INBREEDING COEFFICIENT, HETEROZYGOSITY AND HAEMOGLOBIN POLYMORPHISM IN CATTLE, SHEEP AND GOAT REARED IN KOGI STATE UNIVERSITY LIVESTOCK FARM

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ABSTRACT

Sixty five animals (10 Yankasa sheep, 30 West African Dwarf goats and 25 cattle of mixed breed) were used to evaluate Haemoglobin (Hb) polymorphism, local inbreeding coefficient, and the degree of heterozygosity (genetic diversity) in animals reared at the livestock farm of the Kogi State University, Anyigba using cellulose acetate gel electrophoresis. Results showed that Hb AB was predominant in cattle (0.52) followed by Hb BB (0.32) and Hb AA (0.16) with a gene frequency of 0.42 and 0.58 for the A and B gene locus, respectively. In sheep, only Hb AA was observed in the animals sampled with genotype frequency of 1.00 and gene frequency of 1.00. In goats, Hb AA was more with genotype frequency of 0.80, followed by Hb AB (0.20) while Hb BB was absent. The gene frequency for the A and B locus were 0.90 and 0.10, respectively in goats. The inbreeding coefficient and expected heterozygosity were: for cattle (0.02 and 0.48), for sheep (0.05 and 0.00) and for goats (0.02 and 0.18), respectively. All the genotypes evaluated were in Hardy-Weinberg equilibrium as there were no significant (P>0.05) differences between the observed and expected number of genotypes. Conclusively, Hb polymorphism was observed in all the animals studied with a higher frequency of Hb AA observed in the sheep and goat while Hb AB was more predominant in cattle. The level of inbreeding is still relatively low in the animals studied but appropriate breeding programmes needs to be instituted to keep it at that level, or better still lower it to the barest minimum.

KEYWORDS: cellulose acetate, electrophoresis, genetic diversity, Hardy-Weinberg equilibrium.

INTRODUCTION

Haemoglobin is a coloured blood protein which is very important for its role in the transportation of oxygen to tissues, and carrying away carbon dioxide from the tissues. Its inheritance follows simple Mendelian fashion (Akinyemi and Salako, 2010).

Due to this characteristic, Hb could be used as a biomarker for selection purposes in farm animals. Differences exist in the structure of Hb mainly in its globin component and this difference (called polymorphism), leads to variants of Hb, and the variation may confer or limit the animal's abilities/productivities.

A population is said to be polymorphic for a character if two or more forms of the character are each represented in high enough frequencies to be readily noticeable (Campbell and Reece, 2002). Egena and Alao (2014) in their review of Hb polymorphism in selected farm animals, observed that variation in Hb have been reported to confer selective advantages in different geographical areas to animals. Such selective advantages include effect on meat quality parameters (Bezova *et al.*, 2007) and on hair and horn length (Akinyemi and Salako, 2010). Linking Hb type to productive abilities and other factors as enumerated above could be a means of using it as a genetic marker for selection purposes and genetic improvement programmes.

Although there are more recent methods of evaluating the genetic merit of animals with the intention of using information so obtained for improvement purposes, such high technology inclined methodologies/equipment are still to a large extent, largely inadequate in most developing countries. This study is a pilot work which focuses on the identification of genetic diversity amongst the White Fulani cattle, Yankasa sheep and West African Dwarf goats reared at the livestock farm of the Kogi State University, Nigeria via electrophoretic detection of polymorphism at the Hb locus.

MATERIALS AND METHODS

Study area: Anyigba, the study area is located on latitude $7^{o}15'$ and $7^{o}29'$ North and longitude $7^{o}11'$ and $7^{o}32'$ East with an average altitude of 420 meters above sea level. The study area falls within the tropical wet and dry climatic region and the southern guinea savanna ecological zone with mean annual temperature of $25^{o}C$ and rainfall of 1600 mm. The map of the study area relative to its position in Nigeria, Kogi State and Dekina Local Government Area is presented in Figure 1 (Ifatimehin and Ufuah, 2006).

