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**Tropical Animal Health and
Production**

ISSN 0049-4747


Trop Anim Health Prod
DOI 10.1007/s11250-020-02222-9



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Single nucleotide polymorphisms at heat shock protein 90 gene and their association with thermo-tolerance potential in selected indigenous Nigerian cattle

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Received: 12 September 2019 / Accepted: 17 January 2020
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Abstract

Heat shock protein (*HSP*) 90 gene provides protection and adaptation to thermal assault and certain polymorphisms have been associated to heat tolerance in humans and animals. Single nucleotide polymorphisms (SNPs) of *HSP* 90 gene were used to evaluate the scientific basis of heat tolerance in four zebu breeds of Nigeria. The DNA was extracted from skin tissue of 90 adult bulls representing White Fulani (WF), Sokoto Gudali (SG), Red Bororo (RB), and Ambala (AM). The SNPs were determined in DNAs using PCR, sequencing, and visualization and bio-editing by chromatogram in SeqMan Ngen tool. Subsequently, respective genotypes were constructed and genotypic and allelic frequencies were computed. Also, body parameters related to heat stress (HS) including body temperature (BT), rectal temperature (RT), and respiratory rates (RR) were taken for each animal before biological sampling and heat tolerance coefficient (HTC) was calculated. We detected four SNPs distinct/specific for each breed as follows: change from thymine (T) to guanine (G) at position 116 (T116G) in RB, G to cytosine (C) at 220 (G220C) in SG, G to adenine (A) at two positions, 346 (G346A) and 390 (G390A) in AM and WF, respectively. Heterozygous SNPs showed significantly lower values ($P < 0.0001$) for BT, RT, RR, and HTC than homozygous genotypes at all positions. We hypothesize that animals with heterozygous SNPs in exon 3 of *HSP* 90 may be tolerant to HS. These SNPs can be used as bio-markers for screening large populations of cattle for tolerance to hot tropical conditions in Nigeria and other sub-humid places.

Keywords Heat stress · Insertion-deletion · SNP variants · Transversion · Zebu

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Introduction

Cattle are the most important among livestock species of Nigeria and there is a huge bio-diversity of these animals (Babayemi et al. 2014; Pagot 1992). The most prominent indicine cattle in Nigeria include White Fulani (WF), Sokoto Gudali (SG), Adamawa Gudali (AG), Red Bororo (RB), Ambala (AM), Wadara, Azawak, Muturu, Keteku, N'Dama, and Kuri. The WF Fulani animals (locally known as Bunaji) are the majority (about 37%) and most widely spread local breed (Meghen et al. 1999). The WF breed is valued for genetic predisposition of hardiness among its animals and it is superior to other breeds in terms of diseases resistance, heat tolerance, ability to thrive under varying thermal environment, and adaptation to local conditions (Alphonsus et al. 2012; Blench et al. 1998). The Gudali animals (SG and AG) together form 32% of the national cattle herd of Nigeria and are predominantly found in the north-western part (NNLRS 1999). The SG (locally known as Bokooloji) is named after the place where it is believed to be its origin, Sokoto. The RB (Rahaji) is another prominent cattle breed and constitute 22% of total herd. These animals are rarely found beyond Kaduna in North-Central in the wet season except for the isolated population on Mambila Plateau in North-East Nigeria (Meghen et al. 1999). Regarding the phenotypic characteristics of zebu cattle of Nigeria, most of them have large and well-developed humps, small navel flaps, up curving or lyre-shaped medium length horns, some have long-thick horns, majority have pendulous ears, and the colors are multiple and variable (Katie and Alistair 1986; Williamson and Payne 1990). In terms of the body size, most of the indigenous cattle of Nigeria are small to medium with adult live weight of between 200 and 350 kg, the RB animals being moderately larger compared to the rest of the breeds (Katie and Alistair 1986; Williamson and Payne 1990). With respect to socio-economic importance, most of them are multi-purpose depended for beef and milk production as well as draught animals (Kubkomawa 2017). Furthermore, these animals are adapted to arid and semi-arid regions with varying levels of tolerance to humidity-related disease and poor nutrition (Blench et al. 1998; Kubkomawa 2017; Yakubu et al. 2015).

