Evaluation of haemoglobin variants of Red Sokoto goats and Yankasa sheep in Beji, Niger State, Nigeria: Effect on body weight and reproductive parameters

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Abstract *Corresponding author email: acheneje.egena@futminna.edu.ng

Blood was sampled from 76 Red Sokoto (RS) goats and 76 Yankasa Sheep (YS) to evaluate Haemoglobin (Hb) genotypes and their effect on body weight and some reproductive parameters of the animals. The blood samples were analyzed by cellulose acetate electrophoresis. The electrophoretic test revealed the presence of three co-dominant alleles (A, B and C) which produced four haemoglobin genotypes (AA, AB, BB and BC) in RS goats, and two co-dominant alleles (A and B) which gave rise to two haemoglobin genotypes (AA and AB) in YS. The frequencies of alleles A, B and C were 0.32, 0.61 and 0.07 in RS goats; and 0.945 and 0.055 for alleles A and B in YS. The estimated heterozygosities were 0.406 and 0.118 in RS goats and YS while the estimated inbreeding coefficients were 0.009 and 0.008 in the RS goats and YS, respectively. The observed and expected genotype frequencies deviated significantly from the expected Hardy-Weinberg equilibrium ($P \leq 0.05$; chi-square = 7.82 for RS goats, $P \leq 0.01$; chi-square = 13.13 for YS). There were significant ($P \leq 0.05$) differences observed in the body weight of male YS as a result of the Hb type; individuals with Hb AB were observed to have higher body weight (44.00 kg) compared to those with Hb AA (22.83 kg). No significant ($P > 0.05$) difference was observed however in the body weight of RS goats. Male YS with Hb AB had longer ($P \leq 0.05$) scrotum (26.00 kg) and wider scrotal circumference (33.00 kg) compared to those with Hb AA (18.83 kg; 25.67 kg, respectively). No significant ($P > 0.05$) difference was however observed in the reproductive parameters of RS goats. It was concluded that polymorphism exist at the Hb locus in the YS and RS goats sampled in Beji and this had an influence on body weight and the reproductive parameters evaluated particularly in the sheep. More heterozygotes were also observed in the population studied thereby leading to a low rate of inbreeding coefficient in the animals.

Keywords: Haemoglobin, gene and genotype frequencies, Red Sokoto goat, Yankasa sheep, Beji.

Introduction

Sheep and goats play essential roles in food production and food security in many developing countries of the world. Their great popularity is seen in their ecological adaptation to many different climates and the many uses for which they have been kept by farmers (Agaviezor *et al.*, 2013). Sheep and goats are owned mostly by smallholder farmers as an integral part of the livestock sub-sector in Africa (Tekelye and Kasali, 1992; Workneh, 2000). According to the Ethiopian Agricultural Research Organization (EARO, 2000) and

Ehui *et al.* (2000), sheep and goat contribute immensely to both the subsistence survival and cash income generation of many rural farmers and peri-urban dwellers.

Haemoglobin has been referred to as an “evergreen” red protein (Bettati *et al.*, 2009) which has attracted much attention because of its biochemical, physiological and biophysical properties having relevance to the selection phenomenon of farm animals (Hrinca, 2008; Yakubu and Aya, 2012). Haemoglobin is made up of

four globin chains (two α and two β), and a

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prosthetic group called haem which are found bound to each other (De Souza and Bonilla Rodriguez, 2007). Haemoglobin is the chief carrier of oxygen to the animal tissue for respiration and oxidation purposes, and also the carrier of carbon dioxide from the tissues to the lungs from where it is exhaled into the atmosphere. Haemoglobin has variants and these variants have been reported to be associated with possible environmental adaptability as well as being clinically important as the cause of a variety of genetic disorders of blood in animals (Peters *et al.*, 2004).

Researchers such as Adebambo (2004) and Agaviezor *et al.* (2013) had opined that in animals that have gene specific substances, and where such specific substances could be detected in the blood by appropriate procedures such as electrophoresis; then, the presence or absence of these gene specific substances could be directly correlated to the genotype of the animals. Blood and blood components are therefore undoubtedly, essential biological agents warranting great consideration in the study of any breed of animal.

