

*Full Length Research Paper*

# Haemoglobin genetic types and its association with qualitative traits in West African Dwarf sheep

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This study was carried out to investigate the haemoglobin genetic types and their association with qualitative traits in West African Dwarf (WAD) sheep. The Modified Stratified Sampling Technique (MSST) was used to select the sampling sites within the selected state and animal samples within the sampling sites. A total of 280 adult sheep comprising 140 rams and 140 ewes aged 4 years were used for the study. Data were collected on Mendelian traits such as the horn status, wattle status and hair length on sex basis. Blood samples were collected from the animals for haemoglobin genetic types' determination. The results showed that in ewes, the  $f(HbAA)$ ,  $f(HbAB)$  and  $f(HbBB)$  were 0.36, 0.28 and 0.36, respectively and the  $f(HbA)$  and  $f(HbB)$  was 0.50 in both alleles. In rams, the  $f(HbAA)$ ,  $f(HbAB)$  and  $f(HbBB)$  were 0.68, 0.14 and 0.18, respectively and the  $f(HbA)$  and  $f(HbB)$  were 0.75 and 0.25, respectively. In the pooled data,  $f(HbAA)$ ,  $f(HbAB)$  and  $f(HbBB)$  were 0.68, 0.14 and 0.18, respectively and the  $f(HbA)$  and  $f(HbB)$  were 0.625 and 0.375, respectively. The estimated heterozygosity was 0.47 and the estimated local inbreeding coefficient was 0.054. The hair length indicated sexual dimorphism with 12.79 to 12.98 cm in rams and 4.79 to 4.98 cm in ewes but was not dependent on the haemoglobin genetic types. The result shows that the status of wattles is not influenced by sex. The WAD sheep used had three haemoglobin genotypes under the control of two alleles at the haemoglobin locus.

**Key words:** Ewe, haemoglobin genetic type, inbreeding coefficient, Mendelian traits, ram.

## INTRODUCTION

West African Dwarf (WAD) sheep is owned in small stock by rural peasant farmers in southwestern Nigeria. It is an integral part of family units, features prominently in socio-cultural functions and as emergency source of fund. It is versatile and genetically-adapted to the zone where the basic conditions of its well-being are not compromised (Petazzi et al., 2009). This native livestock is not favored in industrial animal agriculture because of the characteristic

small body size and low productivity. However, its tolerance to diseases and seasonal fluctuations in food and water availability are qualities that can be exploited in industrial animal agriculture.

The genetic quality of this livestock species is under threat of erosion by industrial breeds such that their morphologic and genotypic characterization is essential (FAO, 2011). Consequently, a lot of studies had been

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carried out in the area of morphological characterization (Salako and Ngere, 2002; Yakubu and Akinyemi, 2010; Yakubu and Ibrahim, 2011). Unfortunately, the morphological characteristics do not correspond to the genetic characteristics of blood protein and non-protein polymorphisms because the traits are complex in their mode of transmission and are influenced by the environment (Tsunoda et al., 2010). Blood protein characterization therefore may present higher accuracy procedures for a better measurement of genetic variation in sheep breeds because of their polymorphisms and simple mode of inheritance.

Blood protein characterization using electrophoretic method has been used as a tool for studying relationships among farm animals (Sun et al., 2009; Shahrbabak et al., 2010) and genetic differentiation among breeds and in phylogenetic studies (Ibeagha-Awemu and Erhardt, 2004; Camoglu and Elmaci, 2005). Although DNA-based technologies are now in vogue for genetic characterization, the analysis of genetic markers based on blood protein and non-protein variants remains useful because of its utility, ease, cost, amount of genetic information accessed, simplicity of data interpretation (Arora et al., 2011) and because genetic research in Africa is less fully developed as in Europe (Gifford-Gonzalez and Hanotte, 2011).

Among these, blood proteins is haemoglobin which is the red oxygen carrying pigment in red blood cells of vertebrates. It is a conjugated protein with two pairs of identical sub-units, each with a cleft that contains iron-porphyrin group which is the site of oxygen uptake and release. Osaiyuwu and Salako (2018) found two genotypes of Hb comprising 72% HbAA and 28% HbAB in WAD sheep while Dafur et al. (2019) reported 100% HbAA. Mabruka and Ahmed (2018) reported 22% of HbA and 78% of HbB as gene frequencies of alleles *A* and *B* in WAD sheep. While several other research reports are available on the influence of haemoglobin genetic types on disease resistance, ovulation rates and blood traits in local and exotic breeds of sheep (FAO, 1988; Di Stasio, 1997), little had been done on its relationship with Mendelian traits. It is therefore, the objective of this study to investigate the haemoglobin genetic types and its association with some qualitative traits in WAD sheep.

**MATERIALS AND METHODS**

The Modified Stratified Sampling Technique (MSST) was adopted for the choice of sampling sites within Ekiti State, Nigeria and the animals sampled within the sampling sites. A total of 280 adult sheep comprising 140 rams and 140 ewes aged 4 years were used for the study. The age of the sheep was estimated using permanent teeth eruption (Gerald, 1994). In all the sampling sites, the animals were managed extensively and fed with kitchen wastes only when available.

