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PRELIMINARY OBSERVATIONS ON EFFECTS OF HAEMOGLOBIN GENOTYPE AND ESTIMATE OF GENETIC DISTANCE AT THE HB LOCUS IN WEST AFRICAN DWARF AND RED SOKOTO GOATS*

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Target audience: Academics/Researchers in animal production and molecular genetics.

ABSTRACT

Haemoglobin (Hb) alleles, genotypes, their frequencies and effects of Hb type on some traits were studied in 126 West African Dwarf (WAD) and Red Sokoto (RS) goats. The frequencies of HbA and HbB in RS goats were 0.467 and 0.533, genotype frequencies were HbAA (0.355), HbAB (0.224) and HbBB (0.421). The corresponding values for WAD goats were 0.530, 0.470 and 0.300, 0.460 and 0.240 respectively. Hb type did not significantly affect packed cell volume and total serum proteins. However, Hb type affected haemoglobin concentration in both breeds ($P < 0.05$). HbAA and HbAB type animals had significantly ($P < 0.05$) higher Hb concentration than HbBB animals. Hb type influence may indicate a superior Hb content in erythrocytes associated with the HbA allele. Hb type had no significant effect on four production traits: gestation length, oestrus duration, kid birth weight and average daily gain studied in WAD goats only. Estimate of genetic distance between WAD and RS goats was 0.004 based on Hb locus. This low value may indicate high similarity of alleles at this locus and may have phylogenetic implications. Our current work employing larger sample sizes and many more polymorphic genetic loci will further elucidate this situation. This may pave the way for marker-assisted selection in the genetic improvement of Nigerian goats using biochemical markers.

Key words: Haemoglobin genotypes; genetic marker; genetic distance;goats.

DESCRIPTION OF PROBLEM

A recent livestock census by Resources Management Inventory (1) put the goat population estimate in Nigeria at 34.5 million. This report supports the assertion that Nigeria is more self sufficient in small ruminants than

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in cattle (2). The ability of small ruminants to tolerate harsh climates, the presence of trypanotolerance in some breeds (3), suitabilty to traditional systems on account of small size (4), short generation interval (5) and ability to thrive on poor quality diets provided by scarce grazing on marginal lands (6) all combine to make small ruminants strategic in increasing livestock productivity in rural agricultural systems. Despite these advantages, little attention had been paid to the genetic improvement of small ruminants in Nigeria until recently (7, 8).

Genetically controled biochemical polymorphic loci are not only of genetic and polygenic interest but they can also act as genetic markers for performance traits (9, 10). Genetic distances estimated from polymorphic marker loci may be used to predict heterosis (11) and for breeding policy formulation and conservation (12).

Buvanendran et al. (13) have demonstrated a relationship between haemoglobin polymorphism and resistance to helminth infection while allelic and genotype frequencies of transferrin in Red Sokoto (RS) goats were reported (14). Due to the scanty information on biochemical genetic characterization of Nigerian goat breeds, we conducted this preliminary screening for haemoglobin polymorphic types and investigated possible putative effects on some blood and production traits. Only West African Dwarf (WAD) goats were used to test the effect of Hb type on production traits due to limitation of available data on RS goats. We also estimated genetic distance between the two breeds at the Hb locus using Hb allelic frequencies.

MATERIALS AND METHODS

Animals : This study utilized a WAD goat flock maintained in the Small Ruminant Unit of the Department of Veterinary Surgery and Reproduction, University of Ibadan, Ibadan. They were kept in a purpose-built house made of cement blocks and concrete floor covered with corrugated iron sheets. Pen floors covered with sawdust and wood shavings provided bedding for the animals. The animals were placed on pasture between 0900 and 1600 hours daily. The pasture was a mixture of guinea grass, elephant grass and Cynodon species. This diet was supplemented with a mixture cassava peels, brewery wastes and corn bran (by-product of corn starch production) at a rate of 50 g/head/day. Standard veterinary care was provided by a resident veterinarian. RS goats brought to the Government Abattoir, Bodija, Ibadan for slaughter were sampled. No information was available on their husbandry and management history.

