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Molecular Estimation of Inbreeding Coefficient of Reared Nigerian Indigenous Goats

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ABSTRACT

Heat shock proteins (HSPs), also known as molecular chaperones, are key indicators of stress response in animals. These proteins are highly conserved and are expressed in response to environmental stress, aiding in cellular protection and adaptation. This study aimed to assess the heat stress adaptation ability of two Nigerian goat breeds West African Dwarf (WAD) and Red Sokoto (RS) by evaluating the expression of *HSP70* and *HSP90* genes. Specific primers were used to detect gene expression through PCR and RFLP techniques. Both *HSP70* and *HSP90* genes were expressed in the two breeds under heat stress conditions. Although the expression levels were similar, WAD goats showed slightly higher gene expression (Na = 1.62, I = 0.57) compared to RS goats (Na = 1.59, I = 0.55). Interestingly, this marginal increase in HSP gene expression in WAD goats did not translate to better adaptation. Instead, the findings suggest that RS goats are more effectively adapted to heat stress despite lower gene expression, implying more efficient stress response mechanisms. The *HSP70* gene, in particular, appeared to play a major role in thermal protection, suggesting its potential as a biomarker for selecting heat-tolerant animals. Based on these observations, it is hypothesized that *HSP70* and *HSP90* genes could be linked to thermo-tolerance traits. Further studies are recommended to investigate their associations with adaptability, performance, and stress resilience in Nigerian goat breeds, especially under the challenging thermal conditions of tropical environments.

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INTRODUCTION

Goat production plays a crucial role in food security and livelihoods in Nigeria, where environmental conditions vary from humid rainforest to arid savannah. Heat stress is a major constraint to productivity, as exposure to ambient temperatures above the upper critical limit disrupts homeostasis and impairs growth, reproduction, and milk yield (Dangi et al., 2022). Goats exhibit resilience to high ambient temperatures through behavioural and physiological adaptations, supported by inherent genetic mechanisms.

Breed-specific variation in heat tolerance has been reported, with the West African Dwarf (WAD) goat adapted to humid, forested regions and the Red Sokoto (RS) goat thriving in hotter, drier zones (Bester, 2022). These differences may be partly explained by variation in heat shock proteins (HSPs), a family of molecular chaperones that protect cells from thermal injury. HSP70 and HSP90 are among the most studied members, with key roles in protein folding, repair, and stabilization during stress. Elevated expression or advantageous allelic variants of these genes have been linked to enhanced thermo-tolerance in livestock.

In addition to HSP polymorphisms, inbreeding coefficients derived from molecular marker data can influence adaptive capacity. High inbreeding levels may reduce genetic diversity and limit the potential for environmental adaptation. Despite the importance of these parameters, few studies have examined the relationship between HSP genetic diversity, inbreeding, and heat tolerance in Nigerian goats.

This study addresses this gap by: (1) comparing HSP70 and HSP90 allele frequencies, heterozygosity, and inbreeding coefficients between WAD and RS goats; and (2) assessing whether these genetic indicators correspond to environmental heat stress adaptation, as measured by THI thresholds. We hypothesized that HSP genetic diversity would differ between breeds and that lower inbreeding coefficients would be associated with superior thermo-tolerance.

MATERIAL and METHOD

Ethics Statement

All procedures adhered to the ethical guidelines of the Federal University Oye-Ekiti Animal Ethics Committee (approval number: FUOYE/ 2024/ 04).

Animal Material

The present study was carried out on 45 WAD and 50 Red Sokoto goat breeds in Osun state, Southwestern Zone of Nigeria. All the experimental goats were

carrying an average body weight of 20.21±0.75 kg and 8 months of age. The experiment was carried out during dry season (Dec.–Feb.). All experimental goats were apparently healthy and free from any anatomical and physical abnormalities. All experimental goats were maintained under semi-intensive system of feeding and management.

METHOD

The observations on meteorological variables (relative humidity, temperature) were collected and temperature humidity index (THI) was calculated (Kapila et al., 2023).