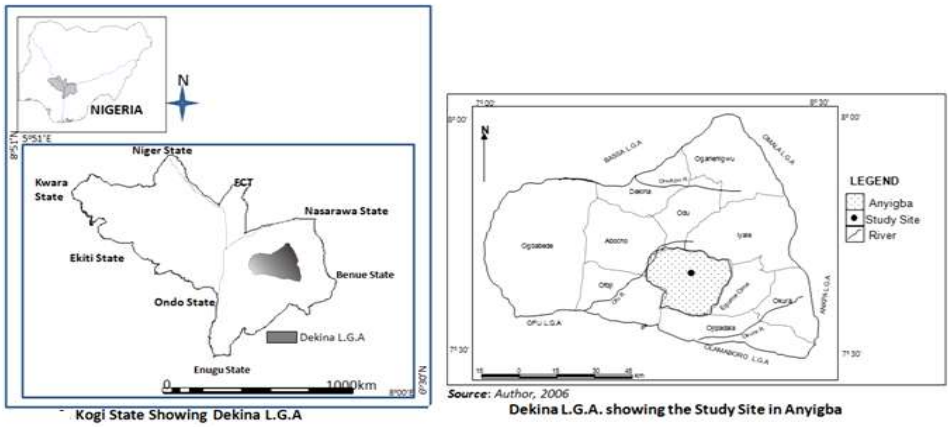

Figure 1: Map of Nigeria showing the relative positions of Kogi State, Dekina Local Government Area and Anyigba town

Sample collection: The animals used for the study were semi-intensively managed. A total of sixty five animals (10 Yankasa sheep, 30 West African Dwarf goats and 25 cattle of mixed breed) were used for the study. Sampled animals were adults within breeding age group. 2 ml of blood was collected from each of the sampled animals by jugular venipuncture into tubes containing Ethylene Diamine Tetra Acetic acid (EDTA) and properly labelled. The blood samples were placed in the refrigerator to keep them from spoilage until when required for laboratory analysis.

Laboratory analysis: The blood samples were lysed directly using distilled water without any prior washing with saline water. The red cell lysates were subjected to electrophoresis using a cellulose acetate gel medium according to standard procedure (Cheesbrough, 2000). The Hb types were identified based on their migration on the electrophoretic substratum detected from the start line towards the cathode zone. Analysis was carried out using a locally fabricated electrophoresis machine (Chikpas Genopack Electrophoresis, Nigeria).

Statistical analysis: The resulting frequencies of the alleles corresponding to the banding pattern were estimated by direct count. Haemoglobin genotype and gene frequencies were estimated as follows:

$$\text{Genotype frequency of AA} = \frac{\text{Number of individuals with AA} \times 100}{\text{Number of individuals sampled}}$$

$$\text{Genotype frequency of AB} = \frac{\text{Number of individuals with AB} \times 100}{\text{Number of individuals sample}}$$

$$\text{Genotype frequency of BB} = \frac{\text{Number of individuals with BB} \times 100}{\text{Number of individuals sampled}}$$

$$\text{Gene frequency of A} = \frac{2AA + AB}{\text{Total number of alleles}}$$

$$\text{Gene frequency of B} = \frac{2BB + AB}{\text{Total number of alleles}}$$

Data on Hb alleles and genotype frequencies were subjected to chi-square analysis to test for goodness of fit for observed and expected frequencies under Hardy-Weinberg Equilibrium (HWE). Heterozygosity (genetic diversity) was estimated as the expected proportion of heterozygotes under Hardy-Weinberg Equilibrium (HWE). The degree of genetic diversity was calculated using the formula;

$$1 - \sum_{i=1}^{k} P_i^2$$

Where P_i is the gene frequency of the i^{th} allele in the k^{th} locus; and i is the number of loci.

Inbreeding coefficient was also estimated to identify the extent of inbreeding in the animals studied. Local inbreeding coefficient was calculated using Lush formula;

$$F = \frac{1}{8M} + \frac{1}{8F}$$

Where M = number of male and F = number of female animals in the population, respectively.

RESULTS

Results of the study showed the existence of three haemoglobin genotypes in the cattle (Hb AA, Hb AB and Hb BB), two in goats (Hb AA and Hb AB), while only one haemoglobin genotype (Hb AA) was observed in the sheep. The haemoglobin distribution of Hb AA, Hb AB and Hb BB for cattle were observed to be 4, 13, and 8, respectively; for the sheep, it was 10, 0 and 0 for Hb AA, Hb AB and Hb BB, respectively while WAD goats were 24, 6 and 0 for Hb AA, Hb AB and Hb BB, respectively (Table 1, 2 and 3). The genotype frequency (Hb %) of the sampled animals (pooled) were: 16, 52 and 32 % for cattle; 100, 0 and 0 % for sheep and, 80, 20 and 0 % for the WAD goat, respectively. The gene frequencies (pooled) were observed to be 0.42 and 0.58 (for cattle), 0.90 and 0.10 (for goat), and 1.00 and 0.00 (for sheep). Chi-square statistics revealed no significant ($P>0.05$) influenced on the observed

and expected genotypes. Estimates of heterozygosity and inbreeding coefficients were 0.48 and 0.02 for cattle, 0.58 and 0.02 for goats, while it was 0.05 and 0.00 for the sheep, respectively.