Elsewhere, the African indigenous cattle are believed to possess inherent ability to withstand various diseases, heat stress (HS) and feed scarcity compared to crossbreds (zebu/sanga \times exotic breeds) or imported exotic breeds or *Bos (B.) taurus* (Hansen 2004; Maule 1990; Mattioli et al. 2000; Porter 1991). For example, the West African N'Dama cattle have been shown to be tolerant to trypanosomiasis (Kim et al. 2017). In Kenya, the Small East African zebu (SEAZ) were reported to be resistant to *Rhipicephalus (R.) appendiculatus* ticks (Latif and Pegram 1992) and to survive well in environments with poor quality forage, water scarcity, and high temperatures (de Clare Bronsvooort et al. 2013; Western and Finch 1986). In Tanzania, the Tanzania shorthorn

zebu (TSZ) animals are believed to be tolerant to ticks, *Theileria parva (T. parva)* parasites, and East Cost Fever (ECF) disease (Chenyambuga et al. 2008; Laisser et al. 2014). However, the scientific basis of the strength of these animals has not been studied explicitly and the inherent capability to HS claimed by many African researchers has not been reported extensively.

There is an increasing impact of HS on cattle and other livestock species (West 2003) leading to poor performance in several traits of economic importance such as reduced fertility, increased respiratory and heart rates, profuse sweating, reduced milk production, and lower milk quality (Kishore et al. 2013). Consequently, a significant loss of income and increase in management costs arise. Losses estimated at \$900 million in the cattle industry per annum have been associated to heat stress (Öner et al. 2017). The concern of HS has become a major issue particularly during recent years when climate change effects are negatively impacting livestock adaptability and survivability and therefore it should be addressed (West 2003). Nigeria is among the tropical countries with severe negative influence of thermal stress on livestock.

The heat shock protein (*HSP*) 90 gene is a member of molecular chaperone sub-families that are known to be critical to thermo-regulation in animals (Onasanya et al. 2017). The genetic variation in *HSP* genes among breeds and the central role that *HSP* 90 gene play particularly in coordinating thermal tolerance suggest that *HSP* 90 gene is a candidate gene for identification of genetic markers and that there is an opportunity to improve thermal tolerance through marker-assisted selection (Basirico et al. 2011). The *HSP* 90 gene is also involved in regulation of cellular homeostasis and folding-unfolding of damaged proteins during thermal assault or any other physiological stress, thereby conferring on stressed animals the adaptive capacity to cope under stressful environmental and physiological conditions (Kapila et al. 2013). Moreover, the expression of *HSP* genes is related to thermal stress and plays vital roles in cell response to environmental stress (Lindquist 1986; Morimoto 1993). Several single nucleotide polymorphism (SNP) variants have been reported in the bovine *HSP* 90 gene. These may be exploited and used to improve thermo-tolerance traits in cattle and to drift heat vulnerable stocks toward superior thermo-tolerant ability. The variants may also become important tools for managing livestock in the face of climate change with the goals of more efficient resource utilization and improved production and reproduction performances (Kapila et al. 2013; Sodhi et al. 2013). To the best of our knowledge, no feasible effort has been made to study the *HSP* genes in livestock especially cattle in Nigeria. For this reason, the aim of this study was to evaluate the SNP polymorphisms at *HSP* 90 gene and find their influence on the thermo-tolerance behavior in Nigerian zebu cattle breeds.

Materials and methods

Animals, measurements of heat-related parameters, and biological sampling

Four local zebu cattle breeds of Nigeria namely WF, SG, RB, and AM were involved in this study. These are the major zebu cattle breeds found in northern parts of Nigeria where random sampling was done. A total of 90 adult bulls/animals representing these breeds and including 25 (WF), 21 (SG), 21 (RB), and 23 (AM) were involved in this study. The animals are raised in traditional farming systems in northern Nigeria where they are sustained on natural pastures comprising of Stylo (*Stylosanthes gracilis*), Leucaena (*Leucaena leucocephala*), and Guinea grass (*Panicum maximum*) or fed crop residues after harvesting. Sampling was done on a designated day after being purchased by slaughterers and transporting them to the slaughter house. Before sampling, measurements of various traits related to HS in animals were taken and these included body temperature (BT), rectal temperature (RT), and respiratory rate (RR). While both BT and RT were measured using a digital thermometer which was placed in armpit or inserted into the anal pore, respectively, RR was measured by observation of flank movement (in breadth per seconds, bps). The measurements were conducted between 9 am and 3 pm during dry season (March to May) when the ambient temperature within and around the lairages ranged from 30 °C in the morning to above 40 °C in the afternoon (Nigeria Meteorological Agency). Within and around the lairages, wind speed ranged between 3.5 and 4.5 miles per hour, wind chill temperature was between 32.5 and 38.5 °C, and humidity was 24% (Nigeria Meteorological Agency). Care was taken not to compromise the animals' welfare by providing water, roof protection from scorching effects of sun intensity, and waiting for at least 48 h before the operations. However, feeds were not provided to adhere to the requirements for slaughter. To avoid terrifying the animals, the persons taking the measurements were allowed to stay with animals in the lairage for a considerable period of time and well ahead of slaughter (at least 3 h). This was important to ensure that the measurements were not compromised in the slaughter house environment. After the measurements, about 200 g piece of skin tissue was excised from the abdominal region immediately after slaughter and after bleeding. The skin sample was quickly sliced into ≤ 0.5 cm (or 1 g in weight) and submerged into 0.5 ml Eppendorf tubes containing RNAlater (QIAGEN, Netherlands). The tubes were packed in a dry iced-cool box (about 4 °C) and were transported to the laboratory at the Federal University Dutse, Jigawa State for storage within sampling day (between 3 and 10 h depending on distance). Sampling was carried out

