

Genetic Relationship among Indigenous Goat Populations in Nigeria based on Cellulose Acetate Electrophoresis Systems

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Abstract

Genetic diversity is the baseline for survivability and improvement of livestock in a wide array of environments. Variations among Red Sokoto, Sahel and WAD goats was determined using 5ml of blood collected by jugular venipuncture from 150 randomly selected goats (50 goats per breed). The samples were subjected to Cellulose Acetate Electrophoresis to determine the genetic variants of Haemoglobin (Hb), Transferrin, Carbonic Anhydrase, Albumin, Esterase 1, Esterase 3 and Alkaline phosphatase. Allele frequencies, similarity indices as well as genetic distances among the breeds were estimated. All the breeds in this study were polymorphic for Haemoglobin, Transferrin, Carbonic Anhydrase, Albumin, Alkaline phosphatase and Esterase 1 except WAD which was monomorphic for Alkaline phosphatase. The frequencies of Hb^A was considerably higher in Red Sokoto and WAD while frequencies of Hb^B were higher in Sahel goat population. The significant deviations from HWE ($P < 0.05$, $P < 0.01$ and $P < 0.001$) was observed for some of the loci within the breeds. Genetic distance (D) obtained in this study ranged between 0.0648 and 0.1579 which indicates little genetic differentiation between the breeds. The results of this current study suggest that genetic improvement will be better achieved if WAD and Sahel goats are crossed.

Keywords: Biochemical characterization, Electrophoresis, Genetic diversity, Hardy-Weinberg Equilibrium, Indigenous goats

1. Introduction

Livestock resources in Nigeria are estimated to include about 35 million goats [1,2]. Goats play a vital role in subsistence Agriculture given their ability to adapt to different environments as multipurpose animals [3]. The indigenous small ruminant populations in Nigeria comprising sheep and goat are important genetic resources because of their adaptive features [4].

Nigeria is known to be home to three major breeds of goats: Sahel, Red Sokoto and the West African Dwarf (WAD) goat [5] which differs in size, coat colour and other morphometric characters. These animals are able to survive under a stressful environmental condition such as high disease incidence, poor nutrition, high temperature and

different husbandry system because of their broad genetic variability.

Recent, advances in biotechnology has paved way for genetic characterization at the molecular levels using techniques such as electrophoresis for polymorphism at protein and enzyme loci and immunogenetic methods for the estimation of variation [6]. Therefore, genetic variation has become improving animal species [6].

Polymorphism is a genetic variant that appears in at least 1% of a population in a herd [7]. It is a discontinuous genetic variation where two or more forms, stages, or types exist in the same species within the same population [8]. It can apply to biochemical, morphological, and behavioural characteristics, but must be discontinuous. Blood protein polymorphisms have been used by several researchers as markers to study evolutionary relationships in mammals. It has been reported by several researchers [9-10] that for an animal that has a gene for a specific substance, the substance

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can be detected in the blood by appropriate procedures, such as electrophoresis and the presence or absence of specific substance is directly related to the genotype. The Objective of this study was to provide information at the biochemical level on the genetic structure and diversity of the major Nigerian goat breeds.

2. Materials and methods

The study was carried out in Ibadan, South-west Nigeria, 120km east of the border with the Republic of Benin. The city is 150m to 275m above sea level with a total area of 3,080km². The mean total rainfall is 1420.06mm with two rainfall peaks in June and September; and mean maximum temperature of 26.46°C, with a relative humidity of 74.55%. (<http://en.m.wikipedia.org/Ibadan>). Blood samples were randomly collected from a total of one hundred and fifty (150) adult goats of both sexes by jugular venipuncture (50 each of Red Sokoto, Sahel and WAD goats) into the heparinized tube and transported in ice. Plasma and erythrocyte fractions were separated from the heparinized whole blood by centrifugation (3500rpm/10mins) at 4°C. Red cells were washed three times with saline solution (0.155M NaCl) and lysed with the fourfold volume of dH₂O. Separate aliquots of plasma and erythrocytes were stored at 4°C until they were analysed.

Through cellulose acetate electrophoresis, inherited biochemical differences in the red blood cells and plasma were tested using a Mini-Protein 3 Gel Electrophoresis System (BioRad#165-3301). Seven structural loci were analysed – Haemoglobin (Hb), Transferrin (Tf), Carbonic anhydrase (Ca), Alkaline phosphate (Alp), Albumin (Alb), Esterase 1(Est-1) and Esterase 3 (Est-3) following the procedure described [11].