**Data collection and laboratory analysis**

Data were collected on Mendelian traits such as the horn status,

wattle status and hair length on sex basis as described by Akinyemi and Salako (2010). Blood samples were collected from the adult sheep of both sexes by jugular venipuncture into well labeled Bijou bottles containing a speck of ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Plasma and erythrocyte samples were separated from the heparinized whole blood by centrifugation. After centrifugation, red cells were washed three times in saline solution (0.155 M NaCl), and lysed with a four-fold volume of distilled H<sub>2</sub>O to release haemoglobin. The separated lysate was stored at 4°C prior to laboratory analysis.

Gel electrophoresis was carried out on cellulose acetate strips to analyze inherited biochemical differences at haemoglobin (Hb) locus. This involved the use of Tris EDTA Borate at pH 8.6 for haemoglobin as described by Riken (2006). The resultant gel was stained with Red Ponceau stain to visualize the protein bands. The frequency of the allele corresponding to each band was estimated by direct count.

**Statistical analysis**

$$\text{Genotypic frequency for HbAA} = \frac{\text{Number of individuals with HbAA}}{\text{Total number of individuals sampled}} \times 100$$

$$\text{Genotypic frequency for HbAB} = \frac{\text{Number of individuals with HbAB}}{\text{Total number of individuals sampled}} \times 100$$

$$\text{Genotypic frequency for HbBB} = \frac{\text{Number of individuals with HbBB}}{\text{Total number of individuals sampled}} \times 100$$

$$\text{Allelic frequency of allele A} = \frac{AA + \frac{1}{2}AB}{\text{Total number of alleles}} \times 100$$

$$\text{Allelic frequency of allele B} = \frac{BB + \frac{1}{2}AB}{\text{Total number of alleles}} \times 100$$

Data on Hb alleles and genotypic frequencies were subjected to Chi-square goodness of fit (one sample test) to know whether the data conform to Hardy-Weinberg equilibrium using the formula:

$$\Sigma(O - E)^2 / E$$

where *O* denotes the observed data and *E* is the expected value.

$$\text{Inbreeding coefficient (F)} = \frac{\text{Number of genotypes in the population sampled}}{\text{Total number of males and females sampled}} \times 100$$

The degree of heterozygosity was calculated as the expected proportion of heterozygotes in a population under Hardy-Weinberg equilibrium.

The PROC GLM and PROC T-test procedures of SPSS (1989) were used to analyze the effects of haemoglobin variants on the Mendelian traits in the WAD sheep.

**RESULTS**

The distribution of genotypic and allelic frequencies of haemoglobin genotypes of WAD sheep is shown in Table 1. In the rams, only 20 and 25 had HbAB and HbBB with

**Table 1.** Distribution of haemoglobin genotypes and gene frequencies in WAD sheep.

Sex	No.	Genotypic frequency			Allelic frequency	
		AA	AB	BB	A	B
Male	140	95 (0.68)	20 (0.14)	25 (0.18)	0.75	0.25
Female	140	50 (0.36)	40 (0.28)	50 (0.36)	0.50	0.50
Total	280	145 (0.52)	60(0.21)	75 (0.27)	0.625	0.375

AA = Haemoglobin AA; AB = Haemoglobin AB; BB = Haemoglobin BB.

**Table 2.** Observed and expected gene frequency of Hb type A and type B in WAD sheep.

Gene	Observed (O)	Expected (E)	(O-E)	(O-E) <sup>2</sup>	(O-E) <sup>2</sup> /E
Type A	350	420	-70	4,900	11.66
Type B	210	140	70	4,900	35
Total	560	560	0	9,800	46.66**

**Table 3.** Distribution of haemoglobin genetic types in association with hair length (cm) in WAD sheep.

Sex/Hb genetic type	Male	Female
AA	12.98±0.35 <sup>a</sup>	4.98±0.38 <sup>b</sup>
AB	12.79±0.29 <sup>a</sup>	4.82±0.30 <sup>b</sup>
BB	12.81±0.32 <sup>a</sup>	4.79±0.34 <sup>b</sup>

Means with the same superscripts along the same columns are similar ( $p>0.05$ ) while means with different superscripts along the same rows are significantly different ( $p<0.05$ ).

**Table 4.** Distribution of haemoglobin genetic types in association with wattles in WAD sheep (n=280).

Sex/Hb genetic types	Male	Female
AA	80 (0.28)	50 (0.18)
AB	35 (0.13)	50 (0.18)
BB	25 (0.09)	40 (0.14)

genotypic frequencies of 0.14 and 0.18, respectively while those with HbAA were 95 with genotypic frequency of 0.68. The gene frequencies for the two co-dominant alleles A and B were 0.75 and 0.25, respectively. In the ewes, 50 had HbAA, 40 had HbAB and 50 had HbBB with genotypic frequencies of 0.36, 0.28 and 0.36, respectively. The gene frequencies for the two co-dominant alleles A and B were the same at 0.50. When the data were pooled, 145 HbAA, 60 HbAB and 75 HbBB with genotypic frequencies of 0.52, 0.21 and 0.27, respectively were obtained. The allelic frequencies for the two co-dominant alleles A and B were 0.625 and 0.375, respectively. The estimated heterozygosity was 0.47 and

the estimated local inbreeding coefficient was 0.054.