for about 10 days to avoid sampling animals from the same location or related ones. The samples were stored at -20 °C before DNA extraction.

DNA extraction

The DNA was extracted from 1 g of the skin tissue according to the procedure in HiPurA™ Multi-Sample DNA Purification (MolBio™ Himedia®, Mumbai, India). The protocol starts with sample homogenization, series of centrifugations, incubation, and washing using HiElute Miniprep spin column with 2 ml collection tubes (MolBio™ Himedia®, Mumbai, India). During extraction, Proteinase K solution was added to the tissue to allow digestion of any proteins and increase purity of DNA. All incubations were done at temperatures between 55 and 70 °C for 20 to 40 min on ACCUBLOCK™ digital dry bath (Labnet International, Edison, NJ, USA) and this was important in completing digestion and increasing the volume of DNA. All centrifuges were done using Thermo Scientific Nanofuge (MCROCL 21/21R) micro-centrifuge (Waltham, USA) from 10,000 rpm to 13,000 for 1 to 3 min. Washing was done using 100% ethanol in the extraction process as well as dilute pre-wash and wash solutions at DNA washing. The DNA was obtained by adding 100 μ l of elution buffer directly to the column, incubating for 5 min at room temperature (15–25 °C) followed by centrifugation at 10,000 rpm for 1 min. Final DNA was incubated at 90 °C to free the DNA from any contamination and was subsequently stored at -20 °C until analyses. The quality (purity) and quantity of DNA were estimated using Thermo Scientific-NanoDrop 2000 Spectrophotometer (Shimadzu Co-operation, Kyoto, Japan) at absorbance ratio between OD₂₆₀ and OD₂₈₀ (OD_{260/280}). The DNA samples with absorbance ratio of 1.6–1.9 were considered enough for further analyses.

Polymerase chain reaction and sequencing

Polymerase chain reaction (PCR) was carried out in a final volume of 15 μ l containing 1.0 μ l of template DNA, 1.0 μ l of each of forward and reverse primers, 7.5 μ l of PCR Master Mix (2 \times) (GeNei™ Red Dye PCR Master Mix, Bangalore, India), and 4.5 μ l of nuclease-free water (MolBio™ Himedia®, Mumbai, India). Primers used in this study F-GCGTCATCACGTGTCATCTT and R-CCTCCTTTGGGGTTCCAGT were published earlier by Kumar et al. (2015) and targeted a span of 450 bp including the coding region in exon 3 of bovine *HSP* 90. Optimization was done for purpose of specificity to suit conditions of the current study. Amplification was performed in a TaKaRa Thermal Cycler Dice™ version III (Takara Bio Inc., Shiga, Japan) and involved 45 cycles of 94 °C (60 s) denaturation, annealing

at 65 °C for 45 s, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. The initial heating of the DNA was done at 94 °C for 5 min. The PCR products were visualized on 2% agarose gel electrophoresis after staining with 1 µg/ml ethidium bromide and were visualized under Bio-Rad Gel Doc™ XR+ Imaging System version 5.1 (Gel Documentation Molecular Imager, Bio-Rad Laboratories, Inc., California, USA).

Sequencing, determination of single nucleotide polymorphisms, and assessment of thermo-tolerance ability in the study animals

The PCR products were purified and subsequently sequenced using an automated ABI DNA Sequencer (Eurofins Genomics Pvt. Ltd., Bangalore, India). The nucleotide sequences were visualized and edited by chromatogram analyses on a SeqMan Ngen Tool (DNASTAR®, Inc., Madison, WI, USA). We aligned our sequences with nucleotide sequence of a similar region from unrelated *B. taurus* DNA obtained in NCBI to detect any variability. Sequence map showing a unimodal peak indicated the homozygous genotype and the overlapping peaks indicated heterozygous genotype and the total genotypes were obtained by direct counting. The favorable SNP genotypes were associated with body parameters to unravel the potential ability of the animals to withstand the negative effects of high heat.

