HAEMOGLOBIN AND POTASSIUM POLYMORPHISM IN AGRO-PASTORAL GOATS HERD FROM SUDAN SAVANNAH ZONE OF NIGERIA

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ABSTRACT

This study was conducted to determine the haemoglobin (Hb) and potassium (K) polymorphism and its distribution in agro-pastoral goats herd of Sudan savanna zone of Nigeria. Five (5l) ml of blood samples were collected each from 250 smallholder agro-pastoral goats and used for this study. The blood samples were collected by jugular venipuncture, using needle and syringe into test tube containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant and samples were properly labeled. The electrophoretic analysis showed three haemoglobin genotypes HbAA, HbAB and HbBB. These genotypes were produced by 2 co-dominant alleles HbA and HbB. The genotypic frequencies were 0.47, 0.43 and 0.099 for HbAA, HbAB and HbBB, respectively. The preponderance of HbA alleles (0.683) was twice its co-dominant allele HbB (0.316). The Chi-square test revealed that the population of goats in the study area was in Hardy-Weinberg equilibrium. The gene frequencies of potassium polymorphism were 0.844 and 0.156 for high (HK) and low potassium (LK) types, respectively. The genotypic frequencies were 0.91 for HK and 0.01 for LK in the overall population. The observed frequencies of potassium types were also in Hardy-Weinberg equilibrium in both locations and in the overall population. thus suggesting that random mating occurred for the system under study and artificial selection has not much been practiced by the smallholder agro-pastoral goats farmers in the study area.

Keywords: Haemoglobin, Potassium, Polymorphism, Agro-pastoral, Goats, Genotypes, Dominant allele, Hardy-Weinberg equilibrium

INTRODUCTION

Genetic variations among the small ruminants that are indigenous to Nigeria have been the focus of 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 (Salako *et al*., 2007; Akpa *et al*., 2011). Advances in the field of biotechnology have led to 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 procedure for measurement of variation (Salako *et al*., 2007). Information from this type of study could be useful as genetic markers for important economic characters and could help in selection of superior animals for breeding purposes. Research reports on Nigeria indigenous goats based on molecular standpoint are limited.

Haemoglobin, an important erythrocyte protein inherited by co dominance in Mendelian fashion has been reported to be a useful marker through which many economic traits, with which it is associated, have been improved in farm animals (Akinyemi and Salako, 2010). Potassium is one of the intracellular elements which constitute the structure of an organism. Its main function is to regulate intracellular density of cells (Palmer, 2015). In ruminant, there are two potassium types, high potassium (HK) and low potassium (LK). The LK is dominant over the HK type. The HK and LK allele frequencies are different among sheep breeds (Shahrbabak *et al.,* 2006), and potassium types have been proved to be genetically controlled (Al-Samarrae and Younis, 2011) and therefore could be a useful tool for selection.

Haemoglobin and potassium types can be determined easily at postnatal period of young animals and these components are not affected by environmental factors (Gurcan *et al*., 2010). Many studies have been conducted using different sheep and goat breeds to determine the level of various blood parameters and to evaluate the relationship between blood biochemical polymorphism and production traits (Shahrbabak *et al*., 2006; Gurcan *et al.,* 2010; Akinyemi and Salako, 2010). These associations have been utilized as a polymorphic marker among domestic animals. This study was conducted to determine the haemoglobin (Hb) and potassium (K) polymorphism and its distribution in agro-pastoral goats herd from the Sudan savanna zone of Nigeria.

MATERIALS AND METHODS

Study Area: The Study was carried out in Jigawa and Katsina States within the Sudan savannah zone of North-West Nigeria. Katsina lies between latitude 11°30 -13°01′ north of the Equator and longitude 07°41′- 10° east of Greenwich Meridian. It is situated at an altitude of 464m (1525ft) above sea level. Generally, the climate varies considerably according to seasons. The seasons are: cool dry (harmattan) season (December to February); hot dry season (March to May); warm wet season (June to September); a less marked season after rains during the months of October to November, characterised by decreasing rainfall and a gradual lowering of temperature. Mean annual rainfall is about 780 mm. Katsina State is hot for most parts of the year, even during the wet season. Mean annual temperatures range between 19° in the cold dry season to 38°, with the highest temperatures normally experienced in April and May, just before the rains (Ati *et al*., 2007). Jigawa State falls within latitude 11°-13°N and Longitude 8°-10°E. The mean annual rainfall varies from 500mm to 1000mm. Rainfall is higher in the Southern part of the State. The mean daily maximum and minimum temperatures are 35°C and 19°C, respectively. The maximum temperature has two peaks occurring in April and October. The lowest temperatures are recorded during the month of December and January. At this period the temperature can fall as low as 10°C or lower at night. The mean relative humidity can be as high as 80% in the month of August and as low as 15% in December. The State has an altitude of between 400 – 600m above sea level. There are two seasons in Jigawa State namely: rainy and dry seasons with the dry season lasting from November to May and the rainy season lasting between June and October (Magashi, 2011).