Blood analyses: Five to eight ml of blood was collected by jugular venipuncture from 50 WAD goats between 0800 and 0900 hours daily. One portion of each sample was expressed into a clean bijou bottle with Na_2-EDTA as an anticoagulant and the rest allowed to clot for sera separation. Sera were separated after 4-6 hours. From 76 RS goats, blood samples were

similarly collected just before slaughter. Packed cell volume (PCV), haemoglobin concentration (Hb conc.) and total proteins (TSP) were determined using standard methods (15).

Haemoglobin typing: Hb alleles were typed using cellulose acetate electrophoresis as described with slight modifications (16). Briefly, 0.5 - 1 ml of whole unsedimented blood was placed into a centrifuge tube and 10 - 15 ml of cold 0.155 M NaCl was added to wash the red cells. The samples were centrifuged at 5 ^{o}C for ten minutes at 900 g and the supernatant discarded. Cold distilled water was added to the sedimented cells to release the haemoglobin by haemolysis. The haemolysates were removed with a tranfer pipette and stored in test tubes at between -10^{o} and -15^{o} C for a maximum of four months before electrophoresis.

Cellulose acetate strips (Oxoid) were prepared and labelled. They were soaked in Tris-EDTA borate buffer (TEB, pH 8.6) and blotted slightly with filter paper to remove excess buffer. Haemolysates were applied with a micropipette and electrophoresis was performed using a Shandon Southern Electophoretic tank with TEB (pH 8.6) as the electrode buffer at 450 V for 30 - 35 minutes. The strips were stained with Ponceau red S for 5 - 10 minutes and progressively destained in 5 % and then 12 % acetic acid solution. The strips were then dried in the oven for 30 minutes at 60^{o} C. The direct gene counting method was used to score Hb bands based on the separation of Hb variants (9). Gene frequencies were calculated using equations provided by Roughgarden (17).

Genetic distance : Using the method of Bodmer and Cavalli-Sforza (18), genetic distance between WAD and RS goats was estimated at the Hb locus using allelic frequencies.

Production traits: Data on gestation length (days), oestrus duration (hours), kid birth weight (kg) and average daily gain (kg) were collected from the production records of the WAD goat flock and categorized into Hb genotype classes.

Statistical analyses: Data on Hb alleles and of genotype frequencies were subjected to chi-square analysis to test for Goodness-of-Fit for observed versus expected frequencies under Hardy-Weinberg equilibrium. One way analysis of variance was performed to determine the effect of Hb type on blood and production traits while mean separation was done using Fisher's Least Significant Difference (19).

RESULTS AND DISCUSSION

Haemoglobin alleles and genotype frequencies: The distribution of Hb genotypes (AA, AB and BB) were 27 (35.5 %), 17 (22.4 %) and 32 (42.1 %) in RS goats while the corresponding values in WAD goats were 15 (30.0 %), 23 (46.0 %) and 12 (24 %) (Table 1). The frequency of HbA allele was 0.467

in RS which was slightly lower than 0.530 in WAD goats, and the reverse was the case for HbB allele (0.533 versus 0.470). The two Hb variants A and B alleles identified in WAD and RS goats and the corresponding detectable genotypes AA, AB and BB are in agreement with the general observation in goats in other parts of the world (20). But two instead of three variants seen in RS goats contradicts Buvanendran et al. (13) as well as the corresponding three genotypes instead of five. This inconsistency may be due to the cellulose acetate electrophoresis resolution system used by these workers and this current work possibly causing some bands to merge confounding interpretation of the electrophoretograms. The use of starch gel or agarose electrophoresis may be more clarifying (10). This is being pursued in further analysis. However, the existence of different lines and distinct populations (12) may warrant more extensive typing.

Table 1: Distribution of Haemoglobin(Hb) Genotypes and Gene Frequencies of Hb Alleles.

