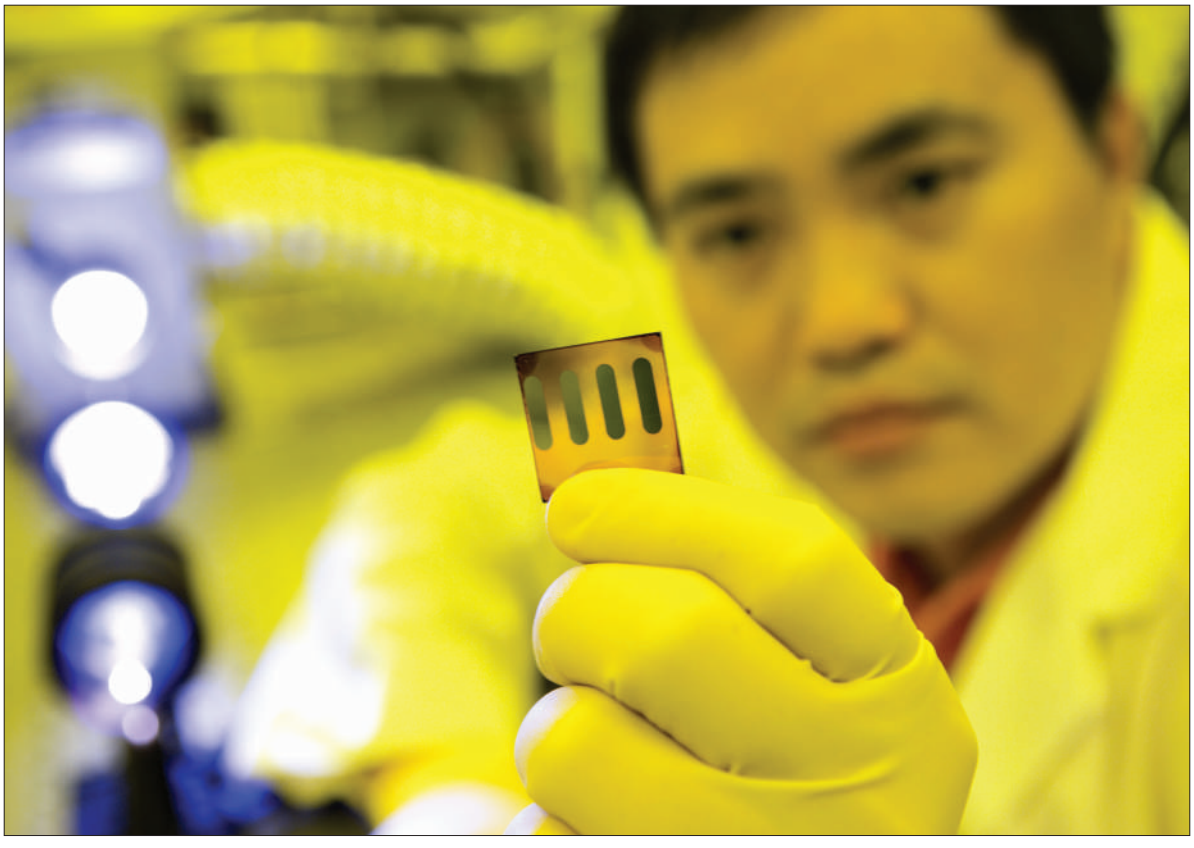

Singapore Journal of

Scientific Research

ISSN: 2010-006x

science alert

http://scialert.net/sjsr

OPEN ACCESS Singapore Journal of Scientific Research

ISSN 2010-006x
DOI: 10.3923/sjsres.2020.160.165

Research Article
Molecular Characterization of Five Populations of Nigerian Indigenous Goat Breeds Using Random Amplified Polymorphic DNA Markers

