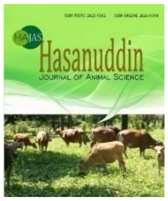

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Heat Shock Protein Gene Expression Analysis in Extensively Reared Indigenous Wad Goats

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ABSTRACT

This study investigated the expression patterns of HSP90 and HSP70 genes, which encode heat shock proteins, using 45 genomic DNA samples isolated from West African Dwarf (WAD) goats in Nigeria. The polymorphism information content (PIC) of each marker was evaluated to determine its level of informativeness. DNA samples were collected from apparently healthy WAD goats in Osun, Oyo, and Kwara States under field heat-stress conditions, with ambient temperature and relative humidity recorded at the time of sampling. Polymerase chain reaction (PCR) amplification was performed using primers specific for each heat shock protein gene, and the PCR products were analyzed by polyacrylamide gel electrophoresis on an ABI 3730 DNA Sequencer. Allelic variation was assessed using *Microsatellite Analyzer* software to generate allele frequencies. The results revealed alleles A and C as the predominant alleles for markers SSR1 (HSP90) and SSR2 (HSP70), respectively. The effective number of alleles and expected heterozygosity were higher for SSR2 (He = 0.40) than for SSR1 (He = 0.36). PIC values ranged from 0.55 (HSP90) to 0.59 (HSP70), indicating that both markers were informative (PIC ≥ 0.50). This study provides the first molecular characterization of HSP90 and HSP70 gene polymorphisms in WAD goats under tropical heat-stress conditions, suggesting their potential as reliable genetic biomarkers for thermotolerance selection and future breeding programs aimed at improving heat resilience in tropical livestock**.**

Keywords: Heat shock proteins; HSP90; HSP70; thermotolerance; West African Dwarf Goat

INTRODUCTION

Goats are vital to the economic livelihoods of thousands of impoverished livestock owners worldwide, who raise them in various climates and terrains to diversify themselves. They are a valuable resource in developing nations, particularly for those living in rural poverty. Small, marginal farmers and landless laborers have traditionally raised goats in semi-arid, arid, hilly, and mountainous areas of emerging nations that are unsuitable for producing traditional crops. These farmers utilize feed ingredients from land that is often unavailable for the production of conventional crops to generate food and fiber at a comparatively low cost. Due to their many uses, goats are sometimes referred to as "poor man's cows."

It may be the only animal that can be used effectively in various socio-economic contexts found in emerging nations. As the effects of climate change on the environment and animal production have become increasingly apparent in recent years, concern regarding heat stress (HS) has grown [1]. The ability of livestock to adapt and survive heat stress is directly impacted by heat stress (HS), which has become a significant problem in the era of climate change [2]. It has been demonstrated that when animals are unable to mitigate the effects of heat stress (HS) load, they may die from hyperthermia [2].

To preserve an animal's health, performance, adaptability, and survivability, the impacts of HS must be mitigated. High ambient temperatures are the primary factor limiting animal production in tropical and subtropical locations [3] [4], whereas extremely low temperatures in temperate regions are similarly detrimental to cattle. The sensation of discomfort and physical strain caused by exposure to extremely hot or cold environments is known as thermal stress. Both heat stress in extreme summers and cold stress in extreme winters are considered forms of thermal stress.

The genes for Heat Shock Proteins (HSPs), including HSP70 and HSP90, are members of the molecular chaperone family and are markedly increased in response to physiological and environmental stress. By generating intracellular and extracellular signals that control cellular processes and general metabolism outside the thermoneutral range, these genes are essential for helping animals survive heat stress [5]. Furthermore, they support the correct folding and unfolding of damaged proteins during heat stress, which helps maintain cellular balance and enhances the animal's ability to adapt to harsh environmental conditions [6]. HSP genes provide defense against cerebral ischemia, circulatory failure, and hyperthermia when over expressed in response to heat stress [5] [6]. Cellular protection (cytoprotection), immunological response, protein synthesis, cytoskeletal protection, protein translocation and steroid hormone receptor regulation, transportation, protein refolding, safeguarding proteins from cellular stress, inhibitory apoptosis, and adaptation during and after thermal assault are all specifically dependent on the HSP 90 gene [6] [7]. It has been demonstrated that the genomic foundation for thermotolerance selection in tropical animals subjected to heat stress is provided by the HSP90 gene. It is generally acknowledged that the biological response to heat stress includes alterations in gene expression. Even yet, the most well-researched examples of genes whose expression is impacted by heat shock are the HSPs. Many HSPs, such as HSP32, HSP40, HSP60, HSP70, HSP90, HSP110, and many more, are found to have increased expression during hyperthermic stress [8][9] [10] [11]. HSPs are essential for intracellular transport, maintaining proteins in an inactive state, and preventing protein degradation [12]. Additionally, HSPs function as molecular chaperones by participating in the assembly of proteins without being integral to the final protein structure [13].

