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Studies of Genetic Distance, Gene and Genotypic Frequencies of Hemoglobin Types of West African Dwarf and Yankassa Sheep

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***Target Audience:** Beef cattle breeders, Researchers, Molecular geneticists*

Abstract

Hemoglobin (Hb) alleles, genotype and their frequencies were studied in 50 West African Dwarf (WAD) and 50 Yankasa sheep. Blood samples were collected from the animals and analysed, hemoglobin (Hb) type were determined by cellulose acetate electrophoresis. The resultant data were subjected to statistical analyses. The gene frequencies estimated in each allele of the population and were tested using Hardy-Weinberg equilibrium. The genetic distance was thereafter estimated according to Bodmer and Cavalli-Sforza method. The frequencies of Hb(A) and Hb(B) in WAD sheep were 0.35 and 0.65 and genotype frequencies were HbAA (0.34), HbAB (0.00) and HbBB (0.66). The corresponding values in Yankassa sheep were 0.75 and 0.25, 0.70, 0.10 and 0.20, respectively. The distribution of the haemoglobin genotypes was tested using Chi-square analysis. The genetic distance at the haemoglobin locus between WAD and Yankassa sheep was estimated and was found to be 0.16, which shows that within the limit of these small populations sampled, there is a similarity of alleles at the locus. Sampling larger number animals in different breeds of sheep will allow for a better genetic studies and marker-assisted selections in sheep improvement programs.

***Keywords:** Genetic Distance, hemoglobin, Sheep, WAD, Yankassa.*

Description of Problem

Farm animals play a crucial part in the livelihood systems and well-being of the poor in the developing world, and thereby in helping to meet the Sustainable Development Goals (SDGs). In addition to food, clothing and other goods, livestock are important for income generation, wealth accumulation, traction and nutrient re-cycling.

In all farm animals, the importance of sheep cannot be overemphasized. *ILCA*(1), *ILCA* (2) and *Fitzurgh et al.* (3) established that over 90% of sheep in sub-Saharan are found in East and West Africa and that they provide about 30% of the meat consumed and 15% of the milk produced in the region. Jollams (4), Adu and Ngere (5) also established that sheep constitute the main and preferred source of meat in the humid zone of West Africa.

In addition to the above, the ability of small ruminants to tolerate harsh climates, the

presence of trypanotolerance in some breeds (6), suitability to traditional systems on account of small size (7), short generation interval (8) and ability to thrive on poor quality diets provided by scarce grazing on marginal lands (9), all combine to make small ruminants strategic in increasing livestock productivity in rural agricultural systems. It is however, unfortunate that in spite of these advantages little attention had been given to the genetic improvement of small ruminants in Nigeria until recently (10) and (11).

Protein electrophoresis had been widely used in studying genetic diversity in several farm animals and poultry (12, 13, 14, 15, 16 and 17). The genetic diversity found in domestic breeds allows breeders to select and develop new breeds in response to changes in demand, environment or climate (17). In an attempt to genetically improve the production of sheep and other small ruminant a lot of trial and error methods of selection of breeds constituting specific crosses are being put in place which were resource wasting because of lack of sufficient information about the level of relatedness of breeds in each species.

The need for the determination of genetic distance among the selected breeds in each species is therefore of paramount importance so as to assess the genetic structure and relatedness to other breeds and to be able to predict potential gains from crossbreeding among the individual populations thereby replacing the previous resource wasting trial-and-error-method.

Materials and Methods

Experimental Site and Management of the animals

The study was conducted at Animal Production Unit, Department of Agricultural Science, Adeyemi College of Education, Ondo. A total of 100 sheep comprising 50 Nigerian WAD and 50 Yankassa sheep were studied. The animals were randomly selected ignoring sex from the Teaching and Research Farm of the institution. The animals were managed semi-intensively by the institution with an extensive paddock for grazing and exercising. All the animals were well fed, clinically healthy and free from internal and external parasites.