Animals Management: The goats were managed under the rural agro-pastoral system of management. The animals were taken out to graze every morning from 8.00 am to 5.00 pm by children and were penned at night. On return from grazing, the animals were penned in small open sided shades by tethering. Before setting out for grazing every morning, the goats were given water and supplement which included groundnut haulms, cowpea haulms and dry grasses. Each animal was tagged with a number for individual identification.

Blood Collection and Analysis: Five (5) ml of blood samples were collected from 250 smallholder agro pastoral goat herds. The blood samples were collected by jugular venipuncture, using needle and syringe into test tube containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant and samples were properly labeled. The blood samples were then washed with normal saline and 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. Cellulose acetate paper strip was used to separate the globin fractions. Electrophoresis was carried out in Shandon electrophoresis tank on cellulose acetate strips 34.5 x 150 with 0.26 M Tris buffer (pH 9.1) at the anode and cathode. The strips were run for 5 minutes at a constant voltage of 250v until a clear separation was observed. Interpretations were made based on the relative mobility of the haemoglobin bands towards the anode. The resulting electrophoregram (Figure 1) showed haemoglobin bands distinct movement toward anodic end of the electrophoretogram.

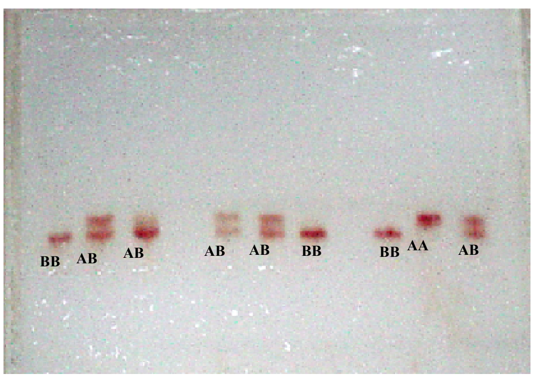

Figure 1: Haemoglobin electrophoregram of agro-pastoral goats herd from Sudan savannah zone of Nigeria

The genotype that migrated faster was labeled HbAA; the slow moving fraction was identified as HbBB. The double band, consisting of both fast and slow band; was labeled HbAB as described by Tella *et al*. (2000) and Das *et al*. (2004). The potassium and sodium concentrations in the blood were determined by colorimetric method using spectrophotometer. The erythrocyte potassium below or equals to 13.00 mmol/l was labeled low potassium, while the erythrocyte potassium above 13.00 mmol/l was tacked high potassium (Galip and Elmaci, 2001).

Statistical Analysis: The data generated from the study were analysed using t-test procedure of SAS (2000) to test the differences between the two locations. The haemoglobin gene and genotypic frequencies were estimated as follows: (i) Genotypic frequency of AA = Number of individuals with AA x 100 ÷ Number of individuals sampled, (ii) Genotypic frequency of AB = Number of individuals with AB x 100 ÷ Number of individuals sampled, (iii) Genotypic frequency of BB = Number of individuals with BB x 100 ÷ Number of individuals sampled, (iv) Allelic frequency of A = AAx2 + AB ÷ Total number of alleles and (v) Allelic frequency of B = BBx2 + AB ÷ Total number of alleles. The expected genotypic frequency was calculated using the formula $p^2 + 2pq + q^2 = 1$, while the genotypic frequencies for potassium types were estimated using the square root method as defined by Cotterman (1954). Based on this method, the homozygote genotypic frequency was calculated by taking the square root of homozygote recessive gene frequency. The HK alleles were dominant over LK alleles. Furthermore, Chi-square test was used to estimate the conformation mode of the goat population to the Hardy-Weinberg genetic equilibrium. The empirical formula used was $\chi^2 = \sum (\text{observed} - \text{expected})^2 \div \text{expected}$.

