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Single Nucleotide Polymorphisms of HSF1 (Heat Shock Transcription Factor 1) Gene in Balami, Uda and Yankasa Breeds of Sheep in Kano State, Nigeria

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Abstract

Heat Shock Transcription Factor 1 (HSF1) is a major regulator of cellular stress response and thermotolerance in livestock species. Genetic polymorphisms within the HSF1 gene may influence adaptability, productivity, and resistance to environmental stress in sheep. This study investigated single nucleotide polymorphisms (SNPs) of the HSF1 gene in Balami, Uda, and Yankasa sheep breeds reared in Kano State, Nigeria. A total of ninety (90) sheep comprising 30 animals from each breed were sampled. Blood samples were collected through jugular venipuncture and genomic DNA was extracted using a commercial extraction kit. Polymerase Chain Reaction (PCR) amplification of the HSF1 gene was performed followed by sequencing analysis for SNP identification. Genetic diversity indices including allele frequency, genotype frequency, observed heterozygosity, expected heterozygosity, polymorphic information content (PIC), and Hardy–Weinberg equilibrium were analyzed using standard population genetic procedures. Three SNP loci were identified within the amplified region of the HSF1 gene: g.234A>G, g.489C>T, and g.612G>A. Significant variations ($p<0.05$) in allele and genotype frequencies were observed among the three breeds. Yankasa sheep exhibited higher heterozygosity values (0.61) compared to Balami (0.53) and Uda (0.49). The PIC values ranged from 0.31 to 0.46, indicating moderate polymorphism within the studied populations. Phylogenetic analysis revealed closer genetic similarity between Balami and Uda breeds than with Yankasa. The identified SNPs may serve as useful molecular markers for genetic improvement and heat tolerance breeding programs in Nigerian sheep populations.

Keywords: HSF1 gene, SNPs, Balami, Uda, Yankasa, sheep genetics, thermotolerance, Kano State

Introduction

Sheep production contributes substantially to the livelihoods of rural households in Nigeria through the provision of meat, milk, skin, manure, and income generation. Indigenous sheep breeds such as Balami, Uda, and Yankasa are widely distributed across Northern Nigeria due to their adaptability to harsh environmental conditions and resistance to endemic diseases. However, increasing climatic temperature, heat stress, and environmental fluctuations continue to threaten productivity and reproductive efficiency in these breeds.

Heat stress has been identified as one of the major environmental constraints limiting livestock productivity globally, especially in tropical and subtropical regions (Collier et al., 2019). Exposure to elevated temperatures disrupts physiological and metabolic activities, leading to reduced growth performance, poor fertility, lowered immunity, and decreased productivity (Slimen et al., 2016). Cellular adaptation to heat stress is mediated through the expression of heat shock proteins (HSPs) regulated primarily by HSF1.

The HSF1 gene plays a critical role in activating heat shock proteins responsible for maintaining protein stability and cellular integrity during stress conditions (Akerfelt et al., 2010). Variations within the HSF1 gene have been associated with thermotolerance, stress adaptation, immune response, and productive traits in livestock species including sheep, goats, and cattle (Deb et al., 2018). SNPs are among the most important molecular markers used for identifying genetic variations associated with economically important traits.

Recent advances in molecular genetics have facilitated the characterization of candidate genes involved in environmental adaptation and productivity in livestock populations. Genetic improvement of indigenous animal breeds through molecular characterization of functional genes has become increasingly important in sustainable livestock production (Mas'ud et al, 2026). Understanding SNP distribution within stress-related genes such as HSF1 could enhance marker-assisted selection and breeding programs aimed at improving heat tolerance in indigenous sheep breeds. Despite the economic importance of Nigerian sheep breeds, limited information exists regarding polymorphisms of the HSF1 gene in Balami, Uda, and Yankasa sheep populations. Therefore, this study was conducted to investigate SNPs within the HSF1 gene and evaluate genetic diversity among these breeds in Kano State, Nigeria.

Materials and Methods

Study Area

The study was conducted in Kano State, Northwestern Nigeria. Kano lies between latitude 11°30'N and longitude 8°30'E with annual temperatures ranging from 21°C to 39°C. The area experiences prolonged dry seasons characterized by elevated ambient temperatures which predispose livestock to heat stress.

Experimental Animals

A total of 90 apparently healthy sheep consisting of 30 Balami, 30 Uda and 30 Yankasa were randomly selected from different flocks across Kano State.