The observed and expected numbers of Hb alleles in WAD sheep are shown in Table 2. The allelic frequencies observed in the population under consideration deviated significantly ( $\chi^2 = 46.66$ ;  $p<0.05$ ) from Hardy-Weinberg equilibrium. Table 3 shows the distribution of haemoglobin genetic types in association with hair length in WAD sheep. The hair length in the Hb genetic types varied from 12.79 to 12.98 cm and 4.79 to 4.98 cm in male and female WAD sheep, respectively. The distribution of haemoglobin genetic types in association with wattles in WAD sheep is presented in Table 4. The  $f(HbAA)$ ,  $f(HbAB)$  and  $f(HbBB)$  were 80, 35 and 25,

respectively in male and 50 and 50 and 40 in female.

## DISCUSSION

The haemoglobin genotypes, two homozygotes AA and BB and one heterozygote AB, detected in this study agree with the general observation that alleles A and B at the same locus are capable of producing three different genotypes AA, AB and BB, in different species of animals (Zaragoza et al., 1987; Tunon et al., 1989). The finding also agrees with Rodero et al. (1996) in Lebrijan Churro breed of Andalusia sheep but not in Merino sheep and Akinyemi and Salako (2010) in WAD sheep. The proportions of the haemoglobin genotypes at 52% HbAA, 22% HbAB and 27% HbBB in this study differ from the 100% HbAA reported by Dafur et al. (2019) in WAD sheep; the 72% HbAA and 28% HbAB obtained by Osaiyuwu and Salako (2018) in WAD sheep; the 88.89% HbAA and 11.11% HbAB in WAD sheep obtained by Akinyemi and Salako (2010); the 0.00% HbAA, 11.11% HbAB and 88.89% HbBB in Lebrijan Churro breed of Andalusia sheep and the 17% HbAA, 20.65% HbAB and 77.17% HbBB for the Grazalema Merino sheep reported by Rodero et al. (1996). However, the result supports the association of the highest frequency of HbAA in females (Akinyemi and Salako, 2010) and the HbAA genotype being the most frequent in WAD sheep (Osaiyuwu and Salako, 2018).

The preponderance of HbAA (52%) genotype can be attributed to an adaptive feature for survivability of the breed as reasoned by Agaviezor et al. (2013) that HbAA has a selective advantage in small ruminants. Also, Tella et al. (2000) reported that the HbAA genotype has selectable advantage in sheep at higher altitudes because its frequency increases towards the forest zone in the Southwestern, Nigeria. The HbAA is more preponderant in rams than in ewes while the HbAB and HbBB are more in ewes which is contrary to the report of Agaviezor et al. (2013) meaning that sex has an effect on the distribution of Hb types in WAD sheep. The  $f(A)$  and  $f(B)$  obtained in this study at 62.5 and 37.5%, respectively differed from 22% of HbA and 78% of HbB obtained by Mabruka and Ahmed (2018) and lower than the corresponding values of 94 and 6% obtained in WAD sheep (Akinyemi and Salako, 2010).

The  $f(HbAB)$  at 0.22 is an indication of the level of genetic diversity at the Hb locus in the investigated sheep population. The estimated degree of heterozygosity (0.47) falls within the 0.30 and 0.80 range reported by Takezaki and Nei (1996) to be appropriate for markers to be used for measuring genetic variation. The rate of inbreeding was low and is in line with the high degree of heterozygosity value, suggesting that the sheep population could be undergoing assortative mating or may be experiencing a Wahund effect.

Comparison of observed and expected allelic frequencies

is a test of the fulfillment of the conditions on which Hardy-Weinberg equilibrium depends. These conditions are random mating among the parents of the individuals observed, absence of migration and mutation which were not fulfilled in this study. This result is consistent with the reports of Musa et al. (2016) but contradicts the observation of Imumorin et al. (1999). The uniformity in hair length within the same sex among the different genetic types indicates that hair length was not dependent on the haemoglobin genetic types.

The hair length showed sexual dimorphism being longer in male than female which agrees with the observation of Akinyemi and Salako (2010). However, the hair length at  $9.27 \pm 4.39$  and  $4.84 \pm 1.28$  cm for ram and ewe, respectively reported by Akinyemi and Salako (2010) were shorter than the values obtained in this study while their postulation that animals with AA haemoglobin type would have longer hair than the other genotypes was not upheld.

The trend observed in the distribution of haemoglobin genetic types in association with wattle in WAD-sheep shows no indication that the different genotypes affect either the presence or absence of wattle in the population. Also, the status of wattle was not sex influenced as it was found in both rams and ewes.

## Conclusion

Haemoglobin polymorphisms in WAD sheep is defined by two alleles, 'A' and 'B' with the  $f(A)$  being 0.625 while the  $f(B)$  was 0.375. The hair length showed sexual dimorphism with longer length in male than female. However, the haemoglobin genetic type did not influence the trait. The status of wattle is not sex influenced as it occurred in some rams and ewes.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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