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Polymorphism of HSP-70 Gene in Extensively Reared Indigenous Goat Breeds

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ABSTRACT
Heat shock protein (HSP) 70 gene is a member of the HSPs sub-family. When animals are subjected to thermal stress, they act as molecular chaperones, providing cellular protection, immune response, protein synthesis, protein folding and unfolding, protection proteins from cellular stress, inhibitory apoptosis and adaptation. Genetic variation is strongly associated with heat stress responses, including variants of heat shock proteins (HSPs), which are required for thermoregulation and stress resistance. Heat shock proteins are highly conserved proteins that are expressed during stress and play an important role in adaptation to environmental stress. The current study was carried out to assess the adaptive capability of different goat breeds (Capra hircus), namely West African Dwarf and Red Sokoto goats, during the peak dry winter. For this purpose, specific primers were used to test the targeted gene (HSP70). PCR, RFLP, and agarose gel electrophoresis were used to determine the expression of the HSP70 gene. Allele frequencies were calculated using gene counting, and the Hardy-Weinberg equilibrium was determined using the Chi-square test. According to this study, the expression of the HSP70 gene was almost identical in RS and WAD goats, implying that RS and WAD goats had the highest genetic similarity (0.9996) and the lowest genetic distance (0.0004). HSP70 may be a potential molecular biomarker in the future for the selection of climate resilient animals.
Keywords: HSP70, Adaptation, Expression, Heat Stress, Heat Tolerance

Introduction

Goats are found in a variety of ecosystems and are thought to be more resistant to extreme weather conditions due to their metabolic size and water conservation capacity [16]. Heat stress occurs when goats are exposed to ambient temperatures above the upper critical limit [20]. Goats are more resilient and adapt to different higher environments by expressing various adaptive strategies [14], and they generally use their thermoregulatory mechanism to relieve stress. Heat shock proteins (HSPs) regulate cellular tolerance to heat stress, and these proteins are responsible for maintaining the organism's balance and acclimating to the stress [9].Inducible HSPs are released intracellularly and extracellularly in response to various environmental stresses [17] & [5] and can be used to detect stress in cells [17]. HSP production regulation is critical to cell survival, and among HSPs, HSP70 plays an important role in cell thermo-tolerance and animal survival [6]. Understanding the cellular regulation of heat stress and the expression pattern of the HSP70 gene will shed light on the mechanism of heat stress adaptation in goats. Because this protein plays numerous roles at the cellular and tissue levels, Hsp70 gene expression has been found to be positively correlated with variations in thermo tolerance in various organisms. The current study was therefore carried out to examine heat shock protein 70 gene expression in different WAD and RS goat breeds during the winter season in Nigeria's South Western Zone.

Materials and Methods

Experimental Design

This study was conducted in the laboratory of the Federal University of Technology Akure, Nigeria from December 2019 to February 2020. Ninety-five (95) adult goats comprised of 45 WAD and 50 RS goats extensively reared by local breeders from different areas of Osun, Oyo and Kwara State respectively were used. The observations on meteorological variables (relative humidity and temperature) were collected and temperature humidity index (THI) was calculated.

Sampling and DNA extraction

Samples (blood samples) were collected from the jugular vein in 10 mL tubes containing EDTA as an anticoagulant. They were stored at -20 °C until DNA extraction, which was conducted according to [12]. It was run on a gel electrophoresis, and the

quality and quantity of the extracted DNA was determined using Nano-Drop as described by [11].

PCR Amplification

Amplification was performed using the primer HSP70-F 5- TGGCGAAAAACATGGCTATC -3 and HSP70-R 5- CTAATCCACCTCCTCAAT -3 [10] at the laboratories of "Macrogen Inc"/South Korea. The amplification reaction was 2µL, including 1µL of template DNA (75 ng), 1 µL of from each forward and reverse primer, 12.5 µL of master (Promega M7502) mix (2x) and 9.5 µLDNase (free water). The PCR conditions were as described by [23] initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 30s (determined gradually), extension at 72 °C for 1 min, and a final extension at72 °C for 10 min, with a ladder (EnzyQuest/SKU: NM018S) of 8 kb base pairs of DNA. Ethidium bromide (1%) was used as a detection method for the PCR products. The ratio of 260/280 was approved in 1.75–1.85 as the best quantification ratio for DNA samples [3].