Table 1: Genotype, gene frequency, inbreeding coefficient, expected heterozygosity and Chi-statistics of cattle

		Genotype frequency			Gene frequency	
	Number	**AA**	**AB**	**BB**	**A**	**B**
Male	9	1.00 (0.11)	7.00 (0.78)	1.00 (0.11)	0.50	0.50
Female	16	3.00 (0.18)	6.00 (0.38)	7.00 (0.44)	0.375	0.625
Total	25	4.00 (0.16)	13.00 (0.52)	8.00 (0.32)	0.42	0.58
			Chi-statistics			
Observed		4.00	13.00	8.00		
Expected		4.41	12.18	8.41		
Deviation		-0.41	0.82	-0.41		
Chi-square		0.038	0.055	0.020		0.113ns

Local inbreeding coefficient **(**F) = 0.02; Heterozygosity expected = 0.48; ns= not significant.

Table 2: Genotype, gene frequency, inbreeding coefficient, expected heterozygosity and Chi-statistics of goat

		Genotype frequency			Gene frequency	
	Number	**AA**	**AB**	**BB**	**A**	**B**
Male	11	9.00 (0.82)	2.00 (0.18)	0.00 (0.00)	0.91	0.09
Female	19	15.00 (0.79)	4.00 (0.21)	0.00 (0.00)	0.89	0.11
Total	30	24.00 (0.80)	6.00 (0.20)	0.00 (0.00)	0.90	0.10
			Chi-statistics			
Observed		24.00	6.00	0.00		
Expected		24.30	5.40	0.30		
Deviation		-0.30	0.60	-0.30		
Chi-square		0.004	0.067	0.30		0.37ns

Local inbreeding coefficient **(**F) = 0.02; Heterozygosity expected = 0.18; ns= not significant.

Table 3: Genotype, gene frequency, inbreeding coefficient, expected heterozygosity and Chi-statistics of sheep

		Genotype frequency			Gene frequency	
	Number	**AA**	**AB**	**BB**	**A**	**B**
Male	6	6.00 (1.00)	0.00 (0.00)	0.00 (0.00)	1.00	0.00
Female	4	4.00 (1.00)	0.00 (0.00)	0.00 (0.00)	1.00	0.00
Total	10	10.00 (1.00)	0.00 (0.00)	0.00 (0.00)	1.00	0.00
		Chi-statistics				
Observed		10.00	0.00	0.00		
Expected		10.00	0.00	0.00		
Deviation		0.00	0.00	0.00		
Chi-square		0.00	0.00	0.00		0.00ns

Local inbreeding coefficient (F) = 0.05; Heterozygosity expected = 0.00; ns= not significant.

DISCUSSION

The control of the three Hb genotype by the two co-dominant alleles A and B as observed in this study has been reported in Nigerian cattle, sheep and goat (Akinyemi and Salako, 2010; Akinyemi and Salako, 2012; Agaviezor *et al.,* 2013; Yakubu *et al.,* 2014). The absence of Hb BB in goats is similar to the observation of Kuwar *et al.* (2001) in Nepalese Hill goats; Johnson *et al.* (2002) in Omani Dhofari goats, and Yakubu *et al.* (2014) in West African Dwarf goats of Nigeria. Osaiyuwu *et al.* (2013) reported that the degree of polymorphism of haemoglobin system of goat breeds is defined by the number of alleles, the ratio between them, the inter-allelic combinatory capacity, number of genotypes expressed, their distribution and the range of variability. Hb AA had selective advantage over Hb AB and Hb BB in the goat. Sam (2012) reported Hb AA to be superior to other Hb genotypes in Red Sokoto goats in reared in Western Nigeria. The predominance of Hb A could be due to its properties (biophysical and biochemical), and its physiological peculiarities.

The very low frequency observed for Hb BB and Hb AB in sheep is contrary to the report of Akinyemi and Salako (2012) who observed very high percentages of the two genotypes among indigenous Nigerian sheep breeds. The frequency of Hb A was very high in the absence of the other haemoglobin types (Table 3). Pieragostini *et al.* (2006) observed that Hb A is found more frequently in sheep living above 40^{o}C latitude. This might be due to specific abilities such as a high relative affinity for oxygen and is therefore very important for survival of the sheep in mountainous areas at latitude above 3000m

(Tsunoda *et al.,* 2006). It is possible that vegetation or climatic factors might have an influence on Hb type. There are reports indicating that even when no deliberate selection pressure was applied at the locus, Hb A genotype increases toward the forest zone. This was reported in Yankasa sheep (Tella *et al.,* 2000). The absence of Hb AB and HB BB in the sheep sampled is not a conclusive proof however, that other form of haemoglobin does not exist generally in sheep in the area where the study took place. The small sample size (mostly due to mortality which might have been brought about by a decrease in fitness) could have been responsible for the occurrence of only Hb A genotype.