Blood was taken from the jugular veins of experimental animals and placed in Ethylene diamine tetra acetic acid (EDTA) tubes to avoid clotting. Following that, the samples were transported to the laboratory on ice and stored at – 20 degrees Celsius. 200µL of the blood sample was utilized for DNA extraction using Bioline International's Isolate II Genomic DNA extraction Kits. With 100µL of elution buffer, the final elution was diluted. According to protocol, the purified DNA sample was also stored at -20ºC for long-term storage. The presence of genomic DNA in the final eluted solution from the last DNA extraction stage was confirmed using agarose gel electrophoresis. The samples were run alongside a DNA ladder at 100 volts for 30 minutes on a 0.75 percent agarose gel containing ethidium bromide. The concentration of extracted DNA was determined using an Ultra-Violet Spectrometer from PG Instruments Ltd. The primer sequences were stated below;

*HSP70*Forward 5′ TCATCGGAGATGCAGCCAAGAA 3′

Reverse 5′ AGATCTCCTCGGGGAAGAAGGT 3′

*HSP90*Forward 5′ AAATAAGTCGACATGCCTGAGCAAACCCAG 3′

Reverse 5′CTTCATCTGCAGTTAGTTAGTCTACTTCTTCCAT 3′

Using the programmed Thermocycler, the amplification process was carried out in 200ul microcentrifuge tubes (Mastercycler pro by Eppendorf). 15microliters of PCR master mix, 1microliter each of forward and reverse primers, 3microliters of DNA template, and 10microliters of sterilized distilled water were used to make a 30microliter reaction mix. The components were properly mixed before being centrifuged for 5 seconds at 11,000 (rpm). After 4 minutes of denaturation at 94ºC, 40 cycles of the following reaction were performed: denaturation at 94ºC for 30 seconds, annealing at 62ºC for 30 seconds, and extension at 72ºC. At 72ºC, the final extension was done for 2 minutes. 10µL of the PCR amplicon was electrophoresed at 100 volts in a 0.75 percent agarose gel with ethidium bromide in 1x TBE buffer with DNA ladder, as stated by Joseph and David (2001). The gel

was placed in a gel documentation machine (VWR's Genosmart2) so that the bands in the gel could be seen under Ultra-Violet illumination. 20uL of PCR products were digested with ten units of restriction enzyme (Invitrogen, USA) specific for each gene. For 5 hours, the reaction mixture was incubated in a water bath at 37°C. The restriction fragments were split in an agarose gel to discriminate between the A and B alleles. The restricted fragments were examined and electrophoresed in a 4 percent agarose/1X TBE gel stained with ethidium bromide after restriction digestion. The molecular sizer was a 100-bp ladder. The bands were visible under UV light, and the gel documentation system photographed the gels (Enduro, Inc). Gene counting was used to calculate allele frequencies. To see if the population was in Hardy-Weinberg equilibrium, a Chi-square test was used.

Data Analysis

The banding pattern on the gel was converted to numerical values, with 1 representing the presence of a band and 0 representing the lack of a band. The software NTSYS-pc, version 2.0, was used to estimate genetic relatedness between genotypes using Jaccard's similarity coefficient, and UPGMA was used to cluster the genotypes (unweighted pair group method using arithmetic averages). The strength of clusters was assessed using Boot program and bootstrap methodology.

RESULTS and DISCUSSION

Figure 1 and 2 revealed PCR – RFLP techniques used to genotype and detect polymorphisms of HSP 70 and HSP 90 genes in two breeds of Nigerian goats. The PCR of all tested goat DNA (45 WAD & 50 Red Sokoto) gave specific amplified fragments at the expected band size 400, 300 and 200 bp respectively for HSP 90 gene while HSP 70 gave specific amplified fragments at the expected band size (300-bp) in two breeds of goats.

The ladder 39 – 58 of electrophoresis gel for HSP 90 gene are upregulated and those animals utilized HSP 90 for adaptation to heat stress condition. From ladder 1 – 38 and 58 – 95 of electrophoresis gel for HSP 90 genes are down regulated and those animals are resistant to heat stress with less utilization of HSP 90. The ladder with thick/ double bands is upregulated for HSP 70 and these ladders are alleles of the same genes while thin /one band indicates single allele and downregulated. It could be deduced that those animals used more of HSP 70 genes for adaptation than HSP 90 genes.