Statistical analysis

The analysis was performed in duplicate. Results were expressed as the Mean±SE. A difference with value $P<0.05$ was considered statistically significant. The different group mean were compared by student's' test by the SPSS Version 16.0.1 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

The results showed good evidence of molecular marker linked to two Nigerian goat breeds (West African Dwarf and Red Sokoto goats) considered. Figure 1 revealed the results of electrophoresis gel for the primer HSP 70 gene. The 1-45 samples represent West African Goat while 46-95 represent Red Sokoto goats in all, 95 samples were amplified and their various sizes of bands were 400, 300 and 200 base pairs.

HSP 70 Genes

A PCR – RFLP techniques was used to genotype and detect polymorphisms of HSP 70 genes in two breeds of Nigerian goats. Using the specific primers designed from heat adaptation genes of goats, the PCR of all tested goats DNA (as animals i.e 45 WAD & 50 Red Sokoto goats) for HSP 70 gene gave specific amplified fragments at the expected band size (300-bp) in two breeds of goats. The ladder with thick bands are upregulated (23 ladders for WAD and 20 RS goats) for HSP 70, they have two bands meaning that they are alleles of the same genes while one band indicates single allele and downregulated (22 ladders for WAD and 30 RS goats). It could be deduced that those animals with thick ladders or bands used HSP 70 genes for adaptation. HSP 70 gene revealed genotype AC as highest in WAD and genotype CC as the highest in Red Sokoto breed of goats.

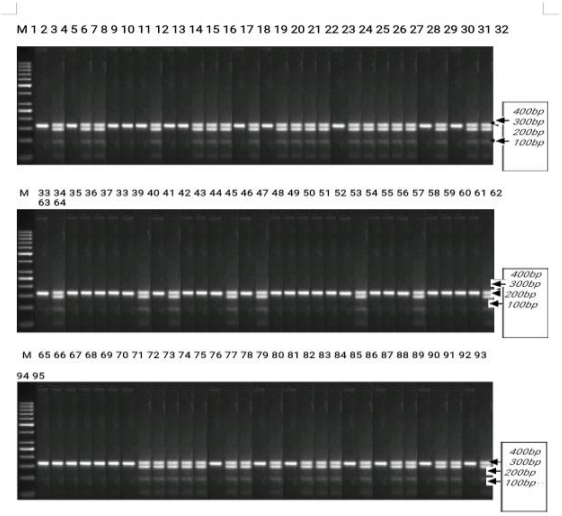

Fig 1: Electrophoresis Gel for the DNA of HSP-70 Gene in WAD and RS Goats

Environmental Conditions Prevailing During the Trial

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

Table 1: Environmental Conditions Prevailing During the Trial

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

Mean±SE = Standard error of mean

In Table 2, genotype frequencies of genes in Nigerian indigenous WAD for HSP70 gene revealed genotypes AA, AC and CC and HSP 70 gene for RS goats revealed genotypes AC and CC. Genotype AA for WAD had frequency of 0.02 (2%) and that of RS goat was not observed, genotype AC had 0.51 and 0.44 frequencies for WAD and RS goat respectively while that of CC were 0.46 and 0.56 respectively. Alleles frequency of A and C of HSP70 are presented in Table 2, For allele A in WAD and RS goats, the value were 0.27 and 0.22while the mean values of allele C in WAD and RS goats were 0.72 and 0.78 respectively.