Polymorphism is defined as the occurrence together of two or more varieties in the same population and at the same time in such proportions that the rarest of them cannot be maintained by genetic mutation alone. Genetic differences observed in farm animals generally lead to variability which is expressed in their reproductive and performance abilities both within and between breeds (Egena and Alao, 2014). Although work exist on the evaluation of Hb genotypes of goats and sheep in Nigeria (Tella *et al.*, 2000; Akinyemi and Salako, 2010; Agaviezor *et al.*, 2013; Osaiwuyi *et al.*, 2013; Yakubu *et al.*, 2014), not much reports exist on polymorphism at the Hb locus in sheep and goats of Niger State and

generally, on the effects of such differences in Hb type on reproductive parameters. Ilesanmi *et al.* (2016) working with Watish Sudanese sheep imported into Nigeria and stationed at Minna, had reported no significant ($p>0.05$) difference in body length, heart girth, ear length, height at withers, teat length, udder length, udder circumference, scrotal length and scrotal circumference of the sheep. The aim of this study therefore was to evaluate haemoglobin types and its effect on body weight and possible association between reproductive parameters of RS goats and YS in Beji, Niger state.

Materials and methods

Experimental location

The experiment was conducted in Beji, Bosso Local Government Area, Niger State, North Central Nigeria. Beji falls within the Guinea Savanna agro-ecological zone. It is found between latitude 09.6126 - 09.6195° north and longitude 06.2669° - 06.2800° east. It lies at an altitude of about 257 m above sea level. The climate is characterized by an alternation of dry season from November to March, and a rainy season from April to October. Thus, the beginning of rainfall is recorded in the month of April; the rain becoming steady between the months of June and July, and the highest is recorded between the months of August and September. The average annual precipitation is between 900 and 1200 mm per year. The temperatures vary according to the month and season. The highest temperatures are recorded in March/June; and the lowest in December/February with average values ranging between 28°C and 38°C (Akpeokhai and Adenle, 2013). Beji was purposely selected for the study because of its proximity to a livestock market and the fact that a lot of sheep and goat keepers exist within the locality.

Research animals

A total number of 152 animals comprising of 76 RS goats and 76 YS of both sexes were randomly sampled for the research. The animals were raised primarily through extensive management system by the farmers. They were approximately two to three years of age; age was determined from attestation given by the livestock keepers.

Parameters measured

Blood samples (5 mL) were obtained from jugular venipuncture using syringe and needle and placed in tubes treated with Ethylene-Diamine Tetra acetic Acid (EDTA) which is an anticoagulant. The samples were properly labelled and transported on ice packs to Dalhatu Araf Specialist Hospital Lafia, Nasarawa State, Nigeria for analysis. The red blood cells were separated, washed in saline solution and lysed with distilled water. Haemoglobin was typed using cellulose acetate electrophoresis. The identification of the haemoglobin types was achieved in accordance with the migration speed of the light spots on the electrophoretical

substratum detected from the start line towards the cathode zone. The direct gene counting method was used to score Hb bands based on the separation of Hb variants (Tella *et al.*, 2000).

Other parameters measured were: Body Weight (BW) of the animals which was obtained using a suspended weighing scale (100 kg capacity); Scrotal Length (SL), was measured as the distance from the point of attachment of the scrotum to the body to the tip of the scrotum; Scrotal Circumference (SC), was measured as the length of the closed curve of the scrotum; Udder Length (UL), was measured as the distance from the point of attachment of the udder to the body to the tip of the udder; and Udder Circumference (UC), was measured as the length of the closed curve of the udder.

Statistical analysis

Genotype and gene frequencies of haemoglobin alleles were estimated according to Hrinca (2008). For RS goats and YS, genotype frequencies were estimated using the formulae below;

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

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

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

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

Gene frequencies for YS were estimated thus;

$$\text{Gene frequency of A} = \frac{\text{AA} \times 2 + \frac{1}{2} \text{AB}}{\text{Total number of alleles}}$$

$$\text{Gene frequency of B} = \frac{\text{BB} \times 2 + \frac{1}{2} \text{AB}}{\text{Total number of alleles}}$$

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Gene frequencies for RS goats were also estimated thus;

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

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

Gene frequency of C = Number of animals carrying BC.

