GENETIC DIVERSITY OF NIGERIAN INDIGENOUS GOAT BREEDS AT THE GROWTH HORMONE (GH) GENE LOCUS

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Abstract

In Nigeria, goats are important as potential source of meat, milk and skin production as well as income. Improving the growth performance of these goat breeds can lead to increase meat, milk and skin production. Growth hormone (GH) gene is associated with growth, bone formation, regulating fat content and other important traits in goat. This study was conducted to assess the genetic diversity of the three Nigerian indigenous goat breeds namely; Sahel (SAH), Sokoto Red (SOR) and West African Dwarf (WAD). Fifteen animals were randomly sampled for this study. Five animals per breed were selected and blood samples were collected into EDTA tubes, thereafter, genomic DNA was extracted. A pair of primers was used to amplify GH gene region. Direct sequencing method was applied for PCR products to study polymorphisms at the GH gene locus of the three Nigerian goat breeds. The observed heterozygosity (Ho) ranged from 0.10 ± 0.10 (SAH) to 0.30 + 0.10 (WAD) while the expected heterozygosity (He) ranged from 0.33 ± 0.01 to 0.44 ± 0.02. The overall Shannon index (I), fixation index (F) and Polymorphic Information Content (PIC) values were 0.63 ± 0.04, 0.50 ± 0.12 and 0.49 ± 0.03 respectively. Gene flow (Nm) between WAD and SOR goats was 40.25, WAD and SAH goats was 19.22 while between SOR and SAH was 37.25. The analysis of molecular variance (AMOVA) within and between populations of goats that 100% of the observed variance occurred within the breeds. The nearest genetic distance was observed between the SOR and SAH breeds (0.0070) indicating closer geographical locations while the largest distance was between WAD and SAH breeds (0.0124). The phylogenetic tree was drawn revealed that the SAH and SOR breeds clustered together while the WAD breed diverged first, confirming the genetic distance results. The present study showed that the distinctiveness of these indigenous goats is fast eroding, therefore effort should be made to control the wearing away of the genetic make-up of the goat populations by adopting improved breeding and management practices.

Key words: Goat breeds, direct sequencing, indigenous, growth hormone

Introduction

Goats are the most numerous domesticated ruminants in Nigeria with an estimated population of 79.38 million (about 6.56%) of the world's goat population (FAOSTAT, 2018). In Nigeria, goats constitute an important source of milk and meat for consumption and source of income (Adebambo et al., 2011).

Goats are spread over a wide range of habitats with a substantial concentration in the tropics and dry zones in developing countries (FAOSTAT, 2006). Therefore, they are expected to show a large range of genetic diversity in adapting to the varying ecosystems. Domestic goat breeds (*Capra hircus*) are generally well-adapted to harsh local environmental conditions (semi-arid or even arid conditions), especially in developing countries throughout the world (FAO, 2008).

FAO (2008) and Hassen et al. (2012), reported that indigenous genetic resources of Africa and the world at large are threatened with extinction, emphasising the importance of maintaining domestic animal diversity. Loss of genetic diversity is detrimental to the conservation as well as utilization since lost genes may be of future economic interest (Hetzel and Drinkwater, 1992) and once lost it cannot be replaced (FAO, 2007).

Growth traits are of great importance to animal husbandry and are complex traits involving multiple genes (polygenic) and possible interactions (Hua et al., 2008). There are several genes that influence the growth and body mass of the animal, among which are growth hormone gene (GH), growth hormone receptor (GHR), Insulin-like growth factor-1 (IGF-1) and others (Ge et al., 2003). Growth hormone (GH) gene is physically located on goat chromosome 19q22 (Pinton et al., 2000), encoded by 1,800 base pairs (bp) long, consisting of 5 exons and 4 introns sequences (Kioka et al., 1989). The GH gene has a direct effect on the synthesis and secretion of growth hormone from the anterior pituitary and is associated with growth (Gadelha et al., 2012) and milk yields traits (Marques et al., 2003). Genetic diversity can define as the variety of alleles and genotypes present in a population. It can be referred to as the total number of genetic characteristics in the genetic makeup of a species that serves as a way for populations to adapt to changing environments (Reed and Richard, 2013).