Statistical analyses

The data were analyzed to obtain the averages for BT, RT, and RR. These were further used to calculate heat tolerance coefficient (HTC) using a linear equation according to Benezra's coefficient of adaptability (BCA) published in Benezra (1954). The HTC is a measure of adaptability of the animals to HS.

$HTC = RR/23 + RT/38.33$; where RR is respiratory rate (bpm), RT is rectal temperature (°C), normal rectal temperature is 38.33 °C and normal respiratory rate is 23 bpm.

Thereafter, statistical analyses were performed using the Generalized Linear Model (GLM) procedure of the statistical analysis system (SAS) Version 9.2 followed by Duncan's multiple range test to separate the means. The SAS model was arranged such that BT, RT, RR, and HTC were the dependent variables whereas SNP genotypes and the breeds were the two fixed variables.

$$Y_{ijk} = \mu + S_i + B_j + e_{ijk}; \text{ where,}$$

Y_{ijk} = observation of the thermo-related measurements;

μ = overall mean;

S_i = effect of i th SNP genotypes on thermo-tolerance measurements;

B_j = effect of j th breed on thermo-tolerance measurements traits; and

e_{ijk} = random error associated with Y_{ijk} observations for thermo-related measurements of the zebu cattle breeds.

Results

Single nucleotide polymorphisms in exon 3 of *HSP 90* gene in four Nigerian zebu cattle breeds

The sequenced region was found within coding region of exon 3 of bovine *HSP 90* and was approximately 450 bp (Fig. 1) between 826 and 1276 positions.

We observed a total of four (4) SNP variants at various positions in the target region, all of them were non-synonymous and leading to amino acid changes (Table 1). In addition, these SNPs were all base exchanges (substitutions), i.e., two transverse and two transition bases. The base exchange SNPs were thymine (T) to guanine (G) at position 116, the G to cytosine (C) at 220, and G to adenine (A) at two positions, 346 and 390, respectively (Fig. 2).

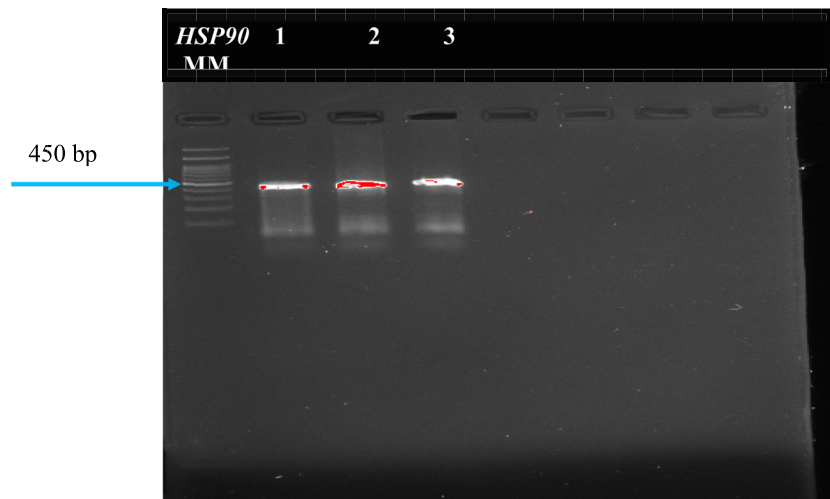
Genotypes and allelic frequencies of SNPs detected in the analyzed samples

From the detected SNPs, we processed the genotypes and calculated the resulting genotypic and allele frequencies. All positions were polymorphic in our DNA samples (Table 2). We were also interested in determining the response of samples to the Hard-Weinberg Equilibrium (HWE) shown by the probability (P) values presented in Table 2. We found that the SNP genotypes at three positions (116, 220, and 390) were in accordance with HWE while the genotypes at position 346 were not in agreement with HWE.

Effect of single nucleotide polymorphism genotypes on the body parameters in the study animals

We related the SNP genotypes to the body measurements collected on the animals before slaughter. Since the SNP genotypes were specific in the four breeds, the association was only considered in each breed. At site 116 with genotypes TT, TG, and GG in RB cattle, the heterozygotic animals (TG) showed lower values for BT, RT, RR as well as HTC. For example, the BT values were 37.910 ± 0.01 , 38.564 ± 0.01 , and 39.842 ± 0.10 °C, respectively, in TG, GG, and TT animals. The differences in body parameters were statistically significant ($P < 0.001$). Similarly, at the polymorphic locus 220 mainly in SG breed, the heterozygous animals (GC genotype) were shown to have lower values for BT, RT, RR, and HTC parameters compared to both homozygous animals. The genotypes at SNP 346G > A in AM samples were not in HWE ($P < 0.05$) and as such it was excluded from further analyses. With respect to the SNP genotypes at site 390, a similar trend can be

Fig. 1 Bands of amplified fragments of *HSP 90* gene (450 bp). MM, molecular marker; lanes 1–3 are representatives of amplified DNAs



observed (the heterozygote animals had lower values for all body parameters compared to both homozygotes). The variation was statistically significant ($P < 0.001$). These results were summarized in Table 3.