Breed	n	Hb Genotypes						Gene Freq.	
		AA	%	AB	%	BB	%	A	B
RS	76	27	35.5	17	22.4	32	42.1	0.467	0.533
WAD	50	15	30.0	23	46.0	12	24.0	0.530	0.470

RS = Red Sokoto; WAD = West African Dwarf

The frequencies of HbA and HbB in WAD and RS goats were lower than in 14 Spanish goat breeds (21). This may simply reflect peculiarities of breeds in the different parts of the world vis-a-vis geographical and associated ecological niches. Using chi-square analyses, it was discovered that the distributions of genotype frequencies on the basis of expected frequencies under Hardy-Weinberg equilibrium was normal in the WAD goat population, while the RS goat population departed very significantly ($Xi = 23.06$; $P < 0.001$) from Hardy-Weinberg equilibrium. This departure fits the theoretical expectation of differences between observed and expected genotype frequencies in a population which is a mixture of subpopulations with different gene frequencies characteristic of Wahlund effect (18).

These are consistent with sampling from an abattoir where animals come from disparate and unknown sources to be slaughtered for sale. Our preliminary study involving one locus did not investigate inheritance and segregation ratios nor strength of heterozygosities at many loci. As a result, more information required to make inferences about the breeding structure and genetic architecture of these breeds using marker loci is being pursued.

Blood traits: Blood traits in both breeds are shown in Table 2. PCV was higher in RS (27.8 ± 9.2 %) than in WAD (25.8 ± 4.0 %) goats. The difference only approached significance. Hb concentration was significantly ($P < 0.05$) higher in WAD (7.0 ± 4.3 g/100 ml) than in RS (4.1 ± 2.6 g/100 ml) goats. Total serum protein tended to be higher in WAD than in RS goats. These differences cannot be easily explained either by genetic factors or nutrition since the WAD goats would appear to be on a higher plane of nutrition. It is more likely that the RS goats though raised semi-intensively were not malnourished. Hb type significanly affected ($P < 0.05$) Hb concentration but did not exert any significant effect on PCV and TSP.

Table 2: Means+SD of Blood Traits in West African Dwarf and Red Sokoto Goats Between Sexes and Breeds*

Blood Traits	Breeds					
	RS			WAD		
	M	F	Total	M	F	Total
PCV(%)	28.2±9.6	27.8±8.3	28.0±8.8	28.0±5.4	25.1±3.2	25.8±4.0
	(23)	(31)	(54)	(7)	(21)	(28)
Hb(g/100ml)	4.7±2.9	4.1±2.3	4.3±2.6[a]	5.7±3.5	8.4±4.6	7.0±4.3[b]
	(33)	(43)	(76)	(13)	(34)	(47)
TSP(g/100ml)	6.2±0.7[a]	6.6±0.7[b]	6.4±0.7	6.6±0.6	6.6±0.6[b]	6.6±0.6
	(27)	(35)	(62)	(11)	(30)	(41)

M = Males ; F = Females

a,b within rows, means with different superscripts are significantly different from each other ($P<0.05$)

* Sample sizes are shown in parentheses under each value

Among the Hb genotypes, animals with HbAA and HbAB showed significantly higher levels of Hb concentration in the blood than those of HbBB genotype (Table 3). This agrees with Jilek and Bradley (22) that HbA or AB were associated with higher mean Hb concentration in ewes. This may indicate a superior Hb content in erythrocytes associated with the HbA allele. The amino acid differences, if any, leading to differential expression of the Hb content in erythrocytes may be attributed to differences in nucleotide coding sequences at the Hb locus.

Production traits: Table 4 summarizes the production traits in WAD goats studied. The mean values for average daily gain was seen to possess the greatest variability while gestation length showed the least. Table 5 shows performance among Hb types for the production traits in WAD goats. Hb type had no significant effect on all production traits. The results confirm that of Dalal et al. (23) on sheep. The present study conflicts with the general evi dence in favour of the effect of HbAB and HbBB alleles on productive performance reported extensively in sheep (22, 24, 25). This could be due to differing genetic reasons for Hb type effect on production

in goats. Our current effort in sampling larger numbers will throw more light on this effect.