HSPs play crucial roles in cell-cycle control signaling and the protection of cells against apoptosis. This study is crucial as it addresses a significant challenge in livestock production: heat stress (HS) and its impact on goat adaptability, survivability, and productivity. Goats play an essential role in the livelihoods of smallholder farmers, particularly in developing countries, where they serve as a primary source of food, fiber, and income. Their ability to thrive in harsh terrains and climatic conditions makes them indispensable to rural economies, particularly for marginalized farmers and landless laborers who rely on them for their livelihoods.

However, with the increasing threat of climate change, rising global temperatures have become a primary concern for livestock production. Heat stress negatively affects goats by compromising their health, reproduction, and overall productivity. To mitigate these challenges, understanding the role of heat shock proteins (HSPs), particularly HSP70 and HSP90, is essential. These proteins play a crucial role in helping goats cope with heat stress by regulating protein folding, cellular protection, and immune response.

By studying the expression of HSP genes in indigenous West African Dwarf (WAD) goats, researchers can identify genetic markers associated with thermo-tolerance. This knowledge can be applied in breeding programs to enhance resilience to heat stress, ensuring sustainable livestock production in regions vulnerable to extreme temperatures. Additionally, insights gained from this research can inform management strategies aimed at improving goat welfare and productivity under challenging environmental conditions.

Ultimately, this study contributes to the broader goal of developing climate-resilient goat farming systems, which is crucial for ensuring food security and alleviating poverty in regions experiencing heat stress. By equipping farmers with the knowledge and tools to breed and manage goats more effectively, the study supports sustainable agricultural practices that benefit both animals and the communities that depend on them.

MATERIALS AND METHODS

The ambient temperature (°C) and relative humidity (RH, in percent) were recorded before blood collection. Moral et al. [14] equations were used to determine the temperature humidity index (THI): $THI = 0.8*T + RH * (T - 14.4) + 46.4$, where T = Ambient Temperature (°C); RH = Relative Humidity (%).

Genomic DNA was extracted from 5 mL blood samples, as described by Regitano and Coutinho [15]. The concentration of DNA was measured via spectrophotometry, and the 260:280 ratio confirmed the quality. DNA integrity was assessed through electrophoresis in agarose gel. Primers were designed for the target regions using Primer3Plus software, available online at http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi. Forward (F) and reverse (R) primers were designed to amplify the HSP70 and HSP90 genes, along with their corresponding amplicon sizes (in base pairs, bp) and annealing temperatures (in degrees Celsius, °C). Reactions to amplify all target regions were conducted with a final volume of 20 µl: 80 ng of the DNA template, 0.165 µM of each primer, 1.5 mM of the reaction buffer containing $MgCl_2$, 0.2 mM of each dNTP, and 0.650 units of Taq DNA polymerase. Polymerase chain reactions (PCR) were performed using a standard PCR program in a Bio-Rad thermocycler model T100. The denaturation step was conducted at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, an annealing step at a temperature of 30 °C based on each primer pair, and a final extension at 72 °C for 50 s. Finally, the PCR products were visualized in an agarose gel and purified using the ExoSAP-IT enzyme.

RESULTS AND DISCUSSION

Alleles frequency

The allele frequencies of A and C indicated by markers 1 and 2 (HSP90 and HSP70 genes) are shown in Table 1. Major allele A, at 76 percent, was identified by SSR_1 (HSP 90 gene), while minor allele C, at 24 percent, was also identified by the same marker. Major and minor alleles C and A were identified similarly by SSR_2 (HSP 70 gene), with corresponding frequency values of 28% and 72%, respectively. The results indicate that the SSR_1 marker (corresponding to the HSP90 gene) corresponds to the major allele A. In contrast, marker SSR2 identified allele C (HSP 70 gene) on the same WAD goats, with a slightly higher percentage (75 versus 72) for allele A as indicated by the SSR_1 marker (HSP 90 gene) (Table 1).