The relationships between breeds and thermo-tolerance ability among the study animals

Furthermore, we considered the differences between breeds in terms of temperature tolerance and we have shown that the WF and the RB breeds showed the lowest BT values, 38.243 ± 0.23 and 38.269 ± 0.17 °C, respectively, compared to the rest of the breeds. Moreover, the SG showed the lowest RT values than the rest of the breeds (37.841 ± 0.08 °C). With respect to the RR, again the WF and RB showed the lowest values, 102.929 ± 0.56 and 102.805 ± 0.4 bpm, respectively. There was a significant variation among the parameters with respect to the breeds. Results are summarized in Table 4.

Discussion

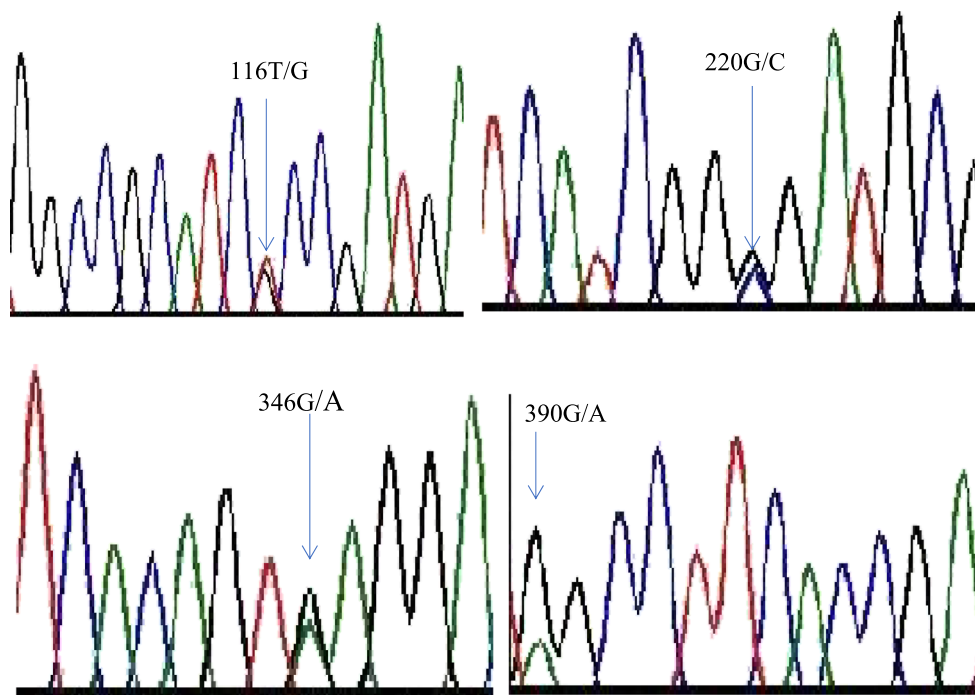
The molecular chaperons sub-families including *HSP 90* gene play important roles in animal survivability. Among the major role of these genes is thermo-regulatory which has enabled various animals to become thermo-tolerant in areas where HS can become a challenge. Most African

countries including Nigeria have long periods of hot humid weather which have been shown to affect animals' capability to perform to their maximum genetic potential. The other African indigenous species of livestock such as cattle, sheep, and goats have been reported by various researchers to be hard and tolerant or resistant to diseases, feed and water scarcity as well as ability to withstand high temperatures (Chenyambuga et al. 2008; de Clare Bronsvort et al. 2013; Hansen 2004; Kim et al. 2017; Laisser et al. 2014; Latif and Pegram 1992; Maule 1990; Mattioli et al. 2000; Porter 1991; Western and Finch 1986). However, in these reports, little is known with regard to the mechanisms of tolerance or resistance and thus this remains an important opportunity which can be explicitly explored. For example, it is important to answer the question how unique are the African animals in terms of the claimed inherent ability to withstand the mentioned stresses. Unfortunately, the complete genomic information of the indigenous African animals is lacking. Until the present time, available information include only how much are the African livestock such as cattle likely to be genetically