Mojabi *et al.* (2001) and Pal and Mummed (2014) reported on studies carried out on Hb polymorphism in cattle although most of the study seem to be in dairy cattle. Gene frequency for the allele A was observed to be higher than that for allele B, in sheep and goat similar to the findings of Agaviezor *et al.* (2013); except in cattle where the allele AB was predominant. However, the gene frequency corresponds with Hardy-Weinberg's equilibrium as no significant differences were observed between the observed and expected genotypes. No conclusion could be drawn however, that sex has an effect on the differences observed in Hb types. This is based partly on the fact that not the same numbers of both sexes were sampled in the study.

The inbreeding coefficient and heterozygosity observed in the study revealed that the extent of inbreeding in the populations studied although quite low is not too encouraging. There's an inverse relationship between inbreeding and expected heterozygosity. If inbreeding coefficient is 0, it means that the observed number of heterozygotes is equal to the expected number, and this equally means that the population is in Hardy-Weinberg's equilibrium. If it is positive and equal to 1, it means that there are no heterozygotes in the population at all implying a completely inbred population (none of the population studied was in this state). Thus, the higher the inbreeding coefficient, the more inbred the population is likely to be while the higher the degree of heterozygosity, the less inbred the population is, and wider is the genetic diversity of the population in question. The values obtained for the two indices in the sheep point to the fact that urgent action needs to be taken to introduce new genetic material into the sheepfold in order to shore up the genetic merit of the sheep. Further decline in heterozygosity will simply usher the sheep population into inbreeding depression with its accompanying negativity and or effect

on productive traits and those traits that have to do with fitness.

There's also the danger of the allele (A) becoming fixed in the sheepfold, and this might not be too advantageous especially if it does not confer any selective advantage in terms of productive, adaptive or reproductive abilities. Fixation of certain alleles and loss of others within the sheep could be due to evolutionary changes either as a result of natural selection for the allele A, random genetic drift, or it might well be that the genes of these blood proteins have been linked with genes that affect economically important traits whose selection (naturally or artificially) might have indirectly change the allele frequency, leading to reduction in diversity at the haemoglobin locus. Genetic drift also causes allele frequencies to fluctuate randomly in each generation. However, if the frequency of an allele ever reaches zero due to genetic drift, it will be permanently eliminated from the population. The other allele, whose frequency is now 1 then becomes "fixed", which means that all individuals in the population will be homozygous for that allele. This continues for all future generations (in the absence of mutation). The average rate at which alleles become fixed is a function of the population size. Hence the larger the population the longer it will take before an allele becomes fixed and vice versa. The tendency toward fixation for allele A in the sheep is therefore not too surprising because of the small population sampled. Perhaps, the nature of the result was also affected by the low number of animals sampled.

The fact that more Hb A genotype was observed compared to Hb AB genotype in goats, is an indication that the population may not currently be undergoing dissortative mating or may not be experiencing a Wahlund effect (Wahlund effect occurs when there are more heterozygotes observed than the normal or as expected in a population). Hence, there is a need to design appropriate breeding and conservation schemes to prevent the erosion of the valuable adaptive traits of the West African Dwarf goats. Cattle were the most diversified at the haemoglobin locus due in part to higher level of heterozygosity and low inbreeding. Inbreeding accumulates in any closed population because of the mating of closely related individuals. Small, closed and or selected populations can rapidly lose heterozygosity and allelic diversity tending toward homozygosity and the possible onset of inbreeding depression. Within breed and high rates of loss of genetic variation may lead to reduced chances of breed survival due to decrease in fitness brought about by inbreeding depression. Such breeds become

subject to faster changes in gene frequencies leading to greater rate of loss of genes and genetic constitutions. Another negative effect in domestic animals is a decrease in selection response and in potential genetic gains in economic traits. Measurement of the effect of inbreeding on economic traits is therefore important in order to estimate the magnitude of change associated with increase in inbreeding. Once the impact of inbreeding has been estimated, its economic impact upon a particular trait can then be determined.

CONCLUSION

The study has shown the nature of Hb polymorphism in cattle, sheep and goat reared at the Kogi State University livestock farmer. Higher frequency of Hb AA was observed in sheep and goat while Hb AB was more predominant in cattle. The study shows that the level of inbreeding in the animal herds is still relatively low. It is recommended that appropriate breeding programme be instituted to keep it at that low level, or better still lower it to the barest minimum. This study was highly limited by the small number of animals reared at the Kogi State University livestock farm.

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