Genetic diversity is the set of differences manifested between species, breeds and individuals within breeds because of differences in their genetic make-up. Genetic diversity is as a result of the allelic and genotypic variations present in a population which often manifest in either morphological, physiological and behavioural differences among the individuals. Genetic diversity can both be observed as phenotypic variation among individuals within breeds and among different breeds (Talle et al., 2005). Variation among individuals within breeds is essential for selection in animal breeding (Meuwissen, 2009 and Talle et al., 2005) and for future breeding (Meuwissen, 2009).

The extent of diversity is paramount for improvement and utilization of genetic resources. Genetic diversity is therefore the backbone of conservation of plant genetic resources for both present and future use (Quedraogo, 2001).

There are reported studies on genetic diversity in goats using; RAPD (Udeh, 2015), blood protein (Sargent et al., 2006), Microsatellite (Ojo, 2014) and Thyroid Hormone Responsive Spot 14 Alpha (THRSPα) gene (Ajayi et al., 2016).

Growth hormone gene had also been utilized to study genetic diversity in; cattle (Yurnalis et al., 2013), sheep (Cobra et al., 2013), Egyptian goats (Othman et al., 2015). However, there is dearth of works on the genetic diversity of Nigerian indigenous goat breeds at the growth hormone (GH) gene locus, therefore this study was undertaken to evaluate genetic diversity in the three Nigerian indigenous goat breeds growth hormone (GH) gene locus. Studies to assess the genetic diversity within and between breeds Nigerian indigenous goat breeds using growth hormone gene is very important as it provides information useful for conservation and utilization of these valuable animal genetic resources.

Materials and methods

Experimental animals and management

Three Nigerian indigenous goat breeds Sahel, Sokoto Red and West African Dwarf. Animal were sampled from three locations; Katsina, Sokoto and Benue states respectively.

Blood sample collection for molecular analysis

Five millilitres (5 ml) of blood were collected from the jugular vein of each animal, into sample

tubes containing ethylene-di amine-tetra-acetic acid (EDTA) as anticoagulant following the procedures described by Ali (2003).

Genomic DNA extraction: Phenol-chloroform protocol

Genomic DNA was extracted using Phenol-chloroform extraction technique (Sambrook and Russell, 2001). About 400 µl of digestion buffer (lysis buffer) and 10 µl of proteinase-K containing 20% SDS (Sodium Dodecyl Sulphate) were added to each sample and mixed by vortexing. The mixture was then incubated for about 30 minutes at 60 °C. About 400 µl of phenol-chloroform was added, mixed by vortexing. The mixture was then spinned at 13,000 rpm for 10 minutes. The supernatant was decanted and transferred to a new tube. To the supernatant, 1,000 µl of 100% ethanol and 40 µl of sodium acetate, were added to precipitate the DNA. Then the pellet was washed twice in 70% ethanol and air-dried at room temperature. The pellets, contain the DNA, was stored at -20 °C for later use.

Polymerase chain reaction (PCR)

The PCR reaction was performed in a total volume of 20 µl of the Master mix, 2.0 µl of genomic DNA, 1.0 µl of each forward and reverse primers and 16 µl of nuclease-free water (ddH_2O) in a PTC-100 Thermal cycler (Bioneer, Alameda, CA, USA). An initial denaturation for 5 min at 94 °C was done, followed by 35 cycles of 45 secs at 94 °C, annealing for 60 secs at 53 °C, extension for 60 secs at 72 °C and a final extension for 5 min at 72 °C. The primers used for PCR were:

GH1: Forward primer 5'- CCC AGG GAT TAA ACC TGA GTC -3'

GH2: Reverse primer 5'- CCC TAG GGA GAG ACC AGG AG -3'

Agarose gel electrophoresis

The quality of genomic DNA amplified was checked on 1.5% agarose gel electrophoresis stained with ethidium bromide. Electrophoresis was carried out at 80 V for about 60 mins. On completion of electrophoresis, the gel was visualized under UV transilluminator to detect amplification.

