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Genetic variability of Nigerian Sheep breeds based on the leptin gene locus

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***Target Audience:** Farmers, Animal breeders and Biotechnologist*

Abstract

The genetic variability of sheep based on the leptin (exon 2) gene was evaluated. One hundred and sixty sheep comprising of 60, 50 and 50 of Yankasa (YB), Balami (BB) and Uda (UB) sheep respectively were used for the study. The genotypic frequencies of GG and GA were observed with the following valued of 0.5968 and 0.4032, 0.7105 and 0.2895 and, 0.5625 and 0.4375 for YB, BB and UB breeds, respectively. Corresponding frequencies of alleles were 0.7984 and 0.2016, 0.8553 and 0.1447 and, 0.7813 and 0.2188 for G and A respectively. The frequency of G allele and genotype GG was higher than that of A allele and genotypes GA for all the breeds. The Chi square test showed that genotypic distributions in all the population were within Hardy-Weinberg equilibrium for the studied populations (P > 0.05). Level of genetic diversity was observed of the gene locus. The observed heterozygosity for YB, BB and UB were 0.678, 0.752 and 0.658 respectively. The corresponding expected heterozygosity was 0.322, 0.248 and 0.342 for YB, BB and UB respectively. The expected heterozygosity (He) values were consistently lower than the observed heterozygosity (Ho). The higher values of observed heterozygosity indicated a relatively high level of genetic diversity of leptin exon 2 genes among the population. The Polymorphic Information Content (PIC) of 0.27 and 0.28 were observed in Yankasa and Uda breeds, Balami breed had lower value of 0.21. In conclusion the leptin locus for the three populations varied, Y and U population had moderate level of polymorphism information content. The molecular phylogenic analysis suggested that YB and UB breeds had closer association.

***Keywords:** leptin gene, Exon 2, Genetic variability and molecular phylogenic*

Description of Problem

Leptin is a small peptide of about 16 kDa encoded by the obese gene (1). It is encoded as the hormone that regulates body weight by maintaining the balance between food intake and energy expenditure through signaling to the brain to change the stored energy levels (2). Leptin gene also aid in controlling appetite, it has other roles in regulating growth, reproduction, body composition and immunity (3). The Mean Number of Alleles (MNA) detected in each population and the expected heterozygosities are good indicators of the genetic polymorphism within populations. The MNA is the average number of alleles observed in a population, while the expected heterozygosities are the proportion of heterozygote expected in a population. Numbers of alleles per locus per population are obtained by direct counting. Generally, the MNA is dependent on the sample size because of the presence of unique alleles that occur in low frequencies

in populations and also because the number of observed alleles tend to increase with increases with population size (4). The Leptin gene is highly conserved across species and is located on chromosome 7q31.3 in humans and on chromosome 4q32 in cattle (5). Leptin gene DNA sequence includes 15,000 base pairs and contains 3 exons, which are separated by 2 introns. Out of 3 exons and 2 introns, only two exons are translated into protein. Leptin informs the hypothalamus (6) about the amount of fat stored in the body through short and long forms of Leptin receptor.

The indigenous sheep of the Northern Nigeria as described by (7) are the Yankasa, Uda and Balami. Although these breeds generally have heavier birth, adult weights, and grow faster than the West African Dwarf sheep which thrives best in the lower fringes of the derived savannah, and the rainforest regions of southern Nigeria, their productivity is severely limited by the harsh semi-arid environments in which they live (7). The characterization of indigenous sheep breeds and breed diversity are essential for conservation of their gene sources and obtainment their elite flocks for breeding purposes as well as to meet future needs in (8 and 9). Therefore, the objective of the study is to evaluate the genetic variability of Nigerian sheep based on leptin gene.

Materials and Methods

Experimental location

The research was conducted at the Maiduguri Livestock market and abattoir. Research Farm of the University of Maiduguri, Maiduguri, Borno. Maiduguri is located on Latitude 11^{o} 5' N and Longitude 30^{o} 09' and an altitude 354m above sea level in North Eastern part of Nigeria. The temperature of the area ranges from 24^{o}C to 40^{o}C or more (17). The annual rain fall of Maiduguri gleans on balance 552.1mm (21.7) of rainfall per year .The ecological zone of Borno is characterized by vast grassland and few trees. The agricultural activities in the area include arable crop farming, livestock rearing, fishing and hunting (10).