F.U. Udeh and H.M. Ndofor-Foleng

Department of Animal Science, University of Nigeria, Nsukka, Nigeria

Abstract

Background and Objective: Goat is the most prolific ruminant among all domesticated ruminants under tropical and subtropical conditions. The study was conducted to molecularly characterize five populations of the Nigerian breeds of goat using random amplified polymorphic DNA markers. **Materials and Methods:** Five breeds of goat were used for the study and each breed constituted a population. The populations were: Sokoto Red, Sahel, Kano Brown, Bornu White and West African Dwarf goats. The experiment was conducted within 4 geographical zones of Nigeria: South East, North West, North East and North Central. One hundred and twenty (120) blood samples were randomly collected from various locations across the four geographical zones in Nigeria, from the jugular vein of the animals and preserved at -20°C. The DNA samples were isolated and purified from the blood samples and the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was carried out. **Results:** The result showed that total number of scored fragments was 20, out of which 13 were polymorphic, which resulted to 65% polymorphism. Observed number of alleles (Na) was 2.0000 across the entire populations while the effective number of alleles (Ne) was1.9897, 1.9287, 1.9836, 1.9003 and 1.9900 for Sokoto Red, Kano Brown Bornu White, West African Dwarf and Sahel goat, respectively. Nei's heterozygosity (H) across the populations ranged from 0.4736 in WAD population to 0.4975 in Sahel goat. Shannon's information index (I) ranged from 0.6665-0.6905. **Conclusion:** It was concluded that there is low genetic diversity and loss of heterozygosis in the populations. The study also indicated that there were low species richness and evenness among the five populations of the Nigerian indigenous breeds of goat.

Key words: Molecular characterization, RAPD marker, goat breeds, shannon index, genetic diversity

Citation: F.U. Udeh and H.M. Ndofor-Foleng, 2020. Molecular characterization of five populations of Nigerian indigenous goat breeds using random amplified polymorphic DNA markers. Singapore J. Sci. Res., 10: 160-165.

Corresponding Author: F.U. Udeh, Department of Animal Science, University of Nigeria, Nsukka, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Goat is reared for milk, meat, wool and leather production[1]. Goat is the most prolific ruminant among all domesticated ruminants under tropical and subtropical conditions. Goat meat holds tremendous demand irrespective of cultural and religious barriers. Therefore characterization of goats for trait improvement is advantageous[2]. According to FAOSTAT[3], Nigeria has a total of about 53.8 million goats which constituted about 6.2% of world's goat population and about 85% of rural households and poor farmer are the major goat keepers[4].

The ability of goats to tolerate harsh climates, the presence of trypanotolerance in some breeds, suitability to traditional systems on account of small size, short generation interval[5] and ability to thrive on poor quality diets provided by scarce grazing on marginal lands all combine to make goats strategic to increasing livestock productivity in rural agricultural systems[6]. Despite these advantages, not much attention has been paid to the genetic characterization and possible genetic improvement of goats in Nigeria. Production of goat is solely in the hands of resource-poor farmers and Hausa-Fulani nomads who rear them under the traditional subsistent husbandry systems, which are notable for both very low input and output. Ibe[7] opined that many developing countries fall short of attaining protein sufficiency mostly due to poor genetic stock leading to low output rate per animal and low productivity.

Molecular markers are important tools in tagging desirable loci underlying the traits which have breeding importance. The study of genetic variation plays an important role in developing rational breeding strategies for economical animal species[8]. In recent years, a range of innovations in molecular genetics have been developed for the study of genetic characterization and evaluation of populations using DNA marker. Genetic analysis of livestock species have been performed using polymorphic markers such as restriction fragment length polymorphisms (RFLPs) and microsatellites[9,10], but their use is limited since designation of these genetic markers is expensive, technically demanding and is time consuming[11,12].

The RAPD technique has also been used in analysis of genetic diversity between different breeds of animals such as cattle[13], goat[14] and sheep[15]. In Nigeria, goats and other local breeds of livestock have been characterized phenotypically but their genetic characterization are still lacking. Adebambo *et al.*[16] and Okpeku *et al.*[17] have both employed molecular markers in characterization of West African Dwarf (WAD) and Red Sokoto (RS) breeds of goat. But, there is limited information on the genetic diversity and characterization of other existing breeds of goat in Nigeria. Genetic characterization among goat breeds in Nigeria is necessary to provide useful genetic information essential for developing effective management plans for the conservation and improvement of their genetic resources. Therefore, this study was conducted to genetically characterize five populations of Nigerian local breeds of goat.