Data on Hb alleles and genotype frequencies were subjected to chi-square analysis to test for goodness of fit for the observed and expected frequencies under Hardy-Weinberg equilibrium. Heterozygosity (H) was estimated as the expected proportion of heterozygotes under Hardy-Weinberg equilibrium. It was estimated using the formula;

$$\text{Heterozygosity (h)} = 1 - \sum X_i^2$$

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

The inbreeding coefficient (F) was also estimated to have an idea of the extent of inbreeding in the sheep and goats populations using Lush formula;

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

Where M and F represents the number of male and female animals sampled.

Other analyses were carried out using the

PROC GLM and PROC T-test procedures of SAS (1993) to determine the effects of haemoglobin variants on morphometric and reproductive parameters evaluated in the animals.

Results

Table 1 shows the Hb genotypes, gene frequencies, degree of heterozygosity and local inbreeding co-efficient of goats and sheep sampled in Beji, Niger state. The electrophoretic test showed the existence of four haemoglobin genotypes in RS goats; two homozygotes (Hb AA and Hb BB), and two heterozygotes (Hb AB and Hb BC) with genotype frequencies of 0.11, 0.29, 0.46 and 0.14, respectively. For the YS, two haemoglobin genotypes were detected; the homozygous (Hb AA) and the heterozygous (Hb AB) with genotype frequencies of 0.93 and 0.07, respectively. The genotype frequencies of Hb AA, Hb AB, Hb BB and Hb BC in male RS goats were 0.13, 0.25, 0.44 and 0.18, respectively.

Table 1: Distribution of haemoglobin genotypes and gene frequencies of RS goats and YS

Specie	Sex	No.	Genotype Frequency				Gene Frequency		
			Hb AA	Hb AB	Hb BB	Hb BC	A	B	C
RS goat	Male	16	2 (0.13)	4 (0.25)	7 (0.44)	3 (0.18)	0.34	0.57	0.09
	Female	60	5 (0.09)	20 (0.33)	29 (0.48)	6 (0.10)	0.30	0.65	0.05
	Total	76	7 (0.11)	24 (0.29)	36 (0.46)	9 (0.14)	0.32	0.61	0.07
YS	Male	21	18 (0.86)	3 (0.14)	-	-	0.89	0.11	-
	Female	55	55 (1.00)	0 (0.00)	-	-	1.00	0.00	-
	Total	76	73 (0.93)	3 (0.07)	-	-	0.945	0.055	-

Hb AA = haemoglobin AA; Hb AB = haemoglobin AB; Hb BB = haemoglobin BB; Hb BC = haemoglobin BC; heterozygosity (goat and sheep) = 0.406 and 0.038; inbreeding coefficient (goat and sheep) = 0.009 and 0.008.

The gene frequencies for the A, B and C alleles were 0.34, 0.57 and 0.09 for the male RS goats. In the female, genotype frequencies were 0.09, 0.33, 0.48 and 0.10. The gene frequencies for the A, B and C co-dominant alleles in the pooled sample were 0.32, 0.61 and 0.07, respectively. For the YS, male genotype frequencies of Hb AA and Hb AB were 0.86 and 0.14 and the gene frequencies for the two co-dominant alleles A and B were 0.89 and 0.11, respectively. In the female, the genotype frequencies were 1.00 and 0.00 while the gene frequencies for the A and B co-dominant alleles in the pooled sample were 0.945 and 0.055, respectively. The observed and expected number of genotypes of haemoglobin in RS goats and YS (Table 2) shows that the genotype frequencies deviated significantly ($P \leq 0.05$; $\chi^2 = 7.82$ for RS goats; $P \leq 0.01$;

$\chi^2 = 13.13$ for YS) from Hardy-Weinberg equilibrium. The estimated degree of heterozygosity (H) for RS goats and YS were 0.406 and 0.038, while the estimated local inbreeding coefficient (F) was 0.009 and 0.008, respectively.