Allele frequencies for each protein and enzyme systems were computed by direct gene counting method. Genetic parameters such as allelic frequencies, Nei's Genetic distance and identity [12], heterozygosity, a test of Hardy-Weinberg Equilibrium, F-Statistics and phylogenetic reconstruction from distance matrix were performed using Tools for Population Genetic Analysis (TFPGA) [13] and Pogene [14].

3. Results and discussion

The results of allele frequencies of seven loci tested in 3 indigenous goat breeds are presented in Tables 1. Of the seven loci tested, six were polymorphic in Red Sokoto (RS) and Sahel populations while only 5 were polymorphic in West African Dwarf (WAD) population.

Table 1. Allele Frequency for seven Polymorphic Loci in Nigerian Indigenous Goat Breeds

LOCUS	N	Allele	Observed no of alleles	POPULATIONS			Overall
				RED SOKOTO	WAD	SAHEL	
Hb	150	A	159	0.8500	0.8500	0.2400	0.6467
		B	141	0.1500	0.1500	0.7600	0.3533
Ca	147	F	133	0.4681	0.5900	0.3000	0.4524
		S	161	0.5319	0.4100	0.7000	0.5476
Tf	148	A	96	0.4388	0.2755	0.2600	0.3243
		B	200	0.5612	0.7245	0.7400	0.6757
Alb	150	A	150	0.5600	0.4400	0.5000	0.5000
		B	150	0.4400	0.5600	0.5000	0.5000
Est-1	148	A	45	0.2245	0.1633	0.0700	0.1520
		B	251	0.7755	0.8367	0.9300	0.8480
Est-3	150	B	300	1.0000	1.0000	1.0000	1.0000
Alp	148	A	100	0.5000	-	0.5000	0.3378
		B	196	0.5000	1.0000	0.5000	0.6622

HB – Haemoglobin, CA – Carbonic Anhydrase, TF – Transferrin, ALB – Albumin, EST 1- Esterase 1, EST 2 – Esterase 3, ALP – Alkaline Phosphatase; A, B, F and S – alleles

Allele frequencies for Hb^A and Hb^B were observed to be 0.85 and 0.15 in WAD and RS populations with overall values of 0.65 and 0.35 respectively. The highest frequency was recorded for Est-1^B (0.93) in Sahel goats. Three genotypes we observed in all the loci studied except Est 3 which was monomorphic in all the populations. The Alp locus in this study had only two genotypes Alp^{AB} and Alp^{BB} respectively. The highest genotypic frequency was recorded for Hb^{AA} in RS (0.84).

In earlier studies [15-17] three genotypes we also reported for various goat populations. In a study of haemoglobin polymorphism in RS goats, [18] reported that Hb^A was more abundant than Hb^B. [19] reported Hb^{AA} as predominant in the 3 breeds of Nigerian goats studied, which was also the case in this current study. However, the absence of Hb^B was reported [20] and opined that the differences in the observed frequencies of these genotypes might be enough evidence of differential mortality. [21] believed that Hb^A conferred selective advantage in sheep found in higher altitudes as it is the most predominant allele in highland breeds of Scottish and English sheep.

Three genotypes (Ca^{AA}, Ca^{BB} and Ca^{AB}) controlled by two codominant alleles (Ca^A and Ca^B) were observed in the Ca locus. The most common allele was Ca^B in all the breeds. [22] reported Ca^S which corresponds to Ca^B as the most common in their study of Nigerian Sheep. In the current study the heterotype Ca^{AB} was observed to be more abundant in the populations. [23] reported that there was no significant difference between the different genotypes at the Ca locus with reference to economic traits.

Transferrin is reported to be very polymorphic in most species, therefore it is often used in pedigree analysis, genetic distance studies and for describing population structure [24]. The Tf^{AB} genotype was higher in RS than in WAD and Sahel goat populations. Similar reports were presented [25] for different goats. In a study of five goat breeds in USA, [26] reported Tf^A and Tf^B, this was similar to the results obtained for the three Nigerian Breeds studied. The Tf^B was more abundant in all the breeds studied whereas Tf^{AB} was prevalent in RS while Tf^{BB} had higher frequencies in Sahel and WAD respectively. The

difference in the distribution of Tf allele and genotype may be of advantage to the RS goat population which is believed to be most prolific of all the breeds studied.