MATERIALS AND METHODS

Study area: The experiment was conducted within four geographical zones of Nigeria: South East, North West, North East and North Central. Nigeria is located in West Africa on the Gulf of Guinea (latitude 10°00′ N, longitude 8°00′ E) with a total area of 923,768 km^2 (twice the size of California). Nigeria is bounded by Niger, Benin and Cameroon Republics on the North, West and East, respectively[18]. The study was conducted in 2017 and it lasted for seven months (from May to December, 2017).

Experimental animals: Five breeds of goat were used for this study: Sahel goat (SH), Kano Brown (KB), West African Dwarf goat (WAD), Red Sokoto goat (RS) and Bornu White goat (BW). Blood samples were collected from the jugular vein of the animals through a process known as venipuncture. Approximately, 5 mL of blood was collected aseptically from each animal into an EDTA container, using 23 gauge sterile needle and syringe and was stored at -20°C using ethylene-di-amine-tetra-acetic acid, (EDTA) as anticoagulant. The laboratory analysis was carried out at Department of Animal Science, University of Port Harcourt, Port Harcourt, Nigeria.

DNA extraction/isolation and RAPD-PCR (polymerase chain reaction) conditions: Total DNA was isolated from whole blood samples using a ZymoBead™ Genomic DNA Kit, following the procedure as recommended by the manufacturer (zymo RESEARCH CORPORATION, e-mail: info@zymoresearch.com. The RAPD-PCR reaction followed the procedure described by El Hentati *et al.*[19]. Amplifications were performed using a thermal cycler (PTC-100TM Programmable Thermal Controller, MJ Research, Inc., MA, USA) using the primers shown in Table 1.

Table 1: Sequences of the random primers selected for the individual typing of the studied animals

Primers	Sequence 5′-3′
GOA1	CCGCGCCGGT
GOA2	CAGCCTCGGC
GOA3	ACGTCGAGCA

GOA1: Primer 1, GOA2: Primer 2, GOA3: Primer 3

The 20 µL amplification reactions contained 50 ng template DNA, 1.0 µM of each primer, 16 µL Nuclease free water in a bioneer AccuPower® TLA PCR Premix. The Thermal cycler was programmed for 40 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 45 sec and extension at 72°C for 1 min. An initial denaturation step of 5 min at 94°C and a final extension step of 5 min at 72°C were included in the first and last cycles, respectively. The results were analyzed quantitatively by visualizing the gel with Ultra Violet (UV) light and a gel imaging device and the intensity of the bands of interest was measured and compared against standards (markers) loaded on the same gel.

RESULTS

Primer sequences and number of score able bands: The sequences and percentage polymorphism for each primer and for all primers used are presented in Table 2. Seven primers were provided for this analysis. Four primers fail to amplify any fragment. Three primers out of the seven were able to produce scorable bands. The band scoring was directly obtained from RAPD profile in Fig. 1. The result showed that total number of scored fragments was 20, out of which 13 were polymorphic, which resulted to 65% polymorphism. Primer GOA1 had a total of 5 fragments and 4 scorable bands were polymorphic, which resulted to 80% polymorphism and it was the highest. Primer GOA3 had a total of 8 reproducible bands scored, out of which, 6 were polymorphic and had 75% polymorphism. Finally, primer GOA2 had 7 fragments, within which 3 were polymorphic and it had the lowest value of 43% polymorphism.

Genetic diversity indices among five Nigerian local goat breeds: Observed number of alleles, effective number of alleles, Nei's heterozygosity and Shannon's information index were employed to characterize five populations of Nigeria breeds of goat as shown in Table 3. The result showed that observed number of alleles (Na) was 2.0000 across the entire populations while effective number of alleles (Ne) varied from 1.9003 in West African Dwarf goat to 1.9900 in Sahel goat. Nei's heterozygosity (H) across the populations ranged from 0.4736 in WAD population to 0.4975 in Sahel goat. Shannon's information index (I) ranged from 0.6665 in WAD to 0.6906 in Sahel goats which were the lowest and highest values, respectively.