Table 3 : Means +SD of Blood Traits in West African Dwarf and Red Sokoto Goats Among Hb Types*

Hb Type	Blood Traits	Breeds		Total
		WAD	RS	
AA	PCV(%)	26.4±1.5(5)	25.4±9.1(21)	25.5±8.4(26)
	Hb(g/100ml)	8.5±5.8(14)	4.2±2.6(27)	5.7±4.4(41)[a]
	TSP(g/100ml)	6.5±0.6(11)	6.3±0.7(22)	6.4±0.7(33)
AB	PCV(%)	25.9±3.8(14)	29.0±7.3(13)	27.4±5.9(27)
	Hb(g/100ml)	7.8±4.3(23)	5.0±3.4(16)	6.7±4.2(39)[a]
	TSP(g /100ml)	6.7±0.6(19)	6.4±0.9(14)	6.6±0.7(33)
BB	PCV(%)	25.4±5.4(9)	30.0±9.1(20)	28.6±8.3(29)
	Hb(g/100ml)	6.5±3.6(10)	4.2±2.1(32)	4.7±2.7(42)[b]
	TSP(g/100ml)	6.4±0.4 (12)	6.5± 0.6(26)	6.5±0.6(38)

a,b within columns, means with the same superscripts are not significantly different from each other.

* Sample sizes are shown in parentheses.

Table 4: Summary of some Performance Traits in West African Dwarf Goats

Traits	Mean±S.D	C.V(%)	Sample size
ED (Hours)	23.8±9.1	38.2	21
GL (Days)	143.0±2.2	1.5	15
KBW (kg)	1.3±0.2	15.48	19
ADG (Kg)	0.033±0.014	42.4	27

ED = Estrus duration; GL= Gestation length; KBW= Kid birth weight; ADG = Average Daily Gain

Table 5 : Means+S.D of some Production Traits in WAD Goats Among Haemoglobin Types

Traits	Haemoglobin Types		
	AA	AB	BB
EL (hours)	23.6±8.0	21.3±11.4	26.7±9.0
GL (days)	142.0±1.9	142.7±0.6	144.6±2.6
KBW (kg)	1.3±0.2	1.2± 0.1	1.3±0.3
ADG (kg)	37.0±1.0	28.0±1.0	35.0±1.0

Genetic distance: The estimated genetic distance between WAD and RS goats was 0.004 using gene frequencies of HbA allele at the Hb locus. This is within the range reported in 14 Spanish goat breeds (21). However, this estimate was based on polymorphism at a single locus compared to the range of Tunon et al. (21) based on six polymorphic marker loci. This distance therefore, is likely to be an overestimate because of large sampling variances (26). On the other hand, it is at best a rough and preliminary estimate, the accuracy of which can be improved by information provided from typing a larger number of polymorphic loci in a larger population of animals (11). It may also indicate a possible evolutionary conservation of alleles at the Hb locus between WAD and RS goat breeds.

CONCLUSIONS AND APPLICATIONS

Our conclusions from this preliminary report may be summarized as follows:

1. Haemoglobin marker locus had no detectable effects on blood traits in both goat breeds and production traits studied in WAD goats.
2. There is a very small genetic distance between WAD and RS goats at the Hb locus based on our sample size. However, we consider this study a preliminary biochemical genetic characterization in Nigerian goats. Further work utilizing many more marker loci and larger samples in progress will more accurately determine phylogenetic relationships, clarify evolutionary development and ascertain relationships between polymorphic marker loci and economic traits.
3. A refined genetic distance can be applied to predict heterosis in designing crossbreeding programmes for goat genetic improvement (11).
4. Future work being planned to include informative matings from large families will form the foundation of gene mapping using linkage in segregating families and exploitation of marker-assisted selection for goat improvement in Nigeria.

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