was HbAB (10; 11).

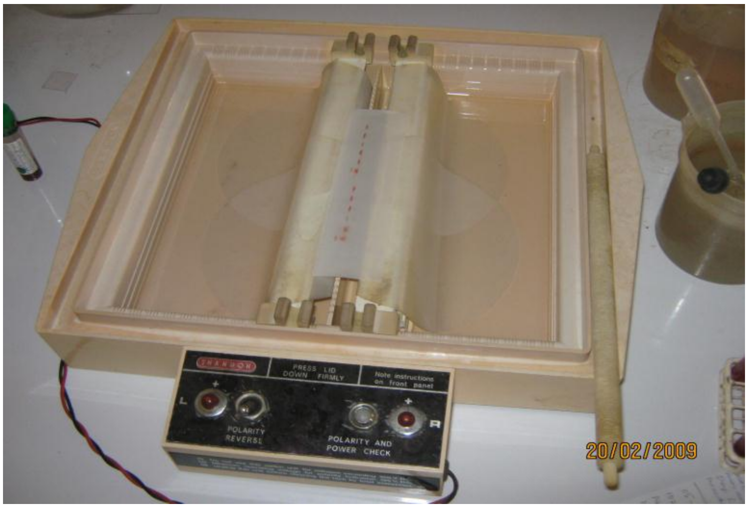

Fig 2- An electrophoresis tank showing acetate strips and band separations as a result of current flowing from a galvanometer.

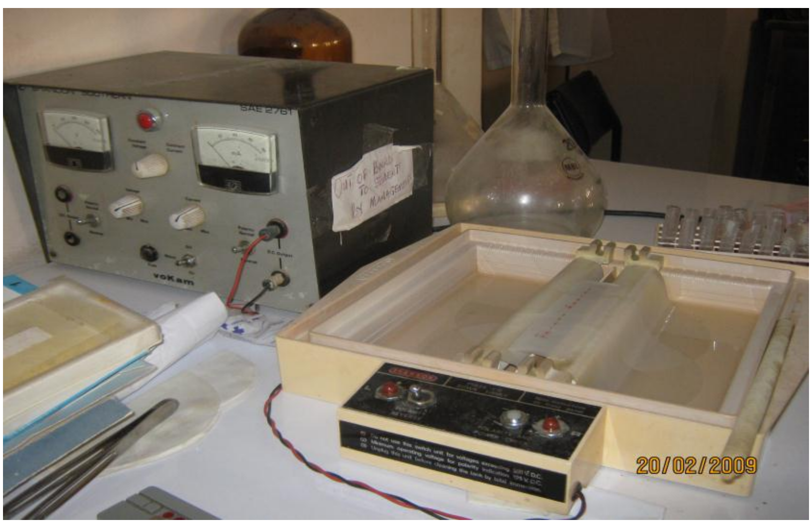

Fig 3- An electrophoresis tank with galvanometer

Strip 1: Haemoglobin types of Nigerian Red Sokoto goats using caprine HbAA and HbBB as controls

a'- Caprine HbBB (Control)
n'- Caprine HbAA (Control)
a,b,d,- Bovine HbBB
c,e,f,g,h,i,j,k,l,m,n- Caprine HbAB
+ - Anode

Strip 2: Haemoglobin types of Nigerian Red Sokoto goats using Human HbAA and HbAS as controls.

a'- Human HbAA (Control)
n'- Human HbAS (Control)
a, b- Caprine HbAA
c, d, h, j, k- Caprine HbBB
e, f, g, i, l, m, n- Caprine HbAB
+ - Anode

Data Computation/Statistical Analysis

Haemoglobin types, gene and allelic frequencies were estimated using a Chi-square Frequency procedure of (12) package and haemoglobin types were determined as earlier stated above.

$$\text{Chi-square } (\chi^2) = \frac{(\text{Observed frequency} - \text{Expected frequency})^2}{\text{Expected frequency}}$$

Model:

$$Y_{ij} = \mu + H_i + e_{ij}$$

Where;

Y_{ij}= Measured variable or value of any observation
μ = Overall mean
H_i= Haemoglobin type effect (1, 2, 3, 4)
e_{ij}= residual effect/error term

Hardy-Weinberg's equilibrium was used for testing the significance of genotypic ratios. This equation is based on the binomial expansion $(p+q)^2 = p^2+2pq+q^2$.

