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Research Article

Expressions analysis of Heat Shock Protein Genes in Nigerian indigenous Goats exposed to Heat Stress

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Abstract: Heat shock proteins (HSPs), also known as molecular chaperons are prominent stress markers, and can be expressed during stress or when animals are under stress. The present study was conducted to evaluate the adaptive capability of different goat (Capra hircus) breeds, i.e. WAD and Red Sokoto under heat stress. The targeted genes HSP70 and HSP 90 were evaluated for this purpose using specific primers. The expression of HSP70 and HSP 90 genes were estimated by PCR and RFLP respectively. The expression of HSP70 and HSP 90 genes were found to be almost identical in two breeds of goats with slightly higher expression observed in WAD goat (Na = 1.62, I =0.57) as against RS (Na =1.59, I =0.55) implying that Red Sokoto goats were more adapted during Heat stress. The results also indicated HSP 70 gene was used mostly for protection against heat stress. These results indicated that, during adverse climatic stress the quantum of expression (HSP70 and HSP 90 genes) were more in WAD. It concluded that Red Sokoto breed was better adapted to heat stress than WAD and HSP70 and HSP 90 genes may be a potential molecular biomarker in the future for selection of climate resilient animals. These genes (HSP 70 and 90) should further be associated with thermo-tolerance traits to unravel their possible effect on thermal-tolerance performance, adaptability and susceptibility of different Nigerian WAD and Red Sokoto goat breeds to environmental stress load and thermal assaults of tropical conditions.

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INTRODUCTION

Goats are distributed in different ecology and supposed to be more tolerant to extreme weather conditions because of their metabolic size and water conservation capacity (Silanikove 2000). Exposure of goats to ambient temperatures above the upper critical limit results in heat stress (Yousef 1985). Goats are more resilient and adjust to different higher environment by expressing different adaptive strategies (Silanikove et at. 2015) and generally utilize its thermoregulatory mechanism to relieve from stress. Cellular tolerance to heat stress is regulated by heat shock proteins (HSPs) and these proteins are responsible for maintaining the balance in organism and to acclimatize the stress (Morange 2006). HSPs are released intra cellularly and extra cellularly in response to various environmental stresses (Sonna et al. 2002, Hecker et al. 2011) in inducible form and can be an indicator of stress in cells (Sonna et al. 2002). The regulation of HSP production is critical to cell survival and among the HSPs, Hsp70 has a significant role in cell thermo-tolerance and animal survival (King et al 2002). Understanding the regulation of heat stress at cellular level and expression pattern of Hsp70 and HSP 90 genes will throw light on the mechanism of heat stress adaptation in goats. Hsp70 and HSP 90 genes expression have been positively correlated with variations in thermo tolerance in different organisms since these proteins play a multi various roles at the cellular and tissue levels. The present study was therefore carried out to detect heat shock protein 70 and 90 genes expression in different goat breeds in South Western Zone of Nigeria.

MATERIALS AND METHODS

The present study was carried out on 45 WAD and 50 Red Sokoto goat breeds in South Western 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 December–February 2023. 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. The observations on meteorological variables (relative humidity, temperature) were collected and temperature humidity index (THI) was calculated.

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 -20^{o}C 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 sprimer sequences were stated below;

Tabel 1 Marker HSP70, HSP90 used in study

Marker		Primer
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). 15 microliters 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 94^{o}C, 40 cycles of the following reaction were performed: denaturation at 94^{o}C for 30 seconds, annealing at 62^{o}C for 30 seconds, and extension at 72^{o}C. At 72^{o}C, the final extension was done for 2 minutes. 10µL of the PCR amplication 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 size 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 (Figures 1 and 2). 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 (un-weighted pair group method using arithmetic averages). The strength of clusters was assessed using Boot program and bootstrap methodology.