The populations studied had Alb being polymorphic in all of them producing three genotypes (Alb^{AA}, Alb^{AB}, and Alb^{BB}) controlled by two alleles (Alb^A and Alb^B). The two alleles observed for Alb in this study had equal occurrence in all the population pooled with Alb^A being 56% in RS and 44% in WAD. [27] reported two bands a fast-moving band designated Alb^F and a slow-moving one Alb^S in Malarabi goat which like in the current study controlled three genotypes (Alb^{FF}, Alb^{FS}, and Alb^{SS}). The authors reported that Alb^S had higher frequency (83.66%) than Alb^F (16.34%). In a similar study, [28] observed similar frequencies in Malabari goats.

The frequency of Est 1^B was found to be higher in all populations and this influenced the frequency of the Est 1^{BB} genotype in all the population studied. In a study [29] it was reported that the enzyme esterase 1 had higher enzyme activity in goats than in sheep and cattle. It is opined that the abundance of the Est 1^B may contribute to the fruity flavour observed in goat milk.

The Est 3 locus was observed to be fixed for Est-3^B in all the populations studied. Whereas the Alp had both the Alp^A and Alp^B, the genotype Alp^{AB} was fixed for the RS and Sahel while only Alp^{BB} was observed for WAD. The observed distributions of these genotypes may be indicative of differences in geographical distributions of these goats. While WAD is predominantly a rainforest animal, RS and Sahel are more abundant in the dryer Savanna regions of the north.

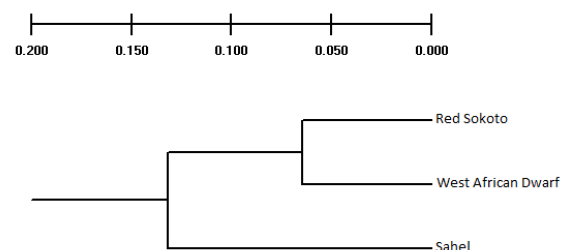


Figure 1. Dendrogram of Genetic Distance between Four Nigerian Indigenous Goats

Table 2. Measures of genetic variation at studied blood protein loci and Deviations from Hardy-Weinberg Equilibrium (DHWE) in Nigeria Indigenous Goat Breeds

Breed/Population	N	MNA*	Heterozygosity*		PIC	DHWE
			<i>Ho</i>	<i>He</i> **		
Red Sokoto	49	1.86 (0.38)	0.4591 (0.35)	0.3733 (0.19)	85.714	2
WAD	49	1.71 (0.49)	0.3017 (0.27)	0.2748 (0.21)	71.429	1
Sahel	50	1.86 (0.38)	0.4257 (0.39)	0.3319 (0.19)	85.714	2
Overall	148	1.86 (0.38)	0.3965 (0.28)	0.3721 (0.18)	85.714	

* = Standard deviation in parenthesis; N = Number of samples; WAD = West African Dwarf, MNA = Mean Number of Alleles; *Ho* = Observed Heterozygosity; ***He* = Expected Heterozygosity computed [30]; PIC = Polymorphic Information Content

Heterozygosity

Table 2. presents the results of measures of genetic variability and deviation from Hardy Weinberg equilibrium. The *Ho* values were between 0.3017 for WAD and 0.4591 for RS. The average observed heterozygosity for all populations was 0.3965. The mean value of *He* for all the populations was 0.3709 and this was higher than the values recorded for each of the population in the current study.

Heterozygosity coefficients which are measures of genetic diversity were similar in the breeds studied. This is an indication of the similar mean number of allele occurring in each breed ranging from 1.71 ± 0.49 to 1.86 ± 0.38 . These values are within the range obtained for sheep breeds using blood protein markers [21, 31-33]. However, the present values are higher than the range of 0.229-0.259 reported for Iranian fat-tailed sheep breeds [34]. The low heterozygosity points to decreasing genetic variability in the Nigerian goat populations at the seven investigated blood protein loci. This may be due to the population structure of the goat sample [35].

The current estimates fall within the recommended average heterozygosity between 0.3 and 0.8 in a population [36], for markers to be useful for measuring genetic variation.

Genetic Identity and Genetic Distance

The results for Nei's genetic distance and identity are presented in Table 4. The results of genetic identity indicated that the Sahel and Red Sokoto population were more genetically alike (0.9667) while the Sahel and WAD populations were the least genetically identical (0.8540).