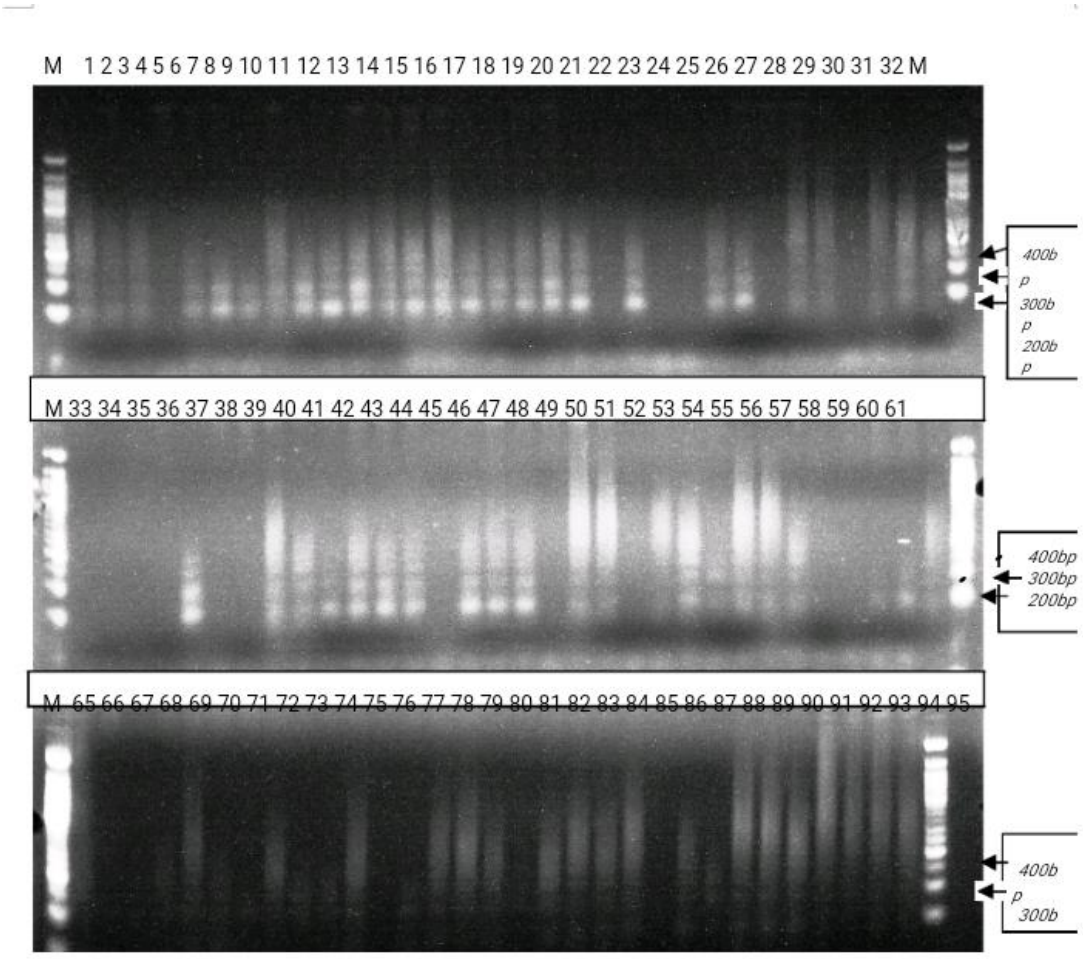

Figure 1. Electrophoresis gel for DNA of HSP-90 gene in WAD and RS goats

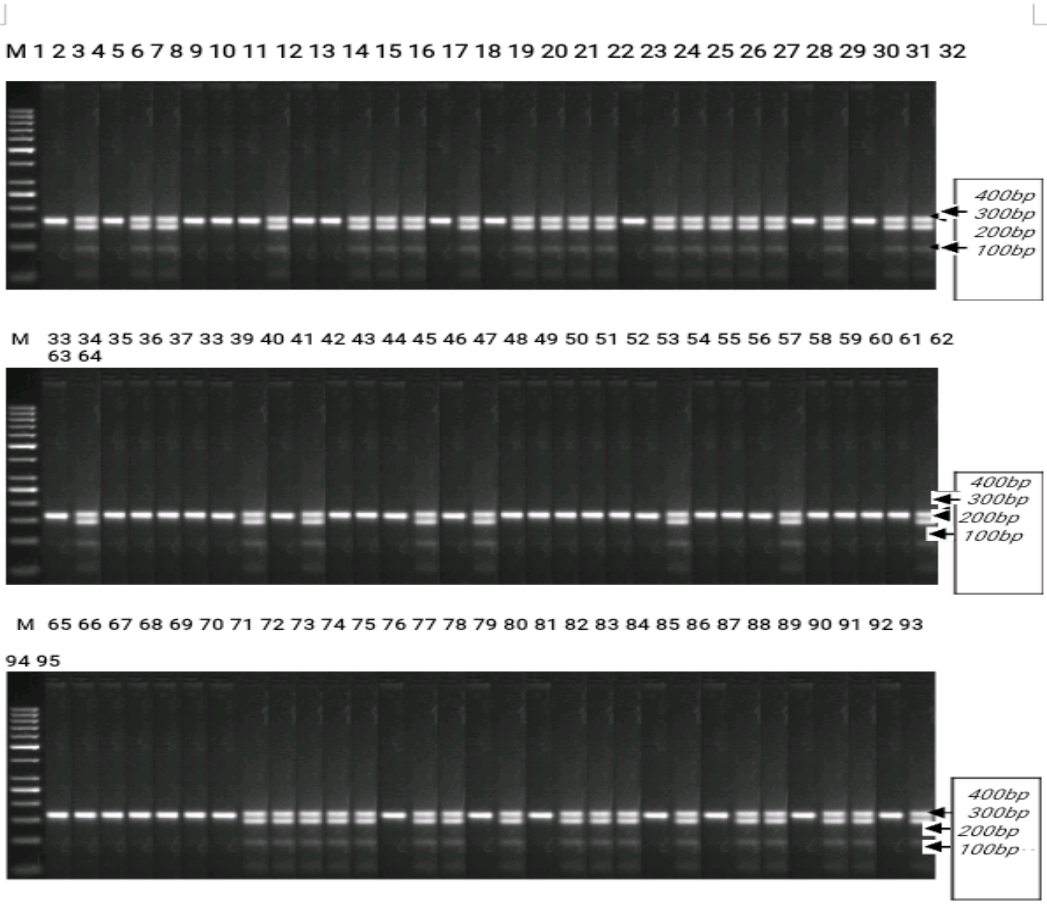

Figure 2. Electrophoresis gel for the DNA of HSP-70 gene in WAD and RS goats.

Prevailing Environmental Conditions

Table 1 shows the prevailing environmental conditions during the experimental period. The values of ambient temperature ranged from 25 – 36^{0}C with an average of 31.44±0.10^{0}C. The relative humidity values ranged from 76.16-89.5% with an average of 83.93±0.11%. The temperature humidity index (THI) during the study period ranged from 74.44-94.54 with an average of 86.26±0.32.

Table 1. Prevailing environmental conditions

Parameters	Range	Mean ± SEM
Environmental conditions		
Ambient Temperature (^{0}C)	25 – 36	31.44±0.10
Relative humidity (%)	76.16 – 89.5	83.93±0.11
Temperature Humidity Index (THI)	74.44 – 94.54	86.26±0.32

SEM = Standard error of mean

Alleles Frequency

HSP90 gene revealed major allele A with 75.56 and 72% and minor allele C with 24.44 and 28 % respectively in WAD and Red Sokoto goats. *HSP70* gene recorded major allele C with 72.22 and 78% and minor allele A with 27.78 and 22% in WAD and Red Sokoto goats respectively in Table 2. The results indicated those major alleles as those one that were majorly utilized for heat stress adaptation by the animals.

Table 2. Allele frequencies of gene in Nigerian indigenous WAD and RS goats

Marker	Allele	WAD (n=45)	RS (n=50)
HSP90	A	0.7556	0.7200
	C	0.2444	0.2800
HSP70	A	0.2778	0.2200
	C	0.7222	0.7800

WAD = West African Dwarf goat, RS = Red Sokoto, A = Allele A, C = Allele C

Tables 3 and 4 show the genetic variation statistics within two breeds of goat. It showed that the observed number of alleles for all loci (HSP 90 and *HSP70*) were the same but the effective number of alleles differed. The effective number of alleles in the WAD goat were 1.52 for *HSP90* and 1.67 for *HSP70*. It can be deduced that the effective number of alleles for all loci of *HSP90* was lower than the *HSP70*. However, the reverse was the case in RS goat where effective number of alleles for *HSP90* was greater than *HSP70*. On mean effective number of alleles for both *HSP90* and *HSP70*, it showed that expression patterns of both breeds and the expression pattern of Heat shock protein genes are breed and specie specific. The Sharman's Information Index was high in RS goat for *HSP90* and WAD for HSP 70 (0.590 and 0.593 respectively) and low in RS goat for *HSP70* (0.53) and *HSP90* (0.56).