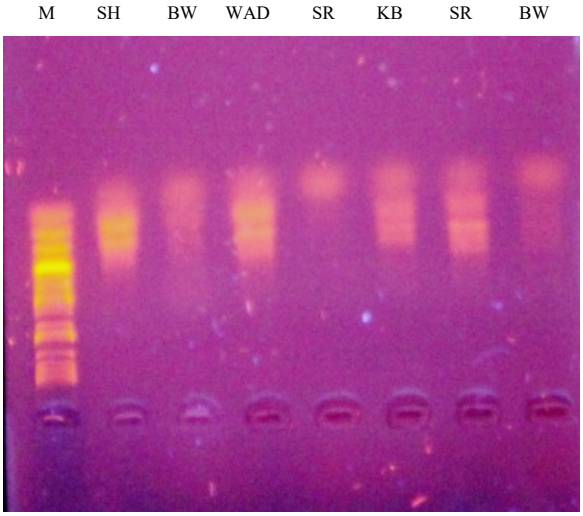

Fig. 1: RAPD profile of five Nigerian goat breeds
M: Molecular marker, SH: Sahel goat, BW: Bornu White, WAD: West African Dwarf, SR: Sokoto Red, KB: Kano Brown

Table 2: Sequence and percentage polymorphism of the primers used

Primers	Sequence	Total no. of scored bands/fragments	No. of polymorphic bands/fragments	Polymorphism (%)
GOA1	CCGCGCCGGT	5	4	80
GOA2	CAGCCTCGGC	7	3	43
GOA3	ACGTCGAGCA	8	6	75
Total		20	13	65

GOA1: Primer 1, GOA2: Primer 2, GOA 3: Primer 3

Table 3: Genetic diversity indices within the five Nigerian indigenous breeds of goat

Breeds/population	Na	Ne	H	I
Sokoto Red	2.0000	1.9897	0.4974	0.6905
Kano Brown	2.0000	1.9287	0.4813	0.6742
Bornu White	2.0000	1.9836	0.4959	0.6890
WAD	2.0000	1.9003	0.4736	0.6665
Sahel	2.0000	1.9900	0.4975	0.6906

Na: Observed number of allele, Ne: Effective number of allele, H: Nei's heterozygosity or gene diversity, I: Shannon's information index

DISCUSSION

The result indicated that the primers varied in their ability to detect genetic diversity among the studied goat populations as they showed different banding pattern across the DNA samples clearer bands recorded in SH and WAD goats indicated farer genetic relationship compared to other breeds with closer relationship depicted by unclear bands.

The polymorphism found within populations varied between primers and breeds which corroborated with the report of Xiang *et al.*[20]. This result agreed with those of Yadav and Yadav[14] and Sulaiman[21] who obtained similar result. The number of amplified fragments in this study agreed with Sulaiman[21] who reported that number of amplified fragments ranged from 8 in primer C5 to 13 in primer D15. Another study by Xiang *et al.*[20] reported that twenty seven polymorphic primers have been used to amplify the genome DNA of 15 Qianbei-Pockmarked goats and 253 bands were detected, of which 141 were polymorphic with a polymorphism frequency range of 30-75%, averaging 55.73%. This finding is in consonant with the result of this study which indicated ranges of (43-80%) percentage polymorphism across the three primer combinations under consideration. The number of amplified fragments in this study agreed with the results of Xiang *et al.*[20] and El Gaali and Satti[22] who reported that each primer amplified 2-14 bands, averaging 9.37 bands in 15 Qianbei-Pockmarked goats and 55 polymorphic bands in Sudanese goat breeds, respectively. The high polymorphism obtained in this study showed that the loci considered were highly diverse and can be employed in genetic improvement and breeding strategies. Fewer reproducible bands obtained in this study may be attributed to lower annealing temperature of 36°C employed. Anuntalabhochai *et al.*[23] reported that a high annealing temperature of 46°C gave greater polymorphism, reproducibility and resolution in RAPD.