Direct sequencing

The PCR products amplified were cut from 1.5% agarose gel and purified using JustSpin Gel Extraction columns (Genaxxon). Sequence analysis and alignment were carried out using Molecular Evolutionary Genetics Analysis (MEGA X) software package (Kumar et al., 2018) and compared using NCBI/BLAST/blastn suite.

Data analysis

Genetic variation

The Genetic diversity indices were estimated using the Genetic Analysis in Excel (GenAlEx) version 6.5 statistical package (Peakall and Smouse, 2012).

Polymorphism information content (PIC)

The polymorphic information content (PIC) per locus was also calculated according to the method described by Botstein et al. (1980) using the Genetic Analysis in Excel (GenAlEx) version 6.5 statistical package (Peakall and Smouse, 2012). A value of $PIC \geq 0.5$ can be considered as highly informative (polymorphic), while value ranges of $0.5 \geq PIC \geq 0.25$ is considered as moderately informative (polymorphic) and values of $PIC \leq 0.25$ is measured as lowly informative (Botstein et al., 1980).

AMOVA

Analysis of molecular variance (AMOVA) was performed to quantify further the extent of population differentiation and the distribution of genetic variation in the sampled population using the Genetic Analysis in Excel (GenAlEx) version 6.5 statistical package (Peakall and Smouse, 2012).

F-Statistics

Wright's F-statistics, genetic differentiation (FST), inbreeding coefficient (FIS), and total inbreeding (FIT), were estimated using the Genetic Analysis in Excel (GenAlEx) version 6.5 statistical program (Peakall and Smouse, 2012).

Genetic distances (Ds)

Standard genetic distance (Ds) and identity between populations were computed according

to Nei and Hi (1979) using the Genetic Analysis in Excel (GenAlEx) version 6.5 statistical program (Peakall and Smouse, 2012).

Dendrogram

Dendrogram was drawn using the Unpaired Group with Arithmetic Mean (UPGMA) methods to estimate Nei's genetic distances between pairs of goats using Molecular Evolutionary Genetics Analysis Version X (MEGA X) software package (Kumar et al., 2018).

Results and discussion

Number of effective alleles, observed and expected heterozygosities across the breeds

Table 1 shows the means (± SE) number of effective alleles, observed and expected heterozygosity for the three goat breeds. The Ne values varied from 1.49 ± 0.02 per locus in Sahelian goats to 1.79 ± 0.06 in West African Dwarf goats. Meanwhile, West African Dwarf goats were had highest values of Ho (0.30 ± 0.10) and He (0.44 ± 0.02) while Sahelian goats had the lowest values of Ho (0.10 ± 0.10) and He (0.33 ± 0.01). The overall Ne, Ho and He values were 1.63 ± 0.06, 0.20 ± 0.05 and 0.38 ± 0.02 for West African Dwarf, Sokoto Red and Sahelian goats respectively. The mean observed heterozygosity (Ho) was lower than those of the expected (He) across the loci in the breeds of goats studied. Takezaki and Nei (1996) reported that, for a genetic marker to be effective and useful in the determination of gene diversity, it has to be between 0.3–0.8. The overall mean number of observed alleles (2.50 ± 0.22) per locus obtained in the study was low. This indicates that, even though there is diversity in the Nigerian goat breeds, they are less genetically diverse when compared to their ancestors (Quaresma et al., 2014). According to Willis et al. (2006), such populations with low effective number of alleles are exposed to inbreeding and are threatened by extinction (Leroy et al., 2013).

The mean (± SE) values of the Shannon index, fixation index and polymorphic Information Content for each breed are as shown in Table 2. The values obtained for the Shannon index, fixation index and polymorphic information content were 0.71 ± 0.10, 0.33 ± 0.20 and 0.37 ± 0.04 for West African Dwarf, 0.63 ± 0.01, 0.47 ± 0.06 and 0.32 ± 0.01 for Sokoto Red and 0.57 ± 0.07, 0.71

Table 1. Number of effective alleles, observed and expected heterozygosities across the breeds.