Table 3. Genetic variation statistics in WAD goats

Marker	Sample Size	Na	Ne	I
HSP90	45	2	1.5857	0.5561
HSP70	45	2	1.6701	0.5908
Mean	45	2	1.6279	0.5735

Na = Observed number of alleles, Ne = Effective number of alleles, I = Shannan's Information index

Table 4. Genetic variation statistics in Red Sokoto Goat

Marker	Sample Size	Na	Ne	I
HSP90	50	2	1.6756	0.5930
HSP70	50	2	1.5225	0.5269
Mean	50	2	1.5991	0.5599

Na = Observed number of alleles, Ne = Effective number of alleles (Kimura and Crow (1964), I = Shannan's Information index (Lewontin (1972))

The values of Ho obtained in this study are 0.48 and 0.51 for WAD goats and 0.56 and 0.44 for RS goats as against the lower values of He which were 0.37 and 0.40 for WAD and 0.40 and 0.34 for Red Sokoto goats respectively, Table 4 and 5. This indicated genetic variation in trans HSP locus in both WAD and RS goats, meaning that selection for Heat stress resistance programme if carefully planned and executed will result in genetic gain towards improved performance in the selected population.

Table 5. Heterozygosity for all loci in WAD Goats

Marker	Sample size	Ho	He	Average heterozygosity	Nei
HSP90	45	0.4889	0.3735	0.3863	0.3694
HSP70	45	0.5111	0.4057	0.3722	0.4012
Mean	45	0.5000	0.3896	0.3793	0.3853

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

Table 6. Heterozygosity for all loci in Red Sokoto Goats

Marker	Sample size	Ho	He	Average Heterozygosity	Nei
HSP90	50	0.5600	0.4073	0.3863	0.4032
HSP70	50	0.4400	0.3467	0.3722	0.3432
Mean	50	0.5000	0.3770	0.3793	0.3732

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

F Statistics and Gene Flow for All Loci

Table 7 shows the F Statistics for all loci with the mean value of Fis of -0.31 and the values of -0.35 and -0.27 were recorded for HSP 90 and 70 gene respectively. The level of genetic variation in WAD and RS goats should theoretically be relatively high within populations and low between populations, hence the reason for different behaviour among the HSP genes.

Table 7. F-statistics and gene flow for all loci

Locus	Sample Size	Fis	Fit	Fst	Nm*
SSR1	95	-0.3576	-0.3554	0.0016	152.7813
SSR2	95	0.2776	-0.2719	0.0045	55.7500
Mean	190	-0.3185	-0.3145	0.0030	82.5262

* Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst

DISCUSSION

This study focused on the investigation of gene expression in various goat breeds during heat stress. THI ranged from 74.44 to 94.54, according to the findings analysis, suggesting that the animals were under stress throughout the experiment (Table 1). The two goat breeds differed in the relative expression of *HSP90* and 70 genes, and the expression pattern showed that Red Sokoto goats had the lowest expression of *HSP90* and 70 genes, while WAD goats had the highest. The Red Sokoto goat's pattern of expression was the lowest, suggesting that it was better able to adjust to heat stress. According to these findings, Red Sokoto goats are the most tolerant of heat stress conditions, while WAD goats are more vulnerable. Proteins of the Heat Shock Protein (HSP) sub-family (molecular chaperone families), which includes the genes *HSP70* and *HSP90*, are known to be significantly expressed in stressful physiological and environmental settings. Through intracellular and extracellular signals that coordinate cellular and total animal metabolism, they help animals respond to external heat loads above thermo-neutral zones (Collier et al., 2018).

Additionally, during thermal attack, the genes control cellular homeostasis and the folding and unfolding of damaged proteins, giving stressed animals the adaptive ability to deal with stressful environmental conditions (Kapila et al., 2023). According to Lee et al. (2016) and Collier et al. (2018), the HSP genes offer a defense mechanism against cerebral ischemia, circulatory shock, and hyperthermia by overexpressing during HS. Specific functions of the HSP 90 gene include immune response, protein synthesis, cyto-skeletal protection, protein translocation and regulation of steroid hormone receptors, transportation, protein refolding, protection proteins from cellular stress, inhibitory apoptosis, and adaptation during and after thermal assault (Sodhi et al., 2013b; Kapila et al., 2023).

When tropical animals are subjected to heat attacks, it has been demonstrated that the HSP 90 gene provides the genomic foundation for thermo-tolerance selection. These results are consistent with the findings of Patir et al. (2020), who found that heat stress causes bovine lymphocytes to produce *HSP70*. Human blood has also been shown to express more HSPA6 in response to heat stress (Sonna et al., 2002, 2022). Similarly, camels have shown that temperature changes impact the amount of expressed *HSP70* (Garbuz et al., 2021, Ulmasov et al., 2023). According to Mizzen et al. (1988), stress tolerance is a crucial trait, although its technique is not well understood.