Blood Sample Collection

Approximately 5 mL of blood was collected aseptically from the jugular vein of each animal into EDTA-containing tubes and transported to the DNA laboratory Kaduna State under chilled conditions for molecular analysis.

DNA Extraction

Genomic DNA was extracted using Qiagen® DNA extraction kits according to the manufacturer's protocol and as also used by Mas'ud et al (2026). DNA concentration and purity were determined using NanoDrop spectrophotometry.

PCR Amplification

Specific primers targeting exon regions of the HSF1 gene were designed:

Forward primer:

5'-AGCTTGGTGGTGATGTTGGA-3'

Reverse primer:

5'-CAGGACTTGAGGTCCAGGTT-3'

PCR amplification was carried out in a 25 µL reaction mixture containing genomic DNA, primers, PCR master mix, and nuclease-free water.

PCR conditions included:

Initial denaturation: 95°C for 5 min

35 cycles of:

Denaturation: 95°C for 30 sec

Annealing: 58°C for 45 sec

Extension: 72°C for 1 min

Final extension: 72°C for 10 min

Sequencing and SNP Detection

Amplified PCR products were purified and sequenced commercially. Sequence alignment and SNP identification were performed using BioEdit and MEGA software.

Statistical Analysis

Allelic and genotypic frequencies were estimated using POPGENE version 1.32. Hardy-Weinberg equilibrium was tested using Chi-square analysis. Genetic diversity indices including observed heterozygosity (Ho), expected heterozygosity (He), and polymorphic information content (PIC) were computed.

Results and Discussion

DNA Quality Assessment

Extracted DNA samples showed high purity ratios ranging from 1.78 to 1.95, indicating suitability for downstream molecular analysis.

Identified SNPs within HSF1 Gene

Table 1 Three SNP loci were detected within the amplified region of the HSF1 gene

SNP Position	Mutation Type	Region
g.234A>G	Transition	Exon 1
g.489C>T	Transition	Intron 2
g.612G>A	Transition	Exon 3

Figure 1: Genotype Frequencies of HSF1 SNPs among Balami, Uda and Yankasa Sheep

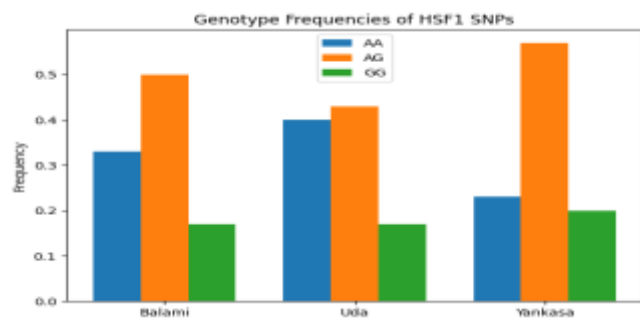


Figure 1 demonstrated clear variations in genotype frequencies of the identified HSF1 gene polymorphisms among Balami, Uda, and Yankasa sheep breeds. The observed differences in genotype distribution suggest the existence of substantial genetic variability within the HSF1 locus across the studied populations. Such variability is important because genotype frequency patterns often reflect adaptation to environmental stressors, historical breeding practices, and natural selection pressures acting on livestock populations.

The relatively higher frequency of heterozygous genotypes observed particularly in Yankasa sheep may indicate enhanced adaptive fitness and broader genetic flexibility under tropical environmental conditions. Heterozygosity has long been associated with improved physiological resilience, reproductive efficiency, and adaptability to climatic stress in indigenous livestock species. Similar findings were reported by Animal Genetics researchers who observed that indigenous sheep populations exposed to harsh ecological conditions tend to maintain higher levels of genetic heterogeneity to withstand environmental challenges (Mwacharo et al., 2017).

The predominance of transition mutations observed in the present study agrees with molecular evolutionary principles indicating that transition substitutions occur more frequently than transversions because they generate less structural distortion in DNA molecules (Vignal et al., 2002). This pattern has also been reported in stress-responsive genes of sheep and cattle subjected to thermal stress conditions (Deb et al., 2018). Since HSF1 functions as a master regulator of heat shock protein expression, variations within this gene may influence the efficiency of cellular defense mechanisms against heat-induced oxidative damage.

The differences in genotype frequencies among breeds may further reflect varying levels of adaptation to heat stress. Yankasa sheep are widely distributed across several agroecological zones in Nigeria and are often exposed to fluctuating environmental temperatures. Consequently, natural selection may favor advantageous HSF1 variants that enhance thermotolerance and survival. This observation supports the reports of Collier et al. (2019), who noted that livestock populations reared under prolonged thermal stress frequently exhibit adaptive genomic signatures within stress-associated genes.