Blood Collection

Blood samples of 5 to 10ml. were collected by jugular venipuncture from 50 WAD and 50 Yankassa sheep between the hours of 8a.m. and 9a.m. into separate ten milliliter (10mL) heparinized vacutainer tubes with heparin as an anti-coagulant. Separate needles and syringes were used to forestall any transmission of disease infection. The blood samples were kept cold by placing them in ice packs and cares was taken to prevent exposure to extreme temperatures and were transported to Animal Breeding and Genetics laboratory, Department of Animal Science, Faculty of Agriculture and Forestry, University of Ibadan for analysis.

Laboratory Analysis

0.5 – 1ml. of whole unsedimented blood were drawn into a vacutainer tubes and 10-15ml. of cold NaCl (0.155ml.) was added to wash the red cells, the samples were centrifuged at 5^0C for 5 minutes at 3000 r.p.m., the supernatant saline was discarded and distilled water was added to the sedimented cells to release the hemoglobin by hemolysis. A transfere pipette was used to remove the hemolysate, a cellulose acetate strips were prepared and labeled with pencil. The strips were later soaked in Tris-EDTA borate buffer (TEB, pH 8.6) and blotted lightly with a filter paper. Haemolysates were applied with a micropipette at the cathode and electrophoresis was performed at pH 8.6 using a Shandom Southern Electrophoresis tube with TEB as electrode buffer at 100V for 15-20 minutes. The strips were later removed and stained with

Poncean Red Stain for 5-10 minutes. The strips were later de-stained first in 5% ethanoic acid(acetic acid) followed by 12% ethanoic acid(acetic acid) solution until the strips were cleared of stain. The strips were later dried in the oven, allelic variants for the hemoglobin (Hb) locus were marked in order of increasing mobility were represented as A and B respectively they were then scored based on the band following the normal procedure as previously described by (15 & 16).

Statistical Analysis

Allele and genotypic frequencies for the locus in each sample were computed by direct gene counting method. The distribution of the haemoglobin genotypes was tested using Chi-square analysis. The genotype frequency for each of the population was estimated using the following expression as used elsewhere by (18).

$$AA = \frac{No\ of\ AA\ x\ 100}{No\ of\ individual\ WAD\ or\ Yankassa}$$

$$BB = \frac{No\ of\ BB\ x\ 100}{No\ of\ individual\ WAD\ or\ Yankassa}$$

$$AB = \frac{No\ of\ AB\ x\ 100}{No\ of\ individual\ WAD\ or\ Yankassa}$$

The gene frequency for each of the population was estimated using the expression, provided by (18)

$$Allele\ A = \frac{2nAA + nAB}{N/2}$$

$$Allele\ B = \frac{2nBB + nAB}{N/2}$$

Where N = total no of individuals sampled in each population
nAA = Observed genotype number for AA
nBB = Observed genotype number for BB
nAB = Observed genotype number for AB

The genetic distance between WAD and Yankassa sheep was estimated according to the method of Bodmer and Cavalli-sforza (19) with the following expression

$d^2 = (P_1 - P_2)^2$

where d^2 = genetic distance
P_1 = gene frequency of allele A
P_2 = gene frequency of allele B

d_2 is simply the square of the difference of the two gene frequencies from the pair populations.

Table 1: Genotypic distribution of hemoglobin (Hb) genotypes in WAD and Yankassa Sheep

	WAD		Yankassa	
Hb Type	No.	Frequency (%)	No.	Frequency (%)
AA	17	34.0	35	70.0
AB	-	0.0	5	10.0
BB	33	66.0	10	20.0
TOTAL	50	100	50	100

WAD = West African Dwarf; Hb = Haemoglobin; No. = Number

Table 2: Gene frequencies of Hb alleles of WAD and Yankassa Sheep

Breed	No.	Gene frequency						A	B
		AA		AB		BB			
		No.	%	No.	%	No.	%		
Yankassa	50	35	70	5	10	10	20	0.75	0.25
WAD	50	17	34	0	0	33	66	0.35	0.65

WAD = West African Dwarf; Hb = Haemoglobin; No. = Number

Results and Discussion

Table 1 shows the genotypes distribution of haemoglobin in WAD and Yankassa sheep.