Sampling size and blood collection

Three breeds (Yankasa, Balami and Uda) of sheep were sampled for the study. A hundred and sixty (60 Yankasa, 50 Uda and 50 Balami) sheep were randomly selected at the Maiduguri abattoir and cattle market (Kasuwan shanu). The animals were brought from different parts of the state for sale and slaughter. Blood samples for DNA extraction were collected through the jugular vein, using 5ml syringe and preserved in Ethylene Diamine Tetra Acetic acid (EDTA) bottles. The bottles were properly labelled with respect to the different breeds. The sample was conveyed in an ice box to the Biotechnology Center, University of Maiduguri for laboratory analysis.

DNA extraction

The quantity and quality of DNA were determined. Concentration of DNA was determined using Nano Drop 200^{o}C Talwan spectrophotometer at absorbance of A260/A230 ratio as described by (11).

Amplification of Leptin gene of sheep DNA was repeated 35 times with temperature changes, each cycle commonly consisting of 3 discrete temperature steps, usually initialization; which consisted of heating the reaction to a temperature of 95^{o}C for 5 minutes and samples were then denatured at 95^{o}C for 30 seconds using Polymerase Chain Reaction (PCR). The annealing temperature was 60^{o}C for 30 seconds. Samples were elongated at 72^{o}C for 5 minutes. Table 1 showed the primers used for PCR amplification.

Table 1: Sequence and position of Primers used for the leptin gene

Gene	Location	Length	Primer Sequence (5-3)	Denature	Annealing
Leptin	Exon 2	260bp	F;CGCAAGGTCCAGGATGACACC R;GTCTGGGAGGGAGGAGAGTGA	95.0^{o}C	60.0^{0}C

The amplified products were subjected to 1.2% agarose gel electrophoresis containing ethidium bromide in Tris-borate EDTA buffer and visualized under UV transillumination. PCR product was loaded in a 1.8% agarose gel prestained with (300) ? lethidium bromide. Electrophoresis was carried out at room temperature for 2 hours at 80 volts using a Bio-Rad Power PacTM electrophoresis machine (Biorad, Hercules, CA, USA). The resulting amplified bands were visualized with UV light (11).

DNA Sequencing and Sequence Analysis

Leptin gene, was sequenced commercially using standard sequencing parameters: ABI 3500XLGenetic Analyzer, CA, USA, POP7TM, Brilliant DyeTM Terminator v3.1. Sequencing was done in both direction and sequence data were normalized and base calling was done on ABI 3500 base calling software.

Genotypic and allelic frequencies of Leptin gene

The genotypic and allelic frequencies were estimated using the formula,

PA= (2NAA + NAB)/2N,

PB= 1-PA; where PA is the frequency of allele A, PB is the frequency of allele B, NAA is the number of genotype AA, NAB is the number of genotype AB;N is the total number of subjects.

Genotypic frequencies, allelic frequencies, Hardy-Weinberg Equilibriums (HWE) Observed heterozygosit-- Expected heterozygosity, Effective allele numbers (Ne) and Polymorphism Information Content (PIC) were statistically analyzed according to the methods of (12).

Phylogenetic analysis

Neighbor-Joining (NJ) trees was constructed using Maximum Composite Likelihood Method and pair wise deletions gap/missing data treatment as described by (13 , 14) . The construction was performed on the basis of genetic distances, depicting phylogenetic relationships among the Leptin gene nucleotide and amino acid sequences of Sheep breeds. The reliability of the trees was calculated by Bootstrap Confidence Values 13 with 1000 bootstrap iterations using MEGA 5.0 software (14).

Results and Discussion

The DNA observed in this work showed fragmentation. In all the samples across the sheep breeds, concentration of DNA was enough for PCR with a ratio of absorbance of 260/280.The fragment of Leptin gene exon 2 (250 bp) was characterized successfully and amplified from each sample DNA (160 samples) used in the present study. The Agarose Gel Electrophoresis of PCR that amplified 250 bp fragment of sheep Leptin gene on exon 2 for the three breeds is presented in Plate 1.PCR-SSCP Leptin gene product was evaluated based on Specific PCR product with a predicted size of 250 bp for exon 2 of Leptin gene was amplified. After PCR-SSCP, two alleles (G and A) were observed. Each animal was classified as either genotype GG or GA with respect to the Leptin genotype. Thus, G250A SSCP showed 2 genotypes namely GG and GA (Plates 2-4).