RESULTS AND DISCUSSION

Electrophoretic Haemoglobin Pattern: The electrophoretic analysis in this study showed three haemoglobin genotypes HbAA, HbAB and HbBB (Figure 1). These genotypes were produced by 2 co-dominant alleles HbA and HbB. The presence of these three haemoglobin genotypes is in agreement with the findings of other studied in goats (Salako, 2002; Hrinca, 2010). However, this report is contrary to the reports of Johnson *et al*. (2002), Ibrahim (2010) and Alphonsus *et al*. (2012) who reported the presence of four haemoglobin types (HbAA, HbAB, HbBB and HbAC) in red Sokoto goats. The absence of HbAC in the present study could be due to the facts that only adult goats were used for this study, and HbAC which is also known as foetal haemoglobin is mostly associated with very young animals (Das *et al*., 2004; Salako *et al*., 2007; Essien *et al*.,2011; Alphonsus *et al.,* 2012). A report by Hrinca

(2010) explained that, the polymorphism degree of haemoglobin system of goat breed is defined by the number of alleles, the ratio between them, the interallelic combinatory capacity, the number of genotypes expressed, their distributions, the grouping mode, variability ranges and dispersion degree. The distribution of the two haemoglobin alleles A and B within the overall population of goat studied was different (Table 1). The allelic frequencies showed that the preponderance of HbA alleles (0.683) was twice its co-dominant allele HbB (0.316). However, the distribution of the haemoglobin alleles in the two locations was similar. The gene frequency of HbA alleles was higher in both Katsina (0.615) and Jigawa (0.656) than the HbB alleles 0.385 and 0.344, respectively. The similarity in the distribution pattern of the Hb-type in the two locations is probably due to similarity in topography and climatic condition of the two locations, the altitude of Katsina and Jigawa are 464 m and 400 – 600 m, respectively. It has been reported that the gene and genotypic frequencies of haemoglobin types differs with altitude and topography of the environment in which the animal lives and that the frequency of HbA is usually higher than that of HbB in high altitude (Alphosus *et al.,* 2012; Akpa *et al.,* 2013). Elisa *et al*. (2010) stated that the expression of Hb-types is related to the suitability of the animal under particular environmental conditions. The genotypic frequencies were 0.47, 0.43 and 0.099 for HbAA, HbAB and HbBB, respectively. This was very close to the previous reports (Ibrahim, 2010; Akpa *et al*., 2011; Alphonsus *et al.,* 2012). The relative high frequencies of the HbA than the HbB suggest that the HbA alleles are favoured by natural selection of the study area (Katsina and Jigawa states). This is in agreement with the previous assertion by Agaviezor *et al.* (2013), that HbA genes are favoured in environment with high altitudes, while HbB are favoured by lower altitudes. In contrast to the present study, Das *et al*. (2004) and Salako *et al*. (2007) reported higher frequency of HbB than HbA at the low altitude of West Bengal India (altitude 42 ft) and Ibadan is (780 ft)above sea level, respectively. The Chi-square test revealed non-significant differences between the observed and expected frequencies of haemoglobin alleles in the overall population and in both locations (Table 2). This indicated that the population of goats was in Hardy-Weinberg equilibrium, thus suggesting that control mating and selection has not been practiced in the agro-pastoral goat herds studied hence no significant change in the gene frequencies.

The percentage frequency of HbAB was the highest 44.80 %, followed by HbAA 43.20 % and the least was HbBB 12 % in the overall population (Table 3), similarly, in Katsina State, the phenotypic frequencies were 43 %, 37 % and 20 % for HbAA, HbAB and HBB, respectively. However, in Jigawa State, the homozygote HbAA was the most frequent (50.00 %), followed by the heterozygote HbAB (43.33 %) and the least was HbBB (6.67 %). This finding is contrary to report of Ibrahim (2010), Das *et al*. (2004) and Hrinca (2010). The results of the present study suggest that the low frequency of the HbBB genotype might be a form of adaptive superiority of HbAA and HbAB over HbBB. Hrinca (2010) explained that extreme temperature (acute cold or sultry heats), the extreme forms of relief (dessert or mountain) and precarious nutrition conditions favour the fixing of the allele HbA, and the temperature situated in the biological comfort zone, the moderate forms of relief (forest steppe, hill, etc.) or the optimal breeding technologies are correlated with a more emphasized fixing of the allele HbB. The present study was conducted in the northwest of Nigeria which is characterised by extreme temperature (which could be as high as 38 – 45 ^{o}C) during the hot season and extreme dry cold. These extreme variations in temperature and other climatic conditions probably favoured the fixing of HbA over HbB alleles. Salako *et al*. (2007) reported that Red Sokoto goats sample in the South West of Nigeria (rain forest zone) had a higher frequency of HbB than HbA, suggesting that they best fit in that zone where the temperature is not so high.

Potassium types, gene and genotypic frequencies: The gene and genotypic frequencies of potassium types in goats is shown in Table 4.