RESULTS AND DISCUSSIONS

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 were up regulated and those animal 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 were up regulated for HSP 70 and these ladders are alleles of the same genes while thin /one band indicates single allele and down regulated. It could be deduced that those animals used more of HSP 70 genes for adaptation than HSP 90 genes.

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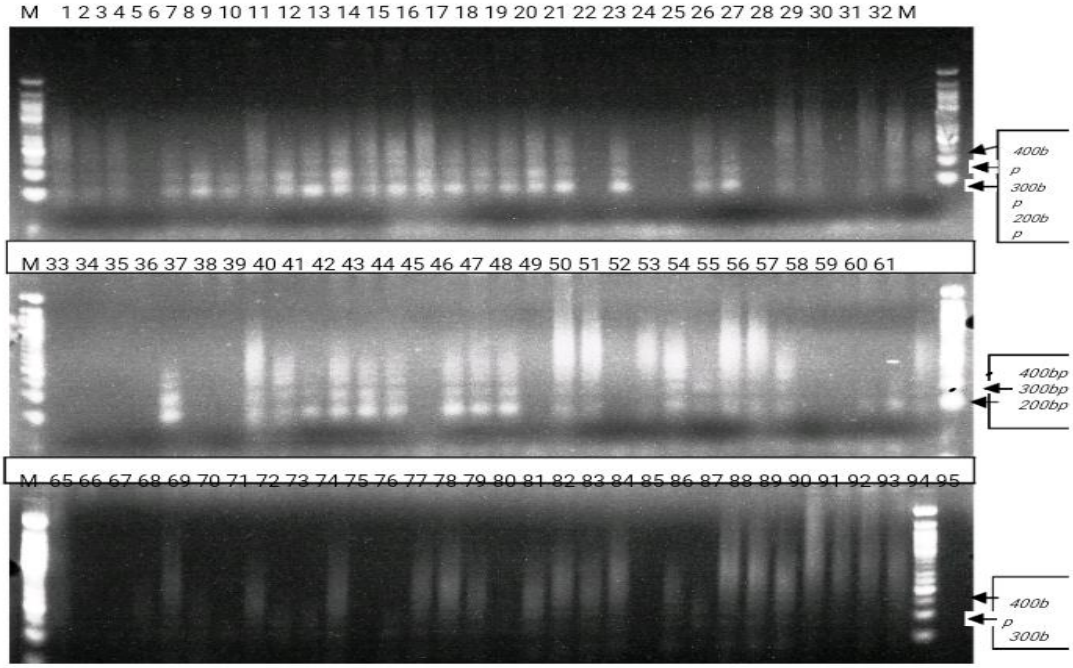