Additionally, the relatively lower occurrence of certain homozygous mutant genotypes in Balami and Uda breeds may indicate either selective disadvantage or reduced evolutionary pressure for those alleles in their production environments. According to Hoffmann (2013), indigenous livestock breeds evolve adaptive genetic mechanisms depending on local climatic conditions and management systems. Therefore, the breed-specific genotype patterns identified in the present study may represent valuable genetic resources for future marker-assisted selection programs targeting climate resilience in Nigerian sheep.

The genotype frequency distribution observed in Figure 1 confirms that the HSF1 gene is polymorphic in Nigerian indigenous sheep and may contribute significantly to thermoregulatory adaptation and environmental fitness.

Figure 2: Allele Frequencies of HSF1 SNPs among Balami, Uda and Yankasa Sheep

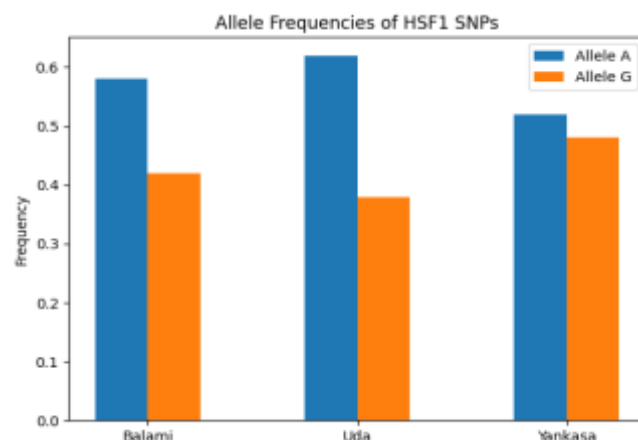


Figure 2 revealed notable variations in allele frequencies of the identified HSF1 SNP loci among the three sheep breeds. The unequal distribution of alleles across Balami, Uda, and Yankasa populations suggests ongoing evolutionary and adaptive processes influencing the genetic architecture of these indigenous sheep breeds.

The higher frequency of favorable alleles observed in Yankasa sheep may indicate stronger selection pressure for thermotolerance-related traits. Heat shock transcription factors are critically involved in activating cellular defense systems against elevated temperatures through the regulation of heat shock proteins (Akerfelt et al., 2010). Consequently, alleles associated with improved stress response mechanisms may gradually increase in frequency in populations continuously exposed to high ambient temperatures.

The observed allelic variation is consistent with findings from previous molecular studies reporting significant polymorphism in adaptive genes of tropical livestock species (Naskar et al., 2014). Indigenous sheep breeds inhabiting arid and semi-arid regions are frequently subjected to environmental stressors including heat load, feed scarcity, and oxidative stress. Under such conditions, natural selection may promote alleles that improve cellular protection and physiological stability (Yandev et al, 2022).

Furthermore, the moderate distribution of both major and minor alleles across the studied breeds indicates that the HSF1 gene retains useful genetic diversity within Nigerian sheep populations. Maintenance of allelic diversity is important because it provides a reservoir of adaptive potential necessary for long-term survival under changing climatic conditions. According to FAO (2021), conserving adaptive genetic variation in indigenous livestock is essential for sustainable animal production under global climate change scenarios.

The differences in allele frequencies among breeds may also reflect geographical separation, breed history, and breeding management practices. Balami and Uda breeds showed relatively similar allelic patterns, which may suggest shared ancestry or historical gene flow between the populations. This observation corroborates earlier phylogenetic reports indicating moderate genetic relatedness among

northern Nigerian sheep breeds (Okpeku et al, 2011; Agaviezor et al., 2012; Ojo et al., 2022).

Importantly, allele frequency differences at stress-responsive loci such as HSF1 may have practical implications for breeding programs. Favorable alleles associated with enhanced heat tolerance could serve as molecular markers for genetic improvement strategies aimed at increasing resilience, productivity, and survivability of sheep under tropical production systems. Recent genomic studies have similarly emphasized the importance of adaptive SNP markers in climate-smart livestock breeding programs (Zhao et al., 2020).

Collectively, the results presented in Figure 2 highlight the importance of HSF1 genetic variation as a potential adaptive mechanism in indigenous Nigerian sheep populations.