Table1. Allele frequencies of the gene in Nigerian indigenous WAD goats

Marker	**Allele**	**WAD (n=45)**
SSR_1	A	0.7556
	C	0.2444
SSR_2	A	0.2778
	C	0.7222

Note:WAD = West African Dwarf goat, SSR_1 = HSP 90 (Marker 1), SSR_2 = HSP 70 (Marker 2), A= Allele A, C= Allele C

Genetic Variation Statistics

Table 2 shows the genetic variation statistics within WAD goats. It showed that the observed number of alleles for all loci (SSR_1 and SSR_2) was the same, but the adequate number of alleles differed. The effective number of alleles in the WAD goat was 1.59 for the SSR_1 marker and 1.67 for SSR_2. It can be deduced that the adequate number of alleles for all loci of SSR_1 was lower than that of SSR_2. The mean adequate number of alleles for SSR_1 and SSR_2 revealed differences in the genetic expression patterns of both markers in the same WAD goats under study. Shannan's information index value of 0.59(SSR_2) was higher than 0.55(SSR_1).

Table 2. Genetic variation statistics in WAD goats

Marker	**Sample Size**	**Na**	**Ne**	**I**
SSR_1	45	2	1.5857	0.5561
SSR_2	45	2	1.6701	0.5908
Mean	45	2	1.6279	0.5735

Note: **Na** Observed number of alleles, Ne Effective number of alleles [Kimura and Crow (1964)], I = Shannan's Information Index [Lewontin (1972)], SSR_1 = HSP90 (Marker 1), SSR_2 = HSP90 (Marker 2)

Heterozygosity for All Loci in WAD Goats

The results revealed that the observed heterozygosity values were significantly greater than the expected heterozygosity values for the two markers (HSP 90 and HSP 70 genes) tested. The highest expected heterozygosity value (Ne_1) of 0.40 was observed in marker 2 (HSP70 gene) compared to a lower value of 0.36 in marker 1 (HSP90 gene) in WAD goats. This result, however, revealed differences in the expression patterns of markers 1 and 2 corresponding to HSP90 and HSP70 genes, respectively (Table 3).

Table 3. Heterozygosity for all loci in WAD Goats

Marker	Sample size	Ho	He	Average heterozygosity	Nei
SSR_1	45	0.4889	0.3735	0.3863	0.3694
SSR_2	45	0.5111	0.4057	0.3722	0.4012
Mean	45	0.5000	0.3896	0.3793	0.3853

Note: Ho: observed heterozygosity, He: Expected heterozygosity, Nei: Nei's (1973) expected heterozygosity

As members of the molecular chaperone family, Heat Shock Protein (HSP) genes—specifically, HSP70 and HSP90—are extensively expressed in response to physiological and environmental stress. By initiating intracellular and extracellular signaling pathways that control cellular processes and general metabolism outside their typical thermal range, these genes are essential for helping animals adapt to heat stress [5].

Proteins encoded by HSP genes lessen heat stress-induced cellular damage. Several stimuli, including high temperatures, physical effort, and oxidative stress, cause them to become activated. In reaction to such difficulties, these proteins are released both inside and outside the cell [16]. Extreme heat triggers an increase in HSP gene expression, which stimulates the synthesis and maturation of new proteins to replace those weakened by stress. Furthermore, when repair is not feasible, HSPs help refold broken proteins or guide them to breakdown pathways. By preventing programmed cell death, the accumulation of these proteins in stressed cells not only facilitates protein repair but also significantly contributes to cell survival [17].

The current study found that West African Dwarf (WAD) goats differed in the expression of two important markers, i.e, HSP70 and HSP90. Results indicated that HSP70 (SSR_2 marker) had a stronger expression pattern than HSP90. Furthermore, HSP70 (0.59) had better Shannon's information index values than HSP90 (0.55), and HSP70 (1.67) had a larger effective number of alleles than HSP90 (1.59). These results are consistent with other studies that have found the HSP70 family to be essential for cellular thermo tolerance [18, 19]. A well-known indicator of the cellular stress response, this gene family is among the most evolutionarily conserved and plays a crucial role in protein folding within cells.