Results and Discussion

The distribution of goats aged 6 months and above according to sex and haemoglobin type is presented in Table 1. There were more females (89.40%) than males (10.60%) for goats that aged 6 months and above in the goat herds studied. Four haemoglobin types were identified from the population under study, HbAA, HbAB, HbBB and HbAC. The HbAB (58.22%) was the most frequent type followed by HbAA (30.52%) and HbaBB (10.33%). The frequency of HbAC (0.93%) in the studied population was the least. The HbAB genotype was the most occurring genotype in the Red Sokoto goat population with 58.2% of the population positive for this genotype and followed by AA (30.5%) with the least value of 10.3% recorded for BB genotype. Generally, the overall results revealed a very high frequency of 124 for HbAB genotype followed by HbAA which have 65 while the HbBB genotype had the least frequency of 22. The same trend was observed when sex was considered with HbBB being notably absent in bucks.The four haemoglobin types observed, that is, HbAA, HbAB, HbBB and HbAC in this study were similar to those documented by (5). The observation in this study indicated that HbA moves faster than HbB and HbAB has both the A and B bands (double bands) (strips 1 and 2) plus the fact that the foetal haemoglobin HbC has a faster mobility than the two adult variants (A and B) and these observation agrees with the findings of other workers (5;10;4). Several authors have reported that HbC is always associated with incidence of anaemia due to illness and environmental stress (4) served as health status indicator. The low frequency of HbC emanating from only two animals could either be associated with this or error in reading the bands due to the band's weak and faint appearance or error due to poor resolution normally experienced for certain experimental protocols in electrophoresis.

(13) also recorded low frequencies of

HbC allele in the goats of Colon and Ischlin in Argentina.

The genotype results obtained presently were contradicted the findings of (4) and (5) that had greater population of HbBB genotypes in both Red Sokoto goats (50%) and Garole Sheep (97.3%) respectively. The reason for these differences could be due to environmental and differences in the animals used in this study. However, results here were similar to those gotten by (5) for Omani goats which had 66% HbAB genotype in their population.

Generally, the overall results revealed a very high frequency of HbAB followed by HbAA. The same trend was observed when sex was considered with HbBB being notably absent in bucks. This could be as a result of the low population of bucks sampled in this study rather than a total absence of the genotype all together in the male gender.

Table 2 shows the gene and genotypic frequencies of haemoglobin types in Red Sokoto goats. The gene frequencies were higher for Hb^A than Hb^B, irrespective of the sex of the goat. The genotypic frequencies revealed a low-high-low pattern for HbAA, HbAB and HbBB with a lower frequency for HbBB. The gene or allelic frequencies of 0.60 for haemoglobin A and 0.40 for haemoglobin B represented a 'high-low' pattern. This was in contrast to the gene frequencies of 0.48 and 0.52 for HbA and HbB respectively for sheep and 0.32 and 0.68 for HbA and HbB respectively for Red Sokoto goats reported by (4). In his findings, a 'low-high' pattern was observed and was also in contrast to the findings of (10). They discovered a gene frequency of 0.014 for HbA and 0.986 for HbB in Garole sheep.

Table 1: The Distribution of goats aged at 6 months and above according to sex and haemoglobin type

Attribute	Observed	Frequency (%)
Sex	321	
Male	34	10.60
Female	287	89.40
Haemoglobin Type (Hb)	213	
HbAA	65	30.52
HbAB	124	58.22
HbBB	22	10.33
HbAC	2	0.93

Table 2: Gene and Genotypic Frequencies of Haemoglobin Types in Red Sokoto Goats

Sample size	Gene frequencies		Genotype Frequencies		
	Hb^A	Hb^B	Hb^{AA}	Hb^{AB}	Hb^{BB}
211	0.60	0.40	0.36 (65)	0.48 (124)	0.16 (22)
Does					
180	0.60	0.40	0.36 (59)	0.48 (99)	0.16 (22)
Bucks					
31	0.60	0.40	0.36 (6)	0.48 (25)	0.16 (0)

Figures in parenthesis indicate the number of animals

Table 3 shows the observed and expected number of haemoglobin genotypes in male and female Red Sokoto goats. The population of Red Sokoto goats was studied in Hardy-Weinberg equilibrium. The expected ratio of Hb variants (HbAA, HbAB and HbBB) in this population of goats was 2:3:1 which was different ($p<0.01$) from the observed ratio of 3:6:1. The observed ratio of 3:5:1 for the does did not differ ($p>0.05$) from the expected ratio of 2:3:1; however, it differed for the bucks which had observed ratio of 1:4:0. The gene and genotypic frequencies of the Hb variants in Red Sokoto goats are highly variable and yet stabilized to fit into expected ratios.