The distribution of Hb genotypes (AA, AB and BB) were 35(70.0%), 10(10.0%) and 20 (20.0%), respectively in Yankassa sheep while the corresponding values in WAD sheep were 17(34.0%), 0(0.0%) and 33 (66.0%) (Table 1). The Chi square analysis for the differences between observed and expected genotype frequencies showed that the deviation was significant ($P<0.05$). Suggesting that the flocks studied is Hardy-Weinberg equilibrium for the haemoglobin (Hb) locus. The frequency of Hb^A allele was 0.75 in Yankassa which was higher than 0.35 in WAD sheep and the reverse was the case for Hb^B allele (0.25 versus 0.65), (Table 2). This was similar to what (12), (13) and (18) reported for RS and WAD goats, sheep and goats and WAD sheep respectively. In similar studies of Hb in sheep, Hb^A has been implicated to have high affinity for oxygen and it is important for survival in areas of altitude above 3000m. Also a possible correlation between Hb polymorphism and genetic resistance to helmith infection in sheep and goat has also been reported (20). The estimated genetic distance between WAD and Yankassa sheep was 0.16. This is a little bit high when compared with the range of 0.003 to 0.097 in 14 Spanish sheep breed as reported by (21) though one should expect this since Spanish sheep are of the same breed while Yankassa and WAD sheep are of different breeds though same species. This shows that within the limit of these small populations sampled there is high similarity of alleles at the Hb locus. This also suggests that the investigated populations were mixture of sub-populations. The genetic distance of 0.16 also shows that Yankassa and WAD sheep are quite distinctive breeds genetically though of the same species and this shows that the crossbreeding of the two breeds

will bring about a better hybrid and therefore provide great opportunity of investment in sheep production. The hybrid will show forth the endowed characteristics of hardiness and high reproductive performance in WAD and good milking ability in Yankassa.

Though these discrepancies in WAD and Yankassa sheep that were highly significant ($P<0.001$) suggests that the samples were mixed of sub-population with different gene frequencies. This confirmed why there were more homozygotes and a short fall in heterozygotes in both populations.

The used of recombinant DNA technology with the restriction of enzymes which cleave DNA at specific recognition sites is more reliable than the electrophoretic analysis used for this study, the DNA-DNA hybridization even appear more reliable than iso-enzyme distances. There is no doubt about it, that these new alternative approaches offered by molecular genetics would give a perfect measurement of genetic distances from the comparison of DNA sequences, though time consuming and expensive it should be investigated.

Conclusion and Applications

The following conclusion could be made from the result of the current study:

1. Considering the genetics distance of 0.16 between the breeds, the results had given a base for a large room of improvement when a large sample size is used in a number of polymorphic loci.
2. The variation indicated that the population had not been previously selected for any genetic improvement.
3. Selection in sheep production on the basis of Hb polymorphism would provide genetic progress towards better genetic resistance to helmith.
4. The true level of variability may be grossly underestimated due to the small sample size used for this study; however the result has provided a genetic resource on which further studies can be built.

References

1. ILCA, (1989). Annual Report. International Livestock Centre for Africa, Addis Ababa, Ethiopia
2. ILCA, (1990) Annual Report. International Livestock Centre for Africa, Addis-Ababa, Ethiopia.
3. Fitzurgh, H. A., Ehui, S. K. and Lahlon-Kassi, A. (1992). Research strategies for development of animal agriculture. World Animal Review 72 (3): 1 - 9.
3. Jollams, L. O. (1960). The West African Dwarf goat. West African Journal of Biological Chemistry 11(3) 74 - 80.
5. Adu, I. F. and Ngere, L. O. (1979). The indigenous sheep of Nigeria. *World Review of Animal Production 15(3) 51 - 62*
6. David-West, K. B. (1983). Problems and prospects of livestock development in Nigeria. *Nig. J. Animal Production 10 (1):16 - 23*.
7. Odubote, I. K. (1994). Genetic analysis of the reproductive performance of West African Dwarf goats in the humid tropics. In: small ruminant research and development in Africa. S.H.B Lebbie and E. Kagwini, Eds., Proc. 3rd Biennial Confr. Of the African Small Ruminant Network, Kampala, Uganda, 5-9 Dec. pp. 33-36.
8. Mack, S. D., J. E. vans Eys and Okali, C. (1985). Small ruminant production under pressure: The example of goats in Southern Nigeria. In: Proc. Workshop in small Ruminant Production Systems in Humid Zone of West African, 23-26 January, 1984,