Standard genetic distance (D) obtained in this study indicates some degree of genetic differentiation between the breeds. According to [37] the D value for local breeds is between 0.000 and 0.058. Different authors have reported different values of

D in different goat breeds. [38] estimated genetic distance between two Nigerian goat breeds at 0.39 using microsatellites markers. Yakubu *et al.* (2010) obtained genetic distance between WAD and Red Sokoto goats as 0.005, 0.005 and 0.18 for wattle, beard and coat colour loci. [39], using DNA microsatellite markers, reported genetic distance between pairs of Nigerian indigenous goat breeds, indicating that the lowest distance was between WAD and Red Sokoto (0.268) and the highest distance was between WAD and Sahel (0.662) goats, respectively.

Table 3. Wright's F-Statistics and Gene Flow for all Loci Studied

LOCUS	F_{IT}	F_{ST}	F_{IS}	Nm^*
HB	0.7374	0.3619	0.5885	0.4408
CA	-0.0314	0.0571	-0.0938	4.1320
TF	0.0135	0.0298	-0.0168	8.1332
ALB	-0.4933	0.0096	-0.5078	25.7917
EST1	-0.1274	0.0312	-0.1638	7.7616
EST3	xxxxx	xxxx	xxxx	xxxx
ALP	-0.5000	0.2500	-1.0000	0.7500
Over all Loci	-0.0677	0.1277	-0.2240	1.7083

* Nm =gene flow estimated from $F_{ST} = 0.25(1-F_{ST})/F_{ST}$; Hb=Haemoglobin, CA=Carbonic Anhydrase, Alb=Albumin, TF=Transferrin, EST-1=Esterase1, ALP=Alkaline Phosphatase

F-Statistics and Hardy Weinberg Equilibrium

The fixation indices for each of the loci studied across population is as presented in Table 3. Overall F_{IT} was estimated at -0.0677 while the heterozygote deficit within population (F_{IS}) was estimated as -0.2240 for all the loci pooled. The measure of breed differentiation (F_{ST}) ranged from 0.0096 in Alb to 0.3619 in Hb with an overall value of 0.1277. The gene flow (Nm) values for each locus studied ranged from 0.4408 to 25.7917 with an overall value of 1.7083 for all the loci. The negative values of F_{IT} observed at Ca, Alb and Alp loci with an overall value of -0.0677 indicates a

deficiency of homozygotes in the population indicating that mates are less related in reference to the average relationship among the population. Negative values of F_{IS} observed imply deficiency of homozygote in the population. This is indicative of an outbred population [17]. A larger proportion of total genetic diversity is explained by the variation within breeds. [17] reported F_{ST} values of 0.01 for Tf to 0.06 for Hb in a population of WAD goats sampled from different locations. Low F_{ST} values are indicative of gene flow between populations. [40] reported F_{ST} values of 5.8% for WAD goats in Kenya suggesting mobility and exchange of genetic materials among the studied populations.

Dendrogram

The Phylogenetic tree constructed (Figure 1) separated the Sahel from other WAD and RS suggesting either early prehistoric separation or separate historical origin. It would have been expected that RS and Sahel breed would be more closely related as may be explained by their geographic location in the northern savanna regions. Furthermore, the estimated dendrogram clustered these Nigerian goat breeds into a sub-population and two major genetic groups. The study suggested that RS and WAD may have had gene exchange, possibly due to uncontrolled or in some occasion deliberate cross-breeding and geneflow between them, which are symptomatic of uncontrolled crossing across much of the country [39]. Based on the higher value of Nei's genetic distance (0.1579), breeding programs involving the crossing of the WAD and Sahel is recommended, since the crosses between breeds which are homogenous but distinctly different in their relationship would produce more hybrid vigour (heterosis) in the crossed progeny.

Table 4. Nei's original measure of genetic identity and genetic distance between Nigerian Goat Populations

Population	Red Sokoto	WAD	Sahel
Red Sokoto	****	0.9573	0.8995
WAD	0.0648	****	0.8540
Sahel	0.1059	0.1579	****

Nei's Genetic Identity (above Diagonal) and Genetic Distance (below Diagonal)

4. Conclusions

In this study we have been able to show the genetic variation between Nigerian indigenous goat breeds

at seven polymorphic loci. Based on the findings presented, we assume that crossbreeding programs involving WAD and Sahel goats will be more beneficial for goat improvement in Nigeria. However, further studies are needed in order to determine at DNA sequence level the true genetic distance and variation among these goat breeds.

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