The observed number of allele was within the range (2-11) reported by Mahmoudi *et al.*[24] in Iranian goat. The value of effective number of allele obtained in this study was higher than the findings of Hoda *et al.*[25] who reported effective number of allele that ranged from 1.234 in Mati to 1.296 in Dukati breeds of Albanian goat. Mahmoudi *et al.*[24] recorded much higher (Ne) values of 5.262, 4.177 and 4.854 in the Markhoz, Najdi and Tali breeds of Iranian goat, respectively. The lower value of (Ne) obtained in this study may be attributed to the effective sample size which was small. This is because effective number of allele (Ne) is the lower bound of total allele frequency which is higher with larger population.

The values of Nei's heterozygosity (H) were generally low, however, were higher than values obtained in Italian goat breeds (range 0.21-0.24) reported by Ajmone-Marsan *et al.*[26], Albanian goat (0.145-0.176) by Hoda *et al.*[25] and Italian goat in the Alps of the Lombardy region (range 0.260-0.290) by Gorni *et al.*[27]. The result agreed with Rahman *et al.*[28] who reported that the highest level of Nei's heterozygosity value was of 0.49, with an average of 0.37 among the 14 goat breeds studied. Also, Biba *et al.*[29] reported deficit heterozygotes of 42.1% (0.421). It also agreed with Adebambo *et al.*[16] who obtained heterozygosity values of 0.46, 0.55 and 0.49 in Maradi, WAD and Maradi×WAD breeds of Nigerian goat. The low values obtained from this study indicated low genetic diversity across populations and loss of heterozygosis. Heterozygosis deficiency indicates departure from Hardy-Weinberg equilibrium[30]. Mahmoudi *et al.*[24] indicated that very low genetic diversity will deprive individuals the characteristics to cope with environmental challenges. They concluded that population with low genetic diversity could be suddenly wiped out or go into extinction. These were clear confirmation that these animals reared by the transhuman Hausa-Fulanis were allowed to breed without control and as such inbreeding may be high among the populations. Hence, the populations have a close genetic relationship Biba *et al.*[29].

Shannon's Information Index revealed low species richness and evenness across the populations. The result showed that Shannon index value obtained in this study were lower than (1.653-2.964) reported by Agaviezor *et al.*[18] in Nigerian sheep breeds and (1.87-3.51) reported by Dixit *et al.*[31] in Indian breeds of goat. The Shannon indices in this study were lower than 3.5, which was set for high species evenness and richness according to Krebs[32]. This might not be unconnected with the level of heterozygote deficiency observed among this population, possibly due to the management system[33]. Canon *et al.*[34] reported significant inbreeding in most of the breeds of sheep and goat studied due to poor breeding management. Low genetic diversity predispose populations to disease attack and expression of deleterious alleles.

On the other hand, heterozygosis deficiency (low genetic diversity) recorded in this study may have also resulted from the following factors: The presence of null alleles which were the alleles that failed to multiply during PCR using the primer sites[35]. The average direct count of heterozygosity in overall loci was less than the expected heterozygosity, which implies that there was loss of heterozygosity in the population under consideration and this resulted to 'allele fixation'[36], hence, relatively uniform values across the entire populations. Heterozygote deficiencies have also been reported in other studies on goats[31].

CONCLUSION

It was concluded that there is low genetic diversity and loss of heterozygosis in the populations. The study also indicated that there were low species richness and evenness among the five populations of the Nigerian indigenous breeds of goat.

SIGNIFICANCE STATEMENT

The study discovers the genetic diversity status of the Nigeria indigenous breeds of goat. The information can be beneficial for the conservation of genetic resources of Nigerian breeds of goats. The results of this study can offer some crucial scientific data useful for breeding programme of Nigerian local breeds of goats. Thus, a new approach on the breeding plans of Nigerian local breeds of goat may have been discovered.

ACKNOWLEDGMENT

The authors wish to appreciate Mrs. Perpetua Adaku Udeh and Mr. Emeka from Faculty of Veterinary Medicine, UNN, for their assistance during sample collection.

Authors also thankful to the Singapore Journal of Scientific Research for publishing this article FREE of cost and to Karim Foundation for bearing the cost of article production, hosting as well as liaison with abstracting and indexing services and customer services.

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