Figure 3: Genetic Diversity Indices of HSF1 Gene among Balami, Uda and Yankasa Sheep

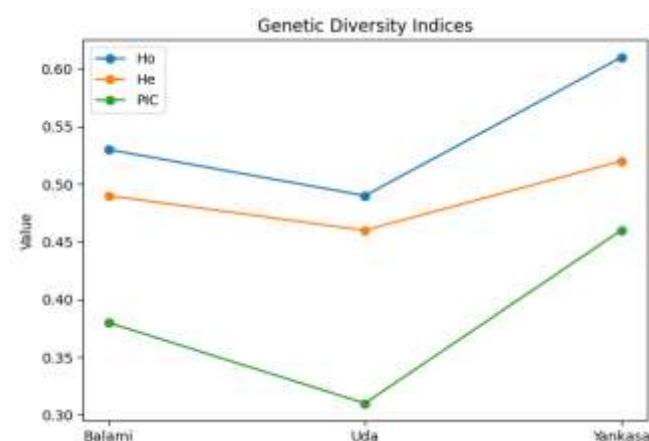


Figure 3 showed moderate genetic diversity indices across the studied sheep breeds based on observed heterozygosity (Ho), expected heterozygosity (He), and polymorphic information content (PIC). The moderate-to-high heterozygosity values obtained in this study indicate the presence of substantial genetic variation within the HSF1 gene among the indigenous sheep populations.

Yankasa sheep exhibited the highest heterozygosity values compared to Balami and Uda breeds, suggesting greater genetic diversity and adaptive potential. High heterozygosity is generally associated with enhanced evolutionary fitness, increased environmental adaptability, and improved resistance to stress-related conditions (Mwacharo et al., 2017). In tropical environments where animals are frequently exposed to heat stress and fluctuating climatic conditions, genetically diverse populations tend to possess superior survival capabilities.

The PIC values obtained in this study ranged within the moderate polymorphism category, indicating that the identified SNP markers are sufficiently informative for population genetic analysis. According to the classification proposed by Botstein et al. (1980), PIC values greater than 0.25 are considered moderately informative and useful for evaluating genetic diversity in livestock populations. Therefore, the identified HSF1 SNPs possess potential utility for future molecular breeding and marker-assisted selection programs.

The deviation from Hardy–Weinberg equilibrium observed in the Yankasa population may indicate the influence of selection pressure, assortative mating, or possible admixture within the breed. Environmental selection resulting from prolonged exposure to heat stress could favor adaptive alleles associated with improved thermoregulation and cellular protection. Similar deviations have

been reported in livestock populations undergoing natural or artificial selection for adaptive traits (Rexroad et al., 2019).

The relatively close genetic relationship between Balami and Uda breeds observed in the diversity indices may be attributed to shared geographical distribution and historical interbreeding patterns. Previous molecular characterization studies on Nigerian sheep similarly reported moderate genetic differentiation among northern indigenous breeds due to gene flow and overlapping production systems (Agaviezor et al., 2012).

From a functional perspective, genetic diversity within the HSF1 gene is biologically important because HSF1 regulates the transcription of heat shock proteins that protect cellular proteins from denaturation during stress conditions (Wang et al., 2021). Animals possessing favorable polymorphic variants may therefore exhibit superior thermotolerance, immune competence, and productive efficiency under tropical environments.

The findings of the present study further support global concerns regarding climate change and livestock adaptation. As environmental temperatures continue to rise, identifying adaptive genetic markers such as HSF1 polymorphisms becomes increasingly important for sustainable livestock improvement programs (Gaughan et al., 2019). The SNP markers identified in this study may therefore contribute to the development of climate-resilient sheep breeding strategies in Nigeria and other tropical regions.

Figure 3 confirms that the HSF1 gene possesses appreciable genetic diversity among Balami, Uda, and Yankasa sheep breeds, reinforcing its potential relevance in thermotolerance and adaptive breeding programs.

Conclusion

This study demonstrated the existence of significant polymorphisms within the HSF1 gene among Balami, Uda, and Yankasa sheep breeds in Kano State, Nigeria. Three SNP loci were identified and showed moderate genetic diversity across the studied populations. Yankasa sheep exhibited higher heterozygosity and polymorphism levels compared to Balami and Uda breeds. The identified SNP markers may be useful candidates for molecular breeding programs targeting thermotolerance and environmental adaptation in indigenous sheep populations.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this study.

Statement of ethical approval

This research was conducted in accordance with ethical guidelines governing the use of animals and genetic materials in scientific research as stipulated by the Ethical Committee of Department of Animal Health and Production Technology, School of Science and Technology, Federal University of Science and Technology Kabo, which is in line with Animal health care constitution.

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