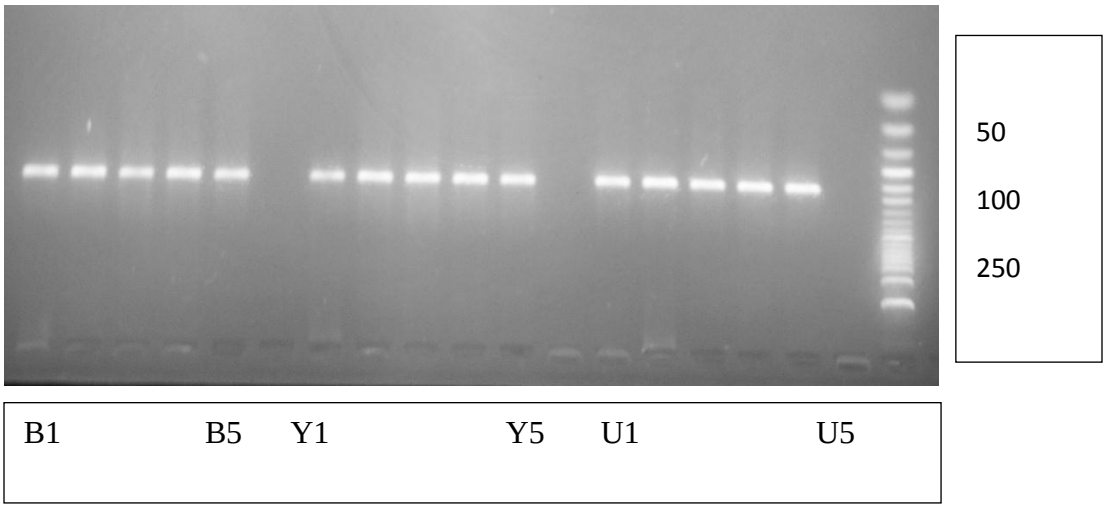

Plate1.Gel Electrophoresis of PCR that amplified 250 bp fragment of sheep leptin gene on exon 2 for the three breeds

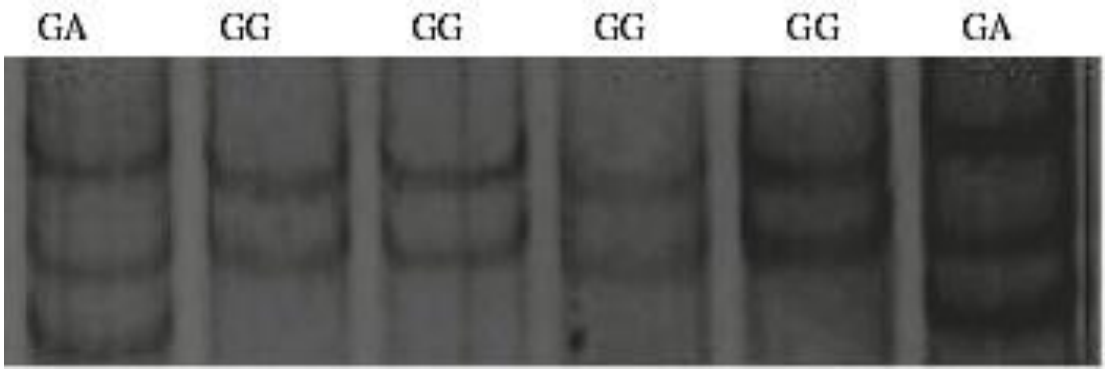

Plate. 2. Gel patterns of PCR–SSCP showing genotype Leptin gene of Yankasa sheep breed

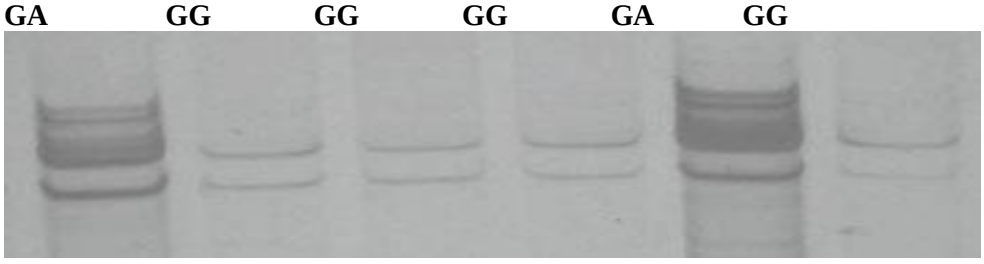

Plate 3. Gel patterns of PCR–SSCP showing genotype Leptin gene of Balami sheep breed