Table 2: Genotype and Allele frequencies of genes in Nigerian indigenous WAD and RS goats

Marker	Genotype	WAD (n=45)	RS (n=50)
HSP-70	AA	0.022	0.00
	AC	0.511	0.440
	CC	0.467	0.560
	Allele		
HSP-70	A	0.2778	0.2200
	C	0.7220	0.7800

WAD = West African Dwarf goat, RS = Red Sokoto goat, HSP 70 AA = Homozygous genotype, AC = Heterozygous genotype, CC = Homozygous genotype.

Tables 3 show the summary of the genetic variation statistics with two breeds of goats, that is WAD and RS respectively. It showed that the observed number of alleles for all loci HSP 70 was the same but the effective number of alleles differed. The effective number of alleles in the WAD and RS goats was 1.67 and 1.52 respectively. Shannon information index for WAD and RS goats were 0.59 and 0.52 respectively. It showed that there were differences in genetic diversity of both breeds.

Table 3: Summary of Genetic Variation Statistics in WAD and RS Goats

Marker	Sample Size	Na	Ne	I
WAD HSP-70	45	2	1.6701	0.5908
RS HSP-70	50	2	1.5225	0.5269

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

The average expected heterozygosity varied from one marker to the other and from one breed of goat to another. It was revealed that the expected heterozygosity for WAD goats were 0.40 while RS goats were 0.34 respectively in Table 4. The value of observed heterozygosity obtained in this study were 0.51 and 0.44 for WAD and RS goats and indicated genetic variation in trans HSP locus in both WAD and RS goats, meaning that selection programme if carefully planned and executed will result in genetic gain towards improved performance in the selected population

Table 4: Summary of Heterozygosity for all loci in WAD and RS Goats

Marker	**Sample size**	**Ho**	**He**	**Average heterozygosity**	**Nei**
WAD	45	0.5111	0.4057	0.3722	0.4012
RS	50	0.4400	0.3467	0.3722	0.3432

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

Table 5 shows the summary of F-statistics and gene flow for all loci while Table 6 shows the genetic identity and genetic distance between WAD and RS goats. Nei's genetic distance was 0.0004 which suggested a low level of genetic

differentiation among pairs of populations. The level of genetic variation in WAD and RS goats should theoretically be relatively low within populations and high between populations. In this study, the low genetic distance observed from WAD/ RS (0.0004) further suggested a high level of genetic identity within and between these populations.

Table 5: Summary of F-Statistics and Gene Flow for All Loci

Locus	Sample Size	Fis	Fit	Fst	Nm*
HSP-70	95	-0.2776	0.2719	0.0045	55.75

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

Table 6: Genetic Identity and Genetic Distance Between WAD and Red Sokoto (RS) Goats

Population	WAD	RS
WAD	-	0.9996
RS	0.0004	-

Above diagonal: Nei's genetic identity, Below diagonal: Genetic distance, WAD = West African Dwarf goat, RS = Red Sokoto goat.

DISCUSSION

Genotype frequencies of genes in Nigerian indigenous goats revealed that genotype frequencies of AA, AC, and CC were identified for the HSP 70 gene. Genotype AA had a frequency of 0.02 (2%) for WAD and was not observed for RS goat, genotype AC had 0.51 and 0.44 frequencies for WAD and RS goat, respectively, while genotype CC had 0.47 and 0.56 frequencies. This demonstrated how genotype frequencies change over time. Evolving populations investigate the genotype frequency space [19].

The mean values for allele A WAD and RS goats were 0.28 and 0.22, respectively, while the mean values for allele C WAD and RS goats were 0.72 and 0.78. This showed that allele C in both breeds is very active during heat stress. HSPs play a role in cell cycle regulation, signaling, and apoptosis prevention. During and immediately after hyperthermia, HSPs become the most abundant proteins produced by cells [7]. Surprisingly, most HSP genes lack introns [7], which could explain how they can be produced in the presence of RNA splicing disruptors like heat.

In this study, the HSP 70 heterozygosity values for WAD and RS goats were 0.51 and 0.44, respectively. The expected heterozygosity for WAD and RS goats were 0.40 and 0.34, respectively.