Figure 1: Electrophoresis gel for DNA of HSP-90 gene in WAD and RS goats

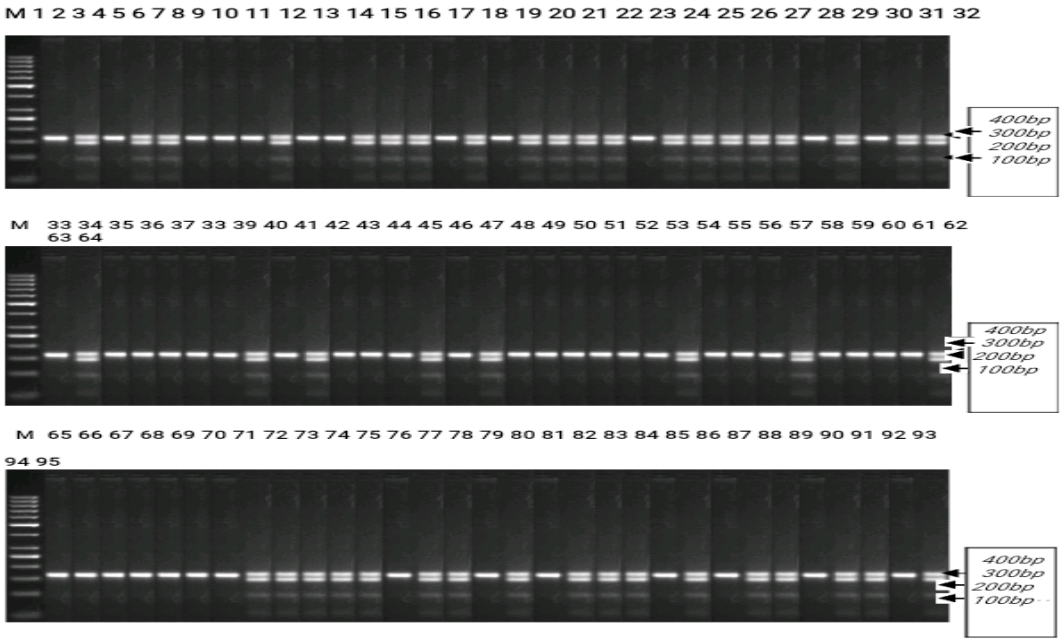

Figure 2: Electrophoresis gel for the DNA of HSP-70 gene in WAD and RS goats.

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 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 [Kimura and Crow (1964)]; I = Shannan's Information index [Lewontin (1972)]

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 were 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 5 and 6). 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 population, 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

Gene expression analysis in different goat breeds under heat stress was the subject of this study. The analysis of results revealed that THI varied from 74.44 – 94.54, indicating that the animals were under stress during the experiment period (Table 1). Relative expression of HSP90 and 70 genes varied amongst the two goat breeds and its expression pattern indicated that WAD goats had highest expression of HSP90 and 70 genes and lowest in Red Sokoto. The Red Sokoto goat had lowest expression pattern indicating better adaptive capability during heat

stress period. These results depicts that WAD goat breed was more sensitive and Red Sokoto goat was most adaptive to heat stress condition.

The Heat Shock Protein (HSP) genes including HSP70 and HSP 90 are members of HSPs sub-family (molecular chaperone families) known to be highly expressed under stressful environmental and physiological conditions. These facilitate responses to environmental heat loads above thermo-neutral zones in animals through intra and extracellular signals that coordinate cellular and whole animal metabolism (Collier et al., 2008). Also, the genes regulate cellular homeostasis and folding-unfolding of damaged proteins during thermal assault thereby conferring on stressed animals the adaptive capacity to cope under stressful environmental conditions (Kapila et al.2013). Through over expression during HS, the HSP genes provide a mechanism for protecting the animal against hyperthermia, circulatory shock and cerebral ischemia (Lee et al., 2006; Collier et al., 2008). In particular, the HSP 90 gene is essential for providing cellular protection (cyto-protection), immune response, protein synthesis, cyto-skeletal protection, protein translocation and regulation of steroid hormone receptors, transportation, re-folding of protein, protection proteins from cellular stress, inhibitory apoptosis and adaptation during and after thermal assault (Kapila et al., 2013; Sodhi et al.,2013b).

The HSP 90 gene has been shown to provide genomic basis for thermo-tolerance selection among tropical animals under thermal assaults. These findings are corroborated with the view of Patir et al. (2007) and (2010) who reported that heat stress is responsible for HSP70 expression in bovine lymphocytes. The higher expression of HSPA6 has also been reported in human blood in response to heat stress (Sonna et al. 2002, 2004) and similar observation has been reported in camel indicating that the temperature change affects the level of expressed HSP70 (Garbuz et al. 2011, Ulmasov et al. 1993). Stress tolerance is a critical characteristic and its methodology is not fully understood (Mizzen et al. 1988).