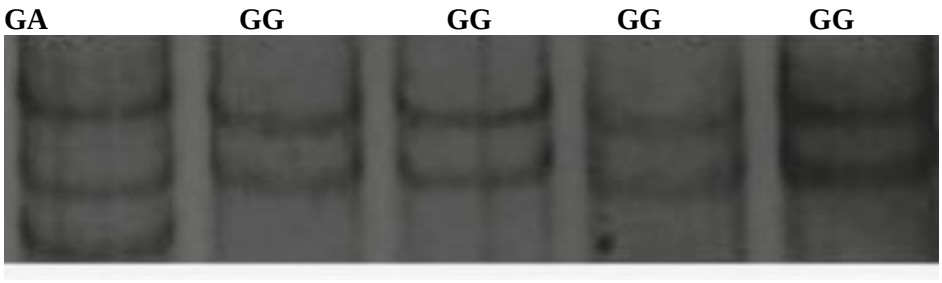

Plate 4.Gel patterns of PCR–SSCP showing genotype Leptin gene of Uda sheep breed

Genetic analysis of Ovine Leptin gene on exon 2 and Chi-square

The Leptin allelic and genotypic frequencies and chi-square (÷2) values are shown in Table 2. The genotypic frequencies of GG and GA were 0.5968 and 0.4032, 0.7105 and 0.2895 and, 0.5625 and 0.4375 for Yankasa, Balami and Uda breeds, respectively. Corresponding frequencies of alleles were 0.7984 and 0.2016, 0.8553 and 0.1447 and, 0.7813 and 0.2188 for G and A. The frequency of G allele and genotype GG was higher than that of A allele and genotypes GA for all the breeds. The Chi square test showed that genotypic distributions in all the population were within Hardy-Weinberg equilibrium for the studied populations ($P > 0.05$). Results of this study are to some extent almost similar to published data on other sheep breeds. Higher G allele and GG genotypic frequencies in sheep agree with previous reports of 15 who observed a higher frequencies of G allele of 0.6500 and GG genotype of 0.6800 compared with 0.450 for A allele and GA genotype of frequency in South African sheep. Similarly, 16 also observed A allele of 0.381 with AA genotype of 0.540 with value of 0.227 for B allele frequency for Iran sheep. The frequencies of AA and AG in the ovine leptin gene of Dorset and Suffolk breeds have been found to be 0.75 and 0.25, and 0.87 and 0.13, respectively (17). 17 found allele frequencies of 0.87 and 0.13 for G and A in Dorset sheep, respectively, which corroborated with the results of this study. 18 reported that the predominant allele in Malpura sheep 'G'allele with a frequency of 0.82 and the counterpart 'T' allele with a frequency of 0.18. This resulted to a dominance of the GG genotype over other genotype. However, the genotype frequencies observed by 19 and 20 were different from the results obtained in this study since they discovered a third genotype AA though at a low frequency. This may be due to differences in geographical location and also methods of analysis.

Table 2: Genotype and allele frequencies at Exon 2(250 bp) locus of Leptin gene

Breed	Genotypic frequencies		N	Allelic frequencies		X^2(HW*)
	GG	GA		G	A	
YB	0.5968(37)	0.4032(25)	60	0.7984	0.2016	2.7554^{ns}
BB	0.7105(27)	0.2895(11)	50	0.8553	0.1447	0.2400^{ns}
UB	0.5625(27)	0.4375(21)	50	0.7813	0.2188	2.5374^{ns}

Where : (YB) = Yankasa breed, (BB) = Balami breed, (UB) = Uda breed ,(HW) = Hardy-Weinberg equilibrium, (ns) = not significant ($P > 0.05$), (N) = Number

Population genetic characteristics of some Nigerian sheep breeds based on Leptin gene

The population genetic characteristics of Nigerian sheep are presented in Table 3. The numbers of effective alleles (Ne) were 1.48, 1.33 and 1.52 for YB, BB and UB Sheep. A relatively high level of genetic diversity at the Leptin gene locus was observed for the sheep breeds in this study. The observed heterozygosity (Ho) for YB, BB and UB populations were 0.678, 0.752 and 0.658, respectively with corresponding expected heterozygosity (He) values of 0.322, 0.248 and 0.342. The expected heterozygosity (He) values were consistently lower than observed heterozygosity (Ho) for all populations. The low (He) indicated a degree of within population mating thus such populations suggested higher rate of inbreeding. In such the high values of Ho showed a relatively high level of genetic diversity among the populations for the Leptin gene. The lower values of He compared with Ho had earlier been reported by (21) in Nigeria indigenous sheep. In contrast, 22 reported higher He compared with Ho in nine populations of Indian sheep. Lower Ho compared with He is an indication of twin of within population mating 23 which could be a proximate indication of inbreeding. (24).