Breeds	N	Na	Ne	Ho	He
WAD	5.00	2.50 + 0.50	1.79 + 0.06	0.30 + 0.10	0.44 + 0.02
SOR	5.00	2.50 + 0.50	1.62 + 0.10	0.20 + 0.00	0.38 + 0.04
SAH	5.00	2.50 + 0.50	1.49 + 0.02	0.10 + 0.10	0.33 + 0.01
Overall	5.00	2.50 + 0.22	1.63 + 0.06	0.20 + 0.05	0.38 + 0.02

SAH = Sahel goats, SOR = Sokoto Red goats, WAD = West African Dwarf goats, Na = no. of alleles, Ne= no. of effective alleles, Ho = observed heterozygosity, He= expected heterozygosity

Table 2. Shannon index, Fixation index, Polymorphic Information Content and Percent Polymorphic Loci Across the goat breeds

Breed	I	F	PIC	% Polymorphic
WAD	0.71 ± 0.01	0.33 ± 0.20	0.37 ± 0.04	100.00
SOR	0.63 ± 0.01	0.47 ± 0.06	0.32 ± 0.01	100.00
SAH	0.57 ± 0.07	0.71 ± 0.29	0.29 ± 0.02	100.00
Overall	0.63 ± 0.04	0.50 ± 0.12	0.49 ± 0.03	100.00

SAH = Sahel goats, SOR = Sokoto Red goats, WAD = West African Dwarf goats, I = Shannon index, F = Fixation index, PIC = Polymorphic Information Content.

± 0.29 and 0.29 ± 0.02 for Sahel goat breeds. The overall values for Shannon index, fixation index and polymorphic information content were 0.63 ± 0.04, 0.50 ± 0.12 and 0.49 ± 0.03 respectively. The highest PIC value (0.37 ± 0.04) was recorded in WAD goats and the lowest PIC value (0.29 ± 0.02) was recorded in Sahel breed. The overall mean PIC was 0.49 ± 0.03. The entire locus was found to be polymorphic as Percent polymorphic loci of 100.00% were reported for all the goat breeds used in this study. The 100% polymorphism is an indication of abundant genetic diversity in the three Nigerian goat breeds.

The pair-wise gene differentiation (F_{ST}) estimates for the three goat breeds are presented in Table 3. The least F_{ST} value of 0.006 was recorded between WAD and SOR goat breeds while the highest F_{ST} value of 0.013 was obtained between WAD and SAH goat breeds. The F_{ST} value obtained between SOR and SAH goat breeds was 0.007. The F_{ST} value showed very low genetic differentiation among the breeds. The very low values of F_{ST} were observed across the three Nigerian goat breeds, ranging from 0.006 to 0.013. The low F_{ST} may be an indication that the Nigerian goat populations have some genetic similarities due to gene flow between the sampled populations. Migration, interbreeding and genetic drift may exert a greater effect on the reduction in genetic differentiation between populations (Laval et al., 2000). This result is however lower than values obtained by other researchers; 0.11 in Nigerian goats (Okpeku et al., 2011), 0.14 in Asian goats (Barker et al., 2001), 0.105 in Chinese goats (Li et al., 2002) and 0.058 in West African Dwarf goats of Kenya (Mujibi, 2005). Weir (1996) and Kalinowski (2002) suggested that $F_{ST} > 0.25$ as highest, moderate when $0.05 < F_{ST} < 0.25$ and the lowest or negligible when the $F_{ST} < 0.05$.