The effect of haemoglobin type on body weight of RS goats and YS is presented in Table 3. No significant ($P > 0.05$) differences were observed in the body weight of the RS goats (buck and doe) as affected by haemoglobin type. For the sheep however, rams with Hb AB had significantly ($P \leq 0.05$) higher body weight than those with Hb AA. The effect of haemoglobin type on reproductive parameters of RS goats and YS (Table 4) revealed that there were no significant ($P > 0.05$) difference between the reproductive parameters of the RS goats (buck and does) as affected by haemoglobin type.

Table 2: Chi-square statistics for the observed and expected number of haemoglobin genotypes in RS goats and YS

Specie		Genotype				Total
		Hb AA	Hb AB	Hb BB	Hb BC	
RS goat	Observed number	7.00	24	36	9.00	76
	Expected number	8.79	29.46	24.69	13.06	76
	Deviation	1.79	5.46	11.31	4.06	0
	Chi-square	0.365	1.012	5.181	1.262	7.82*
YS	Observed number	73	03	0.00	-	76
	Expected number	60.19	14.88	0.92	-	76
	Deviation	12.81	11.88	0.092	-	0
	Chi-square	2.726	9.485	0.92	-	13.13**

Hb AA = haemoglobin AA; Hb AB = haemoglobin AB; Hb BB = haemoglobin BB; Hb BC = Haemoglobin BC; * = significant ($p \leq 0.05$); ** = significant ($p \leq 0.01$).

For the sheep however, rams with Hb AB had longer ($P \leq 0.05$) scrotum and wider scrotal circumference (26.00 cm; 33.00 cm). All the ewes had the same genotype

(Hb AA) hence no statistical analysis was carried out to ascertain the significant nature of the data collected.

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Table 3: Effect of Hb type on body weight (kg) of RS goats and YS

Specie	Sex	Hb AA	Hb AB	Hb BB	Hb BC
RS goat	Male	15.00±2.20	13.00±1.56	13.29±1.18	11.67±1.80
	Female	22.80±3.76	23.40±1.88	23.76±1.56	22.17±3.43
	Pooled	20.37±3.26	21.67±1.76	21.72±1.44	18.67±1.88
YS	Male	22.83 ^b ±1.24	44.00 ^a ±0.00	-	-
	Female	28.40±0.94	00.00±0.00	-	-
	Pooled	27.03 ^b ±0.81	44.00 ^a ±0.00	-	-

^{ab}: means denoted by different superscripts are significantly ($p \leq 0.05$) different; Hb AA = haemoglobin AA; Hb AB = haemoglobin AB; Hb BB = haemoglobin BB; Hb BC = haemoglobin BC.

Discussion

The electrophoretic test showed the presence of four haemoglobin genotypes in RS goats; two homozygotes Hb AA and Hb BB and two heterozygotes Hb AB and Hb BC. These four haemoglobin genotypes were produced by interactions between the three co-dominant alleles A, B and C, respectively. The allele C detected was in association only with the allele B but at a very low frequency (0.07). This observation is similar with the reports of Salako *et al.* (2007) and Alphonsus *et al.* (2012); both reported the existence of four haemoglobin genotypes (AA, AB, BB and AC) in RS goats. The difference between this study and their study is that the allele C was found to be in association with the allele B as against their study where it was in association with the allele A.

The observation of low frequency of Hb C in this study is similar to the reports of Deza *et al.* (2000) on native goats from Central Argentina, Johnson *et al.* (2002) in Omani goats, and Salako *et al.* (2007) in RS goats in Nigeria. The determinant of the synthesis of abnormal haemoglobin (Hb C) might be anaemia due to illness and or environmental stress in the animals (although this was not confirmed through appropriate clinical diagnosis). The allele B had the highest frequencies of 0.57 and 0.65 (buck and doe) in the population and 0.61 when pooled. This corresponds with

the findings of Salako *et al.* (2007), who also reported the allele B to have higher frequency in RS goats. The implication is that RS goats with Hb B are possibly better able to adapt and survive the climatic conditions of Beji and its environs where the study was carried out. This could be due to what is referred to as “better selective advantage” probably brought about by better oxygen carrying ability.