According to (22), a PIC value of < 0.25indicates low polymorphism while a range between> 0.25 and < 0.50 indicate moderate polymorphism and > 0.50 is an indication of high polymorphism .By implication the Yankasa and Uda PIC values of 0.27 and 0.28, polymorphism while Balami with 0.21 had slightly polymorphism. The slight level of polymorphism observed in Balami breed could be attributed to relatively slightly values of He and Ne compared with other breeds. High value of PIC was reported by 25 and related shift in the population is heterogeneous and with minimal degree of natural selection.

Table 3: Genetic variability based on Leptin gene in three indigenous sheep breeds.

Breed	Observed heterozygosity (Ho)	Expected heterozygosity (He)	Effective allele Number (Ne)	Polymorphic information content (PIC)
YB	0.6781	0.3219	1.4748	0.2701
BB	0.7524	0.2476	1.329	0.2169
UB	0.6582	0.3418	1.5193	0.2834

where: (YB) = Yankasa breed, (BB) = Balami breed, (UB) =Uda breed.

The heterozygosity and PIC observed in the study indicated that appreciable genetic variability for sheep populations at the leptin locus. Such genetic variation can be used as a basis for selection to improve livestock production and establish conservation goals for these animals.

Evolutionary relationship of three sheep breeds in Maiduguri

The molecular phylogenetic analysis by Neigbour-Joininig method is shown in Figure 1. The genetic or evolutionary relationships of the amino acid sequences of sheep revealed Yankasa and Uda breeds tend to be closer than Balami. This is suggested by the frequency with which alleles in one species are more closely related to the alleles in a closely related species than to the other alleles in the same species (26 , 27). 21 reported that Uda and Yankasa populations are closer to each other than Balami based on

IGF-1 gene. The closeness of a gene among ruminants may be as a result of threshold in physical and chemical changes due to the same selection pressure which the ruminants undergo. The close similarity of a gene among breeds may be termed as to recent separation in similar selection pressure which the breeds have suffered during evolution (28). Genetic distance and tree construction methods gave insight into the genetic uniqueness of breeds under investigation (29). The tree showed that all the breeds originated from a common source. In addition, Balami diverged first. This was followed by Uda and lastly Yankasa, although separated but are more genetically closely related. However, 30 showed that breeds were clearly separated from each other. These native breeds tend to stand out as two distinct groups. This is in accordance with the well-known evolutionary history of Bovidae subfamily speciation.

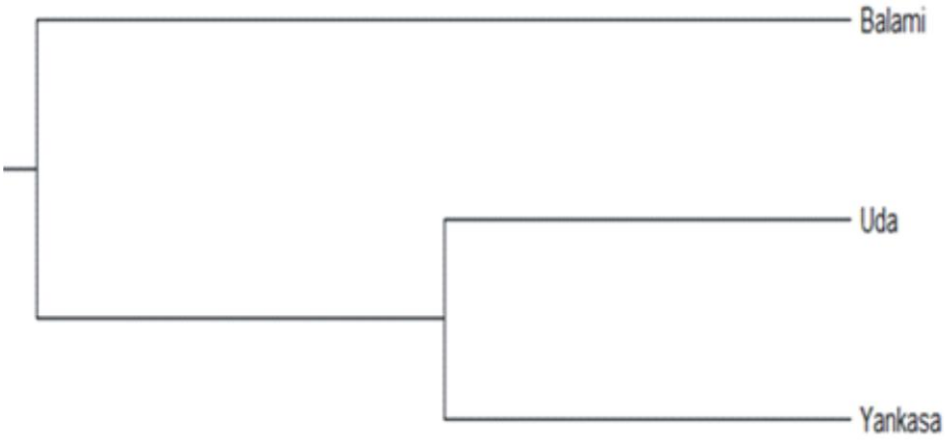

Figure. 1. Evolutionary relationships of Balami, Yankasa and Uda Breeds of sheep

Conclusions and Applications

1. The population genetic indices of gene heterozygosity and effective allele numbers of ovine Leptin locus for the three populations of sheep varied.
2. According to polymorphism Information Content, Yankasa (YB) and Uda (UB) populations had moderate polymorphism levels.
3. On the evolutionary scale Uda and Yankasa were generally closely related suggesting that they have recent common ancestors.

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