The pair-wise gene flow (Nm) estimates between the three goat breeds are presented in Table 4. The highest Nm value 40.2489 was obtained between WAD and SOR and the lowest Nm 19.2204 was between WAD and SAH. The Nm between SOR and SAH was 37.2481. Gene flow refers to the successful transfer of alleles from one population of animals to another. This transfer varies among breeds, individuals and populations over time, and occurs at rates that is sufficient enough to play important evolutionary roles (Arnold, 2015). The main effect of gene flow (Nm) therefore, is the homogenization of allele frequencies between populations. The greater the gene flow between populations, the more the similarity between the population (El Hentati et al., 2012; Han et al., 2016). Gene flow values obtained across the breeds in this study suggests mobility and considerable exchange of genetic material among the three Nigerian goat breeds. The highest value of Nm obtained between WAD and SOR goats in this study, might have resulted from high migration and mobility of SOR goats from the Northern Nigeria to the middle belt zone for pasture. Gene flow values recorded in this study are higher than the values reported by Ojo (2014) who reported the highest gene flow between Red Sokoto and Kano Brown (13.59) and the lowest between Sokoto Red and West African Dwarf goats (3.96). A relative lack of controlled breeding system, parentage control and improved management practices might have facilitated gene flow between geographically nearby breeds (Cañón et

Table 3. Pair-wise gene differentiation (F_{ST}) Estimates between Nigerian goat population

Population 1	Population 2	Gene differentiation (F_{st})
WAD	SOR	0.006
WAD	SAH	0.013
SOR	SAH	0.007

WAD = West African Dwarf, SOR = Sokoto Red, SAH = Sahel.

Table 4. Estimates of Pair-Wise Gene Flow (Nm) Estimates Between the Three goat population

Population 1	Population 2	Nm
WAD	SOR	40.2489
WAD	SAH	19.2204
SOR	SAH	37.2481

WAD = West African Dwarf, SOR = Sokoto Red, SAH = Sahel, Nm = Gene flow.

al., 2006). Okpeku et al. (2011) also suggested that high gene flow could be due to poor breeding management such as uncontrolled mating. Migration plays key roles in modifying allele frequencies due to change in genetic diversity (Tallman et al., 2019).

Analysis of Molecular Variance (AMOVA) was carried out to understand the portioning of the level of genetic variations within and between the three Nigerian indigenous goat breeds (Table 5). The results of the AMOVA revealed that 100% of the of the observed variance occurred within the breeds (the individual difference of animals) while none (0%) of the variance was due to between the breeds. Molecular genetic variation observed in this study was found to be totally due to the within populations (100%) variations while the among population variation contribution was 0%. This result is in consonance with the result obtained by Emeka et al. (2017), using β- Lactoglobulin gene locus in Nigerian goat breeds. The results obtained in this study was at variance with the results of Okpeku et al. (2011) who obtained (71%) variation within populations and (29%) variation amongst populations of West African Dwarf, Sahel and Red Sokoto goats of Nigeria and Ojo et al. (2015), who reported 96% variation within populations and (4%) variation amongst populations using four Nigerian goat populations. According to Toro and Maki-Tanila (2007) the higher genetic diversity observed within a population than between groups may have arisen from overlapping generations with natural selection favouring heterozygosity or subdivision accompanied by genetic drift. Agha et al. (2008) reported that this is more pronounced when the effective population size is very large, which is supported by the poor infrastructure on ground presently for livestock improvement and lack of proper breeding policy in Nigeria.

The result of the Nei genetic identity and distance of the three Nigerian goat breeds is shown in Table 6. The lowest genetic distance (0.0070) was between SOR and SAH with the highest genetic identity (0.9930). The farthest genetic distance (0.0124) was between WAD and SAH goat breeds with the lowest genetic identity (0.9877). High values for genetic distance means low values for genetic similarity and vice versa. The high genetic identity (0.9930) observed in this study confirmed the geographical proximity of SOR and SAH goat breeds. This result showed that SOR and SAH goat breeds were geneticall-y closer, with shortest genetic distance (0.0070) and the highest genetic identity (0.9930). A higher level of gene mixing resulted because of interbreeding. High values for genetic distance means

Table 5. Analysis of Molecular Variance (AMOVA) between and within goat population

Source of variation	Degree of freedom	Sum of squares	Mean sum of squares	Variance component	Percentage variance (%)
Between breeds	2	0.133	0.067	0.000	0%
Within breeds	12	5.600	0.467	0.467	100%
Total	14	5.733		0.467	100%

Table 6. Pair-Wise Population Matrix of Genetic Identity and distance Between of Three Nigerian Goat Breeds