The genotypic frequencies of HbAA, HbAB and HbBB were 0.36, 0.48 and 0.16 corresponding to 65, 124 and 22 animals. The same figures were recorded for both does and bucks. In either case, a"Low–High–Low" pattern for the incidence of haemoglobin type were observed. (10) reported dissimilar results having genotypic frequencies of 0.0, 0.027 and 0.973 for HbAA, HbAB and HbBB respectively with HbBB having an overwhelming majority and HbAA being totally absent. Studies conducted on sheep and goats by (4) were also dissimilar to the findings here. (4) reported sheep genotypic frequencies of HbAA, HbAB and HbBB to be 0.4, 0.1 and 0.5 for ewes and 0.4, 0.25 and 0.35 for rams respectively. While when sex was ignored, they were 0.4, 0.17 and 0.42 respectively. For goats though (4) reported genotype frequencies to be 0.13, 0.36, 0.5 and 0.01 for HbAA, HbAB, HbBB and HbAC. In both sheep and goats however, HbBB seemed to have the majority with the exception of the rams. In all cases with the exception of the goats sampled, HbAB had the least genotypic frequency.

Generally, the research carried out here has brought forth a few interesting results; the low frequency of the HbBB genotype plus its subsequent absence in the small population of males suggest some adaptive superiority of the HbAA over HbBB. (4) in this respect also reported that Red Sokoto goats sampled in the south west of Nigeria (Ibadan) had a higher frequency of HbB suggesting they are fit for lower altitudes (Ibadan approximate, altitude – 780ft above sea level. Source: falling Rain Genomics Inc – 1996–2006). (10) had also previously reported a higher frequency of HbB in Kolkata, West Bengal, India (altitude 42ft). The preponderance of HbA and its greater affinity to oxygen have also been shown to be characteristic of sheep flock of higher altitudes (14).

The population sampled in the current study agreed with all the above conclusions as they are from a geographical location of higher altitude (Katsina 1525ft). (15) put forth the idea that haemoglobin B indicates an Asiatic rather than an African ancestry for most cattle breeds, which makes its frequency higher in the Indian breeds. According to him, the further away the Bos indicus types migrated from India, the lower is the frequency of HbB allele which is also true for the breeds in Southern Europe, except for the high incidence of HbB allele on the Jersey Islands. The same postulate could also be brought forth in the case of the Red Sokot goats, which are thought to have originated from Asia. (16; 17) also reported higher incidence of HbB allele in exotic sheep of India.

The significant differences between haemoglobin types in bucks and in the overall population and the no significant association between haemoglobin types in does could be because of the small sample size of bucks.

Table 3: Observed and expected number of haemoglobin genotype in male and female Red Sokoto goats.

Sample size	Hb Phenotype	Observed No.	Ratio	Expected No.	Ratio	df = 2
Overall						
211	HbAA	65	3	78	2	
	HbAB	124	6	101	3	10.54**
	HbBB	22	1	32	1	
Bucks						
31	HbAA	6	1	11	2	
	HbAB	25	4	15	3	13.94**
	HbBB	0	0	5	1	
Does						
180	HbAA	59	3	66	2	
	HbAB	99	5	86	3	4.0^{NS}
	HbBB	22	1	28	1	

** = Highly significant P<0.01; NS= Not significant

Conclusion and Applications

1. The haemoglobin type or genotype HbAB in the Red Sokoto goat seems to be favoured by natural selection with a preponderance for the HbA allele or gene
2. The goats sampled with the HbBB genotype have shown greater promise for improved production.
3. Larger samples should be used when comparing frequencies of molecules or genes. Larger sample sizes remove biasness and a tendency for results to skewer towards one direction.

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