These variations or differences in expected heterozygosity may be attributed to differences in population structure as well as goat breeds. This indicated the existence of an isolate breaking effect (the mixing of two previously isolated populations) [3]. The observed heterozygosity in this study could be explained by overlapping generations, mixing of populations from different climatic regions, natural selection with heterozygosity in mind, or subdivision with genetic drift, according to [23].

The summary of the genetic variation statistics within breeds of goat

The observed number of alleles for the HSP 70 gene was the same, but the number of effective alleles varied. The effective number of alleles in WAD goats was 1.67, while in RS goats it was 1.52. It was possible to conclude that the effective number of alleles for WAD was greater than the mean value of RS (1.52). This demonstrated that the genetic diversity of both breeds differed Sharman's Index of Information [8].

The inbreeding coefficient (Fis) for the two goat breeds revealed a low level of heterozygosity, implying that a high level of homozygosity would have resulted. This could be the reason for the lack of expected heterozygozity in both goat breeds in this study. The Fis value was -0.278 and the fixation index (Fst) in this study is low in the two breeds cite that there is inbreeding [13].

WAD and RS goats had the highest genetic identities (0.9996) and the smallest genetic distances (0.0004). The genetic distance between Nei populations was 0.0004 indicating a significant low genetic difference and loss of heterozygosity. WAD and RS goats should have a high proportion of genetic variation between populations but a low proportion within populations. The size and signage of Fis reflect its departure from Hardy Weinberg. Equilibrium of genotypes, such that when Fis is zero, the locus is in HWE, and when Fis is positive, there is a heterozygosity deficiency.

A negative Fis score indicated that the level of heterozygote was higher than predicted by HWE [21]. According to the findings of this study, small populations have a high level of inbreeding. However, population selection against inbred individuals could explain these findings [22]. It should be noted that Msdata are more accurate than pedigree data when estimating Fis [4] & [2].

Increased levels of Fis in sheep, according to [21], could be attributed to smaller population sizes, higher levels of selection pressure, or an incorrect measurement method. Similarly, the Fis value in this study matched the Fis value in [23], which ranged from -0.02 in hybrid to -0.017 in Cashmere.

The Fis value in this study matched that of [13], who discovered a population with a negative Fis value, indicating low inbreeding. It also revealed that there is little genetic differentiation within the Saanen, Toggenburg, and British Alpine populations, as well as between the three breeds. This study's Fit (-0.31) and Fis (-0.32) values were compared to range values for five Sicilian sheep breeds (0.08 and 0.03) and Swiss goat populations (Fis 0.014) [25] &[24]. There was a lot of genetic variation in all breeds, with heterozygosity rates above 60%.

Conclusion

In this study, it was discovered that WAD and RS goats had high genetic similarity, low genetic distance, low percentage gene differentiation, and loss of heterozygozity in the studied population.

HSP 70 gene expressions is employed as a marker for heat tolerance in a variety of species, and the information gathered could be useful in improving animal breeding systems' ability to adapt to environmental changes. In both WAD and RS goats, gene expression analyses for the HSP-70 genes should be performed.

More works should be done to amplify the complete sequence of the HSP-70 genes in WAD and RS goats

The low Fst value showed little population difference across and among the experimental animals (breeds) [13]. The individual's mean inbreeding coefficient relative to the subpopulation (Fis) was -0.3185, indicating the presence of heterozygosity excess within goat populations; however,

more research is required to confirm this. The high level of gene flow (Nm) supported the low level of genetic differentiation (0.0030), implying possible population mixing [10].

The 0.0004 genetic difference between Red Sokoto and West African Dwarf goats (table5) was smaller than [1] 0.39 and [11]'s 0.27 for the same breeds. This could have been due to the animals being sampled from a broader geographical area [12].

so that other polymorphisms in the full sequence can be investigated.

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Competing Interests

Authors have declared that no competing interests exist.

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