Ibadan, International Livestock Centre for Africa, Addis Abba, Ethiopia

9. Johnson, W. L; Vans Eys, J. E. and Fitzurgh H. A. (1986). Sheep and goats in tropical and sub-tropical agricultural systems. *J. Anim. Sci. 63 (2):1587-1599.*
10. Ebozoje, M. O. (1998). Preweaning performance of West African Dwarf goats and West African Dwarf X Maradi Halfbreds in Ibadan, Nigeria. Ph.D Thesis, University of Ibadan, Ibadan, Nigeria.
11. Odubote, I. K. and Akinokun, J. O. (1992). Estimates of genetic parameters for economic traits in West African Dwarf goat. *Nig. J. Animal Producion 19 (1):114 - 119.*
12. Imumorin, I. G., Ologun, A. G. and Oyeyemi, M. O. (1999). Preliminary observations on effects of haemoglobin genotype and estimate of genetic distance at the Hb locus in West African, Dwarf and Red Sokoto goats. *Trop. J. Anim. Sci. 19 (1):1 - 9.*
13. Akinyemi, M. O. and Salako, A. E. (2012). Genetics relationship among Nigerian indigenous sheep populations using blood protein polymorphism. Agricultural Science and Technology, 4(2), 107 – 112.
14. Ige, A. O., Salako, A. E., Ojedapo, L. O. and Adedeji, T. A. (2013). Biochemical characterization of indigenous Fulani and Yoruba ecotypes chicken of Nigeria. African Journal of Biotechnology, Vol. 12(50), pp. 7002 – 7008.
15. Osaiyuwu, O. H., Akinyemi, M. O., Omoike, O. A. and Salako, A. E. (2013). Haemoglobin polymorphism in Red Sokoto Goats of Nigerian. Journal of Animal Production advances. 3(9): 278 – 282.
16. Ige, A. O. and Salako, A. E. (2014). Transferrin Genetic Types in Fulani and Yoruba Ecotype of Nigeria indigenous chickens. Iranian Journal of Applied Animal Science, 4 (1): 191 – 196.
17. Fatai, R. B., Akinyemi, M. O. and Osaiyuwu, O. H. (2017). Genetic variation in indigenous Turkey populations in South West Nigeria. Journal of Advances in Agriculture. 7 (2): 1021 – 1029.
18. Salako, A. E., Ijadunola, T. O. and Agbesola, Y. O. (2007). Hemoglobin polymorphism in Nigerian indigenous small ruminant populations – preliminary investigation. *African Journal of Biotechnology. 6 (22): 2636 - 2638.*
19. Roughgarden, J. (1979). Theory of population genetics and evolutionary ecology: An Introduction, Macmillan Publ. Co. Inc. New York.
20. Bodmer, W. F. and Cavalli-Sforza (1976). Genetic, evolution and man. W.H. Freeman & Co. San Francisco.
21. Buvanenedran, V.,Umoh, J. E. and Abubakar, B. V. (1980). An evaluation of body size as related to weight of three West African breeds of cattle in Nigeria. Journal of Agricultural Science, Cambridge, 95: 291 - 224.
22. Tunon, M. J., Gonzalez, P. and Vallejo, M. (1989). Genetic relationships among 14 Spanish breeds of sheep. *Animal Genetics 20 (2): 205-212.*