The findings of the present study showed that expression of HSP70 and HSP 90 genes was breed specific and confirms the view of Dangi et al. (2012), who reported that in Indian conditions, tropical goats have showed significantly higher HSP70 mRNA expression in PBMCs during peak summer season compared to the winter season. On contrary, the goats of temperate region did not show significant levels of HSP70 transcriptional response between peak summer and winter season (Dangi et al. 2012). In yet another study involving heat stress of goat PBMCs in vitro showed significantly higher up-regulation of HSP70 mRNA compared to unstressed cells (Mohanarao et al. 2014). The genetic diversity of HSP 90 gene has been reported to confer a better thermo-tolerance, adaptability, survivability, longevity advantage and increased ability to respond to thermal stress in animals (Singh et al., 2006; Kapila et al., 2013; Sodhi et al., 2013b). The differences among various genetic variants of HSP 90 gene as revealed by differential plot is an evidence of the presence of genetic diversity detected within different genetic groups HSP 90 gene (Bester, 2012; Gori et al., 2012; Yang et al., 2016).

The detected genetic variants of HSP 90 gene could be interrogated as veritable genetic resource for improvement programme of thermo-tolerance, adaptability and survivability advantage to cope with wide range of thermal stress and environmental variations especially in the hot humid tropics (Schwerin et al., 2002a; Kishore et al., 2013) as well as disease tolerance and drug resistance of animals under thermal stress (Zhang et al., 2002b; Aufricht, 2005; Singh et al., 2006). Stress is the result of environmental forces continuously acting upon animals which disrupt homeostasis resulting in new adaptations that can be detrimental or advantageous to the animal (Stott 1981). Among the stressors, heat stress has been of major concern in reducing animal's productivity in tropical, subtropical, and arid areas (Silanikove et al. 1997). The ability

of animal to acclimatize and produce under the specific climate condition signifies the adaptation to a particular environmental niche.

Hsp70 concentration in blood is also a reliable indicator of chronic stress in feedlot cattle (Gaughan 2013). There is considerable evidence that the synthesis of Hsp70 is temperature-dependent (Zulkifli et al. 2003) and thus Hsp70 responses could be considered as a cellular thermometer. The acute phase includes the heat shock response at the cellular level and the chronic phase results in acclimation to the stressor and involves the reprogramming of gene expression and metabolism (Horowitz 2002). In ruminants, there is a loss in productivity as animals pass through the acute phase and return to productivity as they undergo acclimation to the stress (Collier et al. 2006). The species specific difference in HSP70 is due to variation in thermal tolerance (Silanikove 2000, Hightower et al. 1999). Although the present study identified similar pattern of expression in WAD and Red Sokoto goat breed but the expression level in Red Sokoto goats was observed to be relatively lower. Red Sokoto is a breed of desert regions and is highly adapted to heat stress whereas WAD is a breed of Rain forest regions and is comparatively less well adapted to heat stress conditions. There are also considerable points to leverage our findings that portrayed differences between breeds with regard to heat stress in ruminants which has the ability to subdue the metabolism thereby negatively influencing the body heat production and augmenting its effective dissipation (Silanikove 2000, Kadzere et al. 2002). It has been suggested that the expression of Hsp70 was significantly higher during the summer season as compared to the winter in tropical region goats, which might play an important role in thermal stress tolerance against harsh environmental conditions (Dangi et al. 2012). Amelioration of heat stress begins at the cellular level, where there is an interplay of various molecules including activation of heat shock transcription factor 1 that positively correlates to an increased expression of heat shock proteins by binding to promoter region of heat shock elements (HSE) of the HSP genes (Ruell et al. 2009). The present finding was in agreement of studies carried out in bovine lymphocytes (Ptir et al. 2010, Mishra et al. 2010), in bovine PBMCs (Kishore et al. 2013), in caprine PBMCs (Dangi et al. 2012), and in kidneys of goats (Zulkifli et al. 2010). The above results indicated that the expression level of HSP70 and HSP 90 genes were positively correlated to the level of heat stress based on THI parameters.

CONCLUSION

Based on the present results, it concluded that Red Sokoto breed is better adapted to heat stress than WAD goats and HSP70 and HSP 90 genes may be a potential molecular biomarker (Shannan,s information index I = 0.55) in the future for selection of climate resilient animals.

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