HAEMAGLOBIN TYPES IN SHEEP IN OF SEMI-ARID ZONE OF NORTH EASTERN NIGERIA

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ABSTRACT
Genotyping is not a test to diagnose a disease, but rather a test looking for certain information encoded on the prion protein (PrP) gene. This encoded information helps determine the susceptibility of sampled sheep to developing clinical disease from a disease should the animal be exposed. Therefore, haemaglobin types in sheep in the semi-arid zone of North Eastern Nigeria was determined at haemoglobin (Hb) locus level. One hundred and sixty-eight (168) animals were used for the study. Blood samples comprising 56 each of Yankasa, Balami and Uda breeds were collected from households, sheep and goats' markets and abattoir. The blood samples were analysed for haemaglobin types. Blood samples (5 mL per animal) were obtained from the jugular vein using a 10 mL syringe attached to 21gauge x ½ inch needle. The blood samples collected was then placed in tubes coated with ethylene-diamine tetra-acetic acid (EDTA) as anticoagulant. The samples were adequately labeled and taken to Federal Teaching Hospital Gombe haematology's laboratory for analysis. Haemoglobin type was identified using cellulose acetate paper electrophoresis. Genotypic frequencies were estimated, and sheep showed only HbAA genotype. Genotypic frequencies were similar in both sexes of sheep implying that there was no effect in sex on Hb frequency. It is therefore concluded that there were no polymorphisms at Hb locus since only the Hb AA was exhibited.
Keywords: Sheep, Haemaglobin, gene frequency, genotype frequency, Polymorphism.

INTRODUCTION

Sheep breeding is an ancient practice of animal improvement. There has not been any organized form of breeding and animal improvement programmes in Nigeria in the past years. Although in recent years, studies in the field of Nigerian small and large ruminant animals are less reported compared to the non-Nigerian counterparts and other livestock sSpecies (Hrinka, 2013). In addition, researches in the field of biochemical genetics in small ruminant especially sheep are generally scarce globally. Nigerian sheep breeds have been previously studied for genetic variations based on physical production, reproductive and behavioral features (Dafur *et al.,* 2019). Therefore, discovery of genetic polymorphism depicted by proteins that differ electrophoretically provides information for the study of the genetics of the organism at molecular level, cellular, or the entire organism (Alphonsus *et al.,* 2012).

The purpose of the present study on the haemoglobin types of sheep in Gashua, Yobe State Nigeria is to determine the polymorphic forms of sheep hemoglobin as demonstrated by electrophoretic patterns of sheep hemoglobins.

The outcome of the study from the determination of genetic polymorphism from protein differences permits the study of protein evolution and thus the evolution of the organism. Similarly, the study will also enable man to examine the effects of breeding systems on the frequencies of alleles where inbreeding and selection are practiced.

In addition, the study might also provide useful information to breeders, livestock keepers and researchers.

MATERIALS AND METHODS

Area of the Study

The study was conducted in Gashua, Bade Local Government Yobe state located in Northeastern Nigeria. Bade Local Government is bordered by five Local Government areas of Yobe as follows: Jakusko, Dapchi, Karasuwa, Yusufari and Nguru. The area is situated in semi-arid sub-Saharan region of North Estern Nigeria on Lat 12^0 42' 58" and Longitude 10^0 38' 36". The study area experiences averagely low level of rainfall annually over decades. During these periods, the area suffered series of

drought. Although in recent years, there is an observed increasing average rainfall trends but this was accompanied by higher average temperatures of between 48-40 ^{0}C.

Bade local government area has a total land area of 772 square kilometer (km^2) of which is predominantly classified as arable land. The vegetation zone is Sahel. The common trees species found in the area mostly includes; *Acacia seyel, Balanite aegyptiaca*, *Azadira ctaindica, Adonsonia digitata, Faaidherbia albida, Tamarindus indica, Hyphaene thebaica and Anogeissus leiocarpus.*

Breeds of Sheep Used

Three breeds of indigenous sheep found in Nigeria and available in Gashua, Yobe State was used for this study. They include: Yankasa, Balami and Uda. They were available from Gashua environs, abbatoir and, sheep and goat markets.

Study Design and Sampling

The study was carried out between August 2022 and April 2023 where random blood samples were collected from 168 sheep. The samples were collected from Gashua environs, abbatoir and sheep and goat markets where random blood samples were collected from 168 sheep. The total sample size was estimated according to Thrusfield (2005) using 95% confidence and expected prevalence of 89.1% from previous study of James (2014) and desired precision of 5% as follows:

$n = 1.96^2 P_{exp} (1- P_{exp}) / d^2$

Where n = sample size

P_{exp} = expected prevalence (89.1% of James, 2014)

d = desired absolute precision of 5% (0.05).

$n = 1.96^2 \times 0.891 (1-0.891)/ (0.05)^2$

$n = 3.8416 \times 0.891(0.109)/ 0.0025$

$n = 0.3731/ 0.0025$

n = 150 samples approximately

Stratified random sampling was applied to collect the samples from Yankasa, Balami, and Uda sheep used.