Table 1: Gene and Genotypic Frequencies of Haemoglobin types of Goats in Jigawa and Katsina States of Nigeria

Population	Number of Animals	Gene Frequencies		Genotypic Frequencies		
		Hb^A	Hb^B	HbAA	HbAB	HbBB
Overall	250	0.656	0.344	0.432 (108)	0.448 (112)	0.120 (30)
Katsina	100	0.615	0.385	0.43 (43)	0.37 (37)	0.20 (20)
Jigawa	150	0.683	0.317	0.433 (65)	0.50 (75)	0.067 (10)

Figures in parenthesis indicate the number of animals

Table 2: Observed and expected number of haemoglobin genotypes of goats in Jigawa and Katsina States of Nigeria

Population/sample Size	Hb Phenotype	Observed Number	Expected Number	df=2 χ^2
Overall 250	HbAA	108	107.6	0.809^{ns}
	HbAB	112	112.83	
	HbBB	30	29.58	
Katsina 100	HbAA	43	37.82	0.626^{ns}
	HbAB	37	47.36	
	HbBB	20	14.82	
Jigawa 150	HbAA	65	69.97	1.074^{ns}
	HbAB	75	64.95	
	HbBB	10	15.07	

*ns = not significant, *P < 0.001*

Table 3: The phenotypic distribution of goats in Jigawa and Katsina states of Nigeria based on haemoglobin types

Hb types	Observed	Frequency	Observed	Frequency	Observed	Frequency
	Overall		Katsina		Jigawa	
Number	250		100		150	
HbAA	108	43.20	43	43	65	43.33
HbAB	112	44.80	37	37	75	50.00
HbBB	30	12.00	20	20	10	6.67

The overall gene frequencies of the K-types were 0.844 and 0.156 for high potassium and low potassium, respectively. The genotypic frequencies were 0.91 for HK and 0.09 for LK types in the overall population. For the two locations, the distribution of the gene frequencies of the potassium types were similar; 0.83 and 0.17 in Katsina and 0.853 and 0.146 in Jigawa for HK and LK, respectively. Also the genotypic frequencies were similar for the two locations; in Katsina, the genotypic frequencies were 0.91 and 0.09 for HK and LK respectively, and in Jigawa State the frequencies observed were 0.92 and 0.08 for HK and LK respectively. The observed frequencies of potassium types were in Hardy-Weinberg equilibrium in both locations and in the overall population (Table 5). This finding was in agreement with the findings of Cobanoglu *et al*. (2011) who reported allele frequencies of 0.80 and 0.20; 0.85 and 0.15; 0.87 and 0.13 for HK and LK respectively in Saanen, Maltase and Turkish hair goats from Turkey. Although low potassium (LK) type is dominant over its counterpart high potassium (HK) type genetically (Al-Samarrae and Younis, 2011; Hrincă, 2015), majority of the animals (about 90 %) were of HK type in the goat population studied.

Table 4: Gene and genotypic frequencies of potassium types of goats Jigawa and Katsina States of Nigeria

Population	Number of Animals	Gene Frequencies	Genotypic Frequencies		
		HK	LK	K^H	K^L
Overall	250	0.84	0.16	0.91	0.09
Katsina	100	0.83	0.17	0.91	0.09
Jigawa	150	0.85	0.14	0.92	0.08

Table 5: Observed and expected numbers of potassium genotypes of goats in Jigawa and Katsina States of Nigeria

Population/sample Size	Potassium Phenotype	Observed Number	Expected Number	df=1 X^2
Overall (250)	K^H	211	178.00	0.946^{ns}
	K^L	39	65.78	
Katsina(100)	K^H	83	68.80	0.871^{ns}
	K^L	17	28.2	
Jigawa(150)	K^H	128	109.05	6.747^{ns}
	K^L	22	3.19	

Ns = not significant; p<0.001

This is probably due to the close relationship between the K-types and Hb types, where by animals with HbA were reported to have high K. A study by Hrincă (2015) showed that lambs with HbA had significantly more potassium in their blood than those possessing either HbB or HbAB and that excess of high K types was observed amongst carriers of haemoglobin A genes. This suggests that HbA is strongly associated with HK; the fact that the preponderance of HbA allele in the population of goats studied was higher (0.683) than HbB could explain the reason for the observed high frequency of HK than LK type in the population of goats studied. This situation could also suggest that HK-type animals might naturally have selective advantage in the studied area. It had been observed (Shahrbabak *et al*., 2006) that HK allele is predominant in dry region with harsh temperature while LK allele pre-dominates region with normal and humid climate. From the foregoing, it could be suggested that goats with HbA alleles and high potassium (HK) types are adapted to high altitude and extremes whether conditions than those with HbB and low potassium (LK) type.

Conclusion: The Chi-square test revealed non-significant differences between the observed and expected frequencies of haemoglobin and potassium alleles in the overall population of goats studied and in both locations. This indicated that the population of goats was in Hardy-Weinberg equilibrium for both Hb and K-types, thus suggesting that random mating occurred for the system under study and artificial selection has not much been practiced by the smallholder agro-pastoral goats farmers in the study area.

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