Population 1	Population 2	Genetic identity	Genetic distance
WAD	SOR	0.9928	0.0072
WAD	SAH	0.9877	0.0124
SOR	SAH	0.9930	0.0070

WAD = West African Dwarf, SOR = Sokoto Red, SAH = Sahel

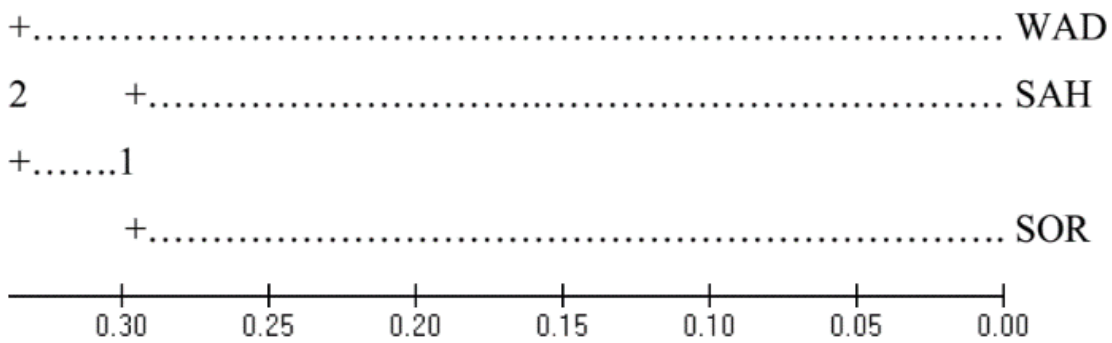

Fig. 1. UPGMA Dendrogram Representing the Genetic Relationship Among the Three Nigerian Indigenous Goat Breeds

(WAD = West African Dwarf, SOR = Sokoto Red, SAH = Sahel)

low values for genetic identity and vice versa. The highest genetic distance obtained between WAD and SAH (0.0124) indicates that the WAD were more distant from the SAH breed, with the lowest genetic identity of 0.9877. Ojo (2014) reported higher values of genetic distance using Microsatellite in Nigerian goat populations; 0.02 (between Red Sokoto and Kano Brown), 0.121 (between West African Dwarf and Kano Brown), higher than the values obtained in the present study.

The genetic dendrogram tree of relationship (Figure 1) was drawn using the UPGMA method (Sneath and Sokal, 1973). WAD diverged first and is more genetically distinct from SOR and SAH. The tree showed that SOR and SAH are within one cluster. The tree reveals that all the breeds originated from a common ancestry. The phylogenetic tree supports the genetic distance estimates where SAH and SOR formed a separate cluster independent of WAD. The dendrogram separated the three Nigerian goat breeds according to their geographical location in the country. WAD diverged first and is more genetically distinct from SOR and SAH. SOR and SAH goats, found within close locations in the northern region, clustered closely together exhibiting a sharp difference from the WAD goats from the southern region. The result indicated that SOR and SAH had shortest genetic distance (0.0070) and closest genetic identity (0.9930) while WAD and SAH had the farthest genetic distance (0.0124) and weakest genetic identity (0.9877). This could be attributed to the geographical adaptation of these breeds, WAD breed were well adapted to southern humid area of Nigeria while SAH breed were adapted to semi-arid region of northern Nigeria. The result of this study demonstrated that geographically adjacent populations were more genetically related, probably because of founder effects and interbreeding, especially around bordering areas. This result can be compared with the report of Shadma (2006) using the UPGMA tree of three goat breeds showed that the Surti breed forms the most distinct breed. The Zalawadi and Gohilwadi clustered together and Surti was clearly differentiated from the other two. The phylogenetic tree confirms the genetic distance and identity estimates obtained in this study.

Conclusion

Genetic diversity study of animal genetic resources gives room for breed conservation and utilisation. The present study showed that the distinctiveness of these indigenous goats is fast eroding, therefore effort should be made to control the wearing away of the genetic make-up of the goat populations by adopting improved breeding and management practices.

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