In the YS, two haemoglobin genotypes were detected; the homozygous Hb AA and the heterozygous Hb AB. The two haemoglobin genotypes were produced by two co-dominant alleles A and B. This is similar to the observations of Akinyemi and Salako (2010), who also detected two haemoglobin genotypes (AA and AB) although in the West African dwarf sheep. The higher frequency of Hb AA than that of Hb BB, agreed with the results of Tella *et al.* (2000) and Akinyemi and Salako (2010), on higher frequencies of Hb AA over Hb BB in West African dwarf Sheep. This means that there is selective advantage for Hb A in the sheep sampled at Beji compared to Hb AB. It would be expected therefore that YS with Hb A will have better survivability in Beji than sheep with the other genotype.

The estimated heterozygosity and inbreeding coefficient for RS goats and YS, indicates that heterozygotes were more than homozygotes and this is complimented by the low rate of inbreeding observed in both

Table 4: Effect of haemoglobin type on reproductive parameters (cm) of RS goats and YS

Specie	Parameter	Male				Female			
		Hb AA	Hb AB	Hb BB	Hb BC	Hb AA	Hb AB	Hb BB	Hb BC
RS goat	Scrotal length	14.00±2.68	12.75±1.89	12.29±1.43	12.00±2.19	-	-	-	-
	Scrotal circumference	22.00±2.68	20.75±1.89	20.29±1.43	20.00±2.19	-	-	-	-
	Udder length	-	-	-	-	20.00±2.50	17.45±1.04	16.72±1.04	15.83±2.89
	Udder circumference	-	-	-	-	28.80±3.32	29.60±1.66	27.48±1.38	2.50±3.03
YS	Scrotal length	18.83 ^b ±1.08	26.00 ^a ±0.00	-	-	-	-	-	-
	Scrotal circumference	25.67 ^b ±0.67	33.00 ^a ±0.00	-	-	-	-	-	-
	Udder length	-	-	-	-	11.91±0.41 _†	-	-	-
	Udder circumference	-	-	-	-	28.05±0.78 _†	-	-	-

^{ab}: means denote by different superscripts are significantly ($p \leq 0.05$) different statistically analyzed (only one genotype was observed); Hb AA = haemoglobin AA; Hb AB = haemoglobin AB; Hb BB = haemoglobin BB; Hb BC = haemoglobin BC.

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animals. These values indicate the population of heterozygotes in a random mating population. It has a clear biological implication as it could be regarded as a measure of the degree of genetic diversity at the haemoglobin locus in the investigated animals. The low degree of heterozygosity (YS) is an indication of the need to introduce new genetic material into the sheep population reared in Beji. This will serve to increase or shore up the degree of heterozygosity (in YS) and at the same time, reduce the level of inbreeding. Heterozygotes usually have better chances of surviving natural selection than homozygotes. This is evident from the results of this study as there are more heterozygous animals in the populations sampled.

The body weight of male YS was affected by the haemoglobin type. The heavier body weight observed in YS having Hb AB is an indication of favourable natural selective advantage for the trait. Mating YS with Hb AB therefore, could lead to improvement in body weight of the sheep. Farmers and sheep keepers will do well therefore to select sheep with Hb AB if they desire heavier sheep. The longer and wider scrotum observed in YS with Hb AB is an indication of sound breeding ability. Brito *et al.* (2004) reported that in the evaluation of the breeding soundness of a male animal, larger testes leads to the production of more spermatozoa than smaller testes. More so, Keith *et al.* (2009) suggested the use of scrotal size and testicular measurements to select for improved sperm production and breeding males. Therefore YS rams with Hb AB should be selected for breeding purpose as they are likely to be more productive than those with Hb AA. The significant nature of the body weight and scrotal length and circumference of the YS is however at variance with the reports of

Ilesanmi *et al.* (2016) as they observed no significant differences in the parameters in Watish Sudanese sheep domiciled in Minna.

Conclusion

Results from the study revealed the presence of four haemoglobin genotypes in RS goats and two haemoglobin genotypes in YS. The number of heterozygotes was more than homozygotes in both animals. Low rate of inbreeding was observed particularly in the sheep. The higher body weight, longer and wider scrotum observed in rams with Hb AB could be used as a good selection criterion for breeding purpose.

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