Human lifespan, survivability, and improved heat stress response have all been linked to polymorphisms in the HSP70 gene [19]. Specific single-nucleotide polymorphisms (SNPs) in the HSP70 promoter region have been linked to important reproductive traits, including pregnancy rates and calf weaning weights [20, 21, 22] as well as shorter productive lifespans in dairy cows [23]. Similar to this, studies on Holstein cows have identified regulatory single-nucleotide polymorphisms (SNPs) in genes such as HSP70A1A and ATPIA1, which play a role in thermoregulation [24, 25]. Additionally, SNPs linked to heat tolerance have been found within the HSF1 gene [26].

SNPs in the 5′ regulatory region of HSP70.2 have been shown to affect semen quality features, including sperm motility and concentration, in pigs. Polymorphisms in the HSP70 gene are associated with varying degrees of heat tolerance in chickens [27]. Functional variations of the HSP70.2 gene also have a significant impact on cellular responses to stress and mRNA stability [28, 29].

Genetic equilibrium can be influenced by factors such as mutation, migration, genetic drift, and selection, which alter genotypic and allelic frequencies over generations. SNPs in HSP70 and HSP90 genes exhibiting high heterozygosity indicate genetic variability, which is essential for selection and breeding programs. A heterozygosity level greater than 0.70 is considered high polymorphism [30]. However, in the present study, both HSP70 and HSP90 showed heterozygosity levels below 0.50, indicating moderate genetic variation.

For association studies, markers having a minor allele frequency (MAF) greater than 0.10 are frequently deemed appropriate. The results of this study are likely influenced by the sampling approach, which focused on phenotypic extremes, as indicated by the observed MAF values, deviations from Hardy-Weinberg equilibrium, and the presence of just two genotypes. Compared to HSP70 (72) and HSP90 (75), the allele frequencies were somewhat higher. The genetic diversity of the HSP70 gene has been highlighted by earlier reports of similar results in chickens [28, 29], humans [19], and poultry [27].

Diversity in the HSP90 gene has been linked to improved survival, adaptation, and thermotolerance in several species [19, 6, 7]. Genetic diversity among various ethnicities is suggested by the differences in HSP90 genetic profiles [31, 32]. The HSP70 gene exhibited higher genetic variability in this study than the HSP90 gene, indicating that it is more important for WAD goats' tolerance to heat stress. The identified genetic variations between these two genes may serve as valuable genetic resources for breeding initiatives that enhance livestock's survival, adaptability, and thermotolerance, particularly in hot and humid tropical climates [29, 33]. Furthermore, in animals subjected to heat stress, these genetic variations may contribute to the development of medication resistance and disease tolerance [19, 27, 34].

Other HSP genes or loci are similar in mammalian species. For example, an earlier study by Gade et al. [25] reported that the HSP70 gene in mammalian species exhibited a high degree of relatedness. HSP genes were highly conserved in both protein-coding and regulatory sequences, exhibiting ordinary homology. Amino acid sequences of the HSP70 gene were highly conserved among HSP sub-families [35].

CONCLUSION

The genes are conserved across a wide range of animals. As such, they can serve as biomarkers for selection and breeding programs aimed at enhancing thermo-tolerance in various livestock animals, particularly WAD goats under thermal stress. The expression patterns could be further investigated for potential specific effects on the thermo tolerance performance of WAD goats in hot tropical environments.

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CONFLICT OF INTEREST

No conflict of interest in this research

ETHICAL CLEARANCE

This study on the expression analysis of heat shock protein (HSP) genes in extensively reared indigenous West African Dwarf (WAD) goats has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) and the Ethical Review Board of the Federal University of Technology, Akure. The research complies with the ethical guidelines for animal welfare and experimentation, ensuring minimal discomfort to the animals involved.

All procedures, including blood sample collection, were conducted following the principles outlined in the guidelines for the Care and Use of Laboratory Animals and the International Ethical Standards for the Use of Animals in Research. Blood samples were obtained through venipuncture by trained veterinary personnel using sterile techniques to minimize pain and distress. Adequate care was taken to ensure that the goats were handled adequately before, during, and after sample collection to prevent unnecessary stress.

The animals used in this study were maintained under standard husbandry conditions, with access to appropriate nutrition, suitable housing, and veterinary care. No invasive procedures or long-term suffering were induced as part of the research. Additionally, animal owners and caretakers provided informed consent before sample collection, ensuring transparency and compliance with ethical standards.

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