The current study's results demonstrated that breed-specific expression of the *HSP70* and HSP 90 genes supports the findings of Dangi et al. (2022), who found that tropical goats in India exhibited noticeably higher levels of *HSP70* mRNA

expression in PBMCs during the peak summer season as opposed to the winter. Conversely, between the height of the summer and the winter, goats from the temperate zone did not exhibit appreciable levels of *HSP70* transcriptional response (Dangi et al., 2022). In a different investigation, goat PBMCs under heat stress in vitro shown a markedly greater up-regulation of *HSP70* mRNA in comparison to unstressed cells (Mohanarao et al., 2014).

According to reports, animals with the genetic diversity of the HSP 90 gene have improved thermo-tolerance, adaptability, survivorship, lifespan advantage, and a greater capacity to react to heat stress (Singh et al., 2006; Sodhi et al., 2013b; Kapila et al., 2023). The presence of genetic variety found within distinct genetic groups of the HSP 90 gene is demonstrated by the differences between the different genetic variants of the gene as shown by the differential plot (Bester, 2022; Gori et al., 2022).

It is possible to consider the identified genetic variations of the HSP 90 gene as a true genetic resource for improving programs of disease tolerance and drug resistance in animals under thermal stress, as well as thermo-tolerance, adaptability, and survivability advantages to cope with a wide range of heat stress and variations in the surroundings, particularly in the hot humid tropics (Schwerin et al., 2022; Kishore et al., 2023). According to Stott (1981), stress is caused by environmental factors that constantly affect animals, upsetting their homeostasis and leading to new adaptations that may be harmful or beneficial. In tropical, subtropical, and dry regions, heat stress has been a significant stressor that lowers animal productivity. An animal's capacity to adapt to a certain environmental niche is indicated by its capacity to acclimate and produce under a given climate.

Another trustworthy marker of ongoing stress in feedlot cattle is the level of *HSP70* in their blood (Gaughan 2023). *HSP70* responses may be seen as a cellular thermometer since there is strong evidence that the synthesis of *HSP70* is temperature-dependent (Zulkifli et al., 2023). The heat shock response occurs at the cellular level during the acute phase, whereas the reprogramming of gene expression and metabolism during the chronic phase leads to acclimatization to the stressor (Horowitz, 2022). According to Collier et al. (2006), ruminants have a decrease in productivity during the acute period and regain it after acclimating to the stress. Variations in temperature tolerance account for the species-specific differences in *HSP70* (Silanikove, 2020).

The expression level in Red Sokoto goats was found to be comparatively lower, despite the fact that the current investigation found a similar pattern of expression in WAD and Red Sokoto goat breeds. While WAD is a species from rain forests and is somewhat less suited to heat stress circumstances, Red Sokoto

is a breed from desert regions and is highly adaptable to heat stress. Additionally, there are important reasons to use our findings that showed breed-specific variations in heat stress in ruminants, which can suppress metabolism, reducing body heat production and increasing its efficient dissipation (Silanikove 2020; Kadzere et al., 2022).

It has been proposed that in goats from tropical regions, *HSP70* expression was considerably higher in the summer than in the winter, which may be crucial for their ability to withstand extreme weather conditions and thermal stress (Dangi et al., 2012). Heat stress alleviation starts at the cellular level, where a number of molecules interact, such as heat shock gene transcription factor 1 activation, which binds to the promoter region of heat shock elements (HSE) of the HSP genes and positively correlates to increased expression of heat shock proteins (Ruell et al., 2019).

The current result is consistent with research conducted on goat kidneys (Zulkifli et al., 2020), caprine PBMCs (Dangi et al., 2022), bovine lymphocytes (Ptir et al., 2020, Mishra et al., 2020), and bovine PBMCs (Kishore et al., 2023). The aforementioned findings showed a favorable correlation between the degree of heat stress as measured by THI measures and the expression levels of the *HSP70* and *HSP90* genes.

CONCLUSION

According to the current findings, Red Sokoto goats are more heat-tolerant than WAD goats, and the genes *HSP70* and *HSP90* may one day be used as genetic biomarkers to choose animals that are climate-resilient (Shannan's information index I = 0.55).

Conflict of Interest Statement

The authors have declared that there are no competing interests.

Authors Contribution

All authors contributed equally to the conception, design, and preparation of this manuscript.

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