Blood sample collection and analysis

Blood samples (5 mL per animal) were obtained from the jugular vein using a 10 mL syringe attached to 21gauge x ½ inch needle. Air was first cleared from the syringe before puncturing at 20^0 angle (Pratt, 1985) and the syringe was aspirated to confirm insertion and collection of blood. The blood samples collected was then placed in tubes coated with ethylene-diamine tetra-acetic acid (EDTA) as anticoagulant. The samples were adequately labeled and taken to Federal Teaching Hospital's haematology laboratory for analysis. Haemoglobin type was identified through electrophoresis according to Wintrobe (1967).

Haemaglobin electrophoresis using cellulose acetate at alkaline pH

The blood sample was centrifuged at 1200g for 5 minutes. Then 20 µL of the packed red cells were diluted with 150µL of haemolyzing reagent, mixed gently and left to stand for at least 5 minutes. With power supply disconnected, the compartment of the electrophoresis tank was filled with Tris – EDTA buffer. Cellulose acetate membrane was immersed into the buffer slowly for at least four minutes to avoid trapping air bubbles and to ensure that it was evenly saturated. It was then removed and excess buffer bloated off between two pieces of absorbent paper but was not allowed to dry out before sample application. The haemolysed sample was applied to the cellulose acetate membrane using a suitable applicator and was allowed to remain in contact. The membrane was then placed upside down across the bridge of the tank on top of the buffer with the line of the applicator on either the cathode or anode pole.

Electrophoresis commenced by connecting power supply and ran at 250-350V until a visible separation was observed (within 20 minutes). Power supply was disconnected; membrane was removed and stained in Panceau S for 3-5 minutes. It was again removed, drained and eluted of the excess stain with three changes of destaining solution (5% acetic acid) for 2 minutes each. It was subsequently dehydrated in absolute methanol for 2-3 minutes and immersed in a cleaning solution for 6-8 minutes, dried at 65^0C for 4-6 minutes, labelled and stored in a protective plastic envelop. The haemoglobin on the membrane was then identified by comparison with a standard chart.

Since only one allele (A) was detected, the haemoglobin genotype and gene frequencies were estimated this way; Genotype frequency AA = number of AA/ total number x 100; based on breed and sex.

For the gene frequency estimation, the equation shown below was used.

$P = (2N_{AA} + N_{AB})/2N$ 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, NAA = 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

Table 1 depicts gene and genotype frequencies according to sex and breeds. Of all the 168 sheep consisting 36 each of Yankasa, Balami and Uda breeds considered across sex, only haemoglobin AA genotype was detected representing a genotype frequency of 1.00. In this study that only homozygote (AA) genotype was detected in sheep, agrees with the reports of Dafur *et al*. (2019) in Jos, Boyi *et al.* (2017) in Gombe State. However, these reports contradict the report by Salako *et al.* (2007) who reported the existence of other genotypes such as HbBB and HbAB apart from HbAA. The existence of only HbAA in this study could be due to adaptive survivability feature. Although different genotypes have been reported to show selective advantages in other geographical locations (Ndamukong, 1995), this however might have led to fixation of HbAA in sheep in the present study. Herselman *et al.* (2006) reported that sheep with haemoglobin HbAA have been found to have higher resistance to gastrointestinal parasites. Haemoglobin polymorphism therefore, is known to vary with resistance; hence could be a yardstick for selection. This resistance has been attributed to higher affinity for oxygen (Di Stasio, 1997).

The present study did not show variation in haemoglobin type by sex since all the sheep considered exhibited same haemoglobin type (HbAA). This indicates sex may have no effect on haemoglobin types.

The present study not only adds to our store of knowledge, but it may have economic value if there is a reasonable association between hemoglobin types and productivity of the animals.

Table 1: Genotype of Nigerian sheep according to sex and breeds

Species:		**N**	**Genotype Frequency**			**Gene Frequency**	
			AA	AB	BB	A	B
	Male	84	84 (1.00)	00 (0.0)	00(0.0)	1.00	0.00
	Female	84	84 (1.00)	00 (0.0)	00(0.0)	1.00	0.00
Breed:							
	Yankasa						
	Male	28	28(1.00)	00(0.0)	00(0.0)	1.00	0.00
	Female	28	28(1.00)	00(0.0)	00(0.0)	1.00	0.00
	Balami						
	Male	28	28(1.00)	00(0.0)	00(0.0)	1.00	0.00
	Female	28	28(1.00)	00(0.0)	00(0.0)	1.00	0.00
	Uda						
	Male	28	28(1.00)	00(0.0)	00(0.0)	1.00	0.00
	Female	28	28(1.00)	00(0.0)	00(0.0)	1.00	0.00

CONCLUSION

It can be concluded that genotypic frequencies were estimated, and sheep showed only HbAA genotype. Genotypic frequencies were similar in both sexes of sheep implying that there was no effect in sex on Hb frequency; and that there were no polymorphisms at Hb locus since only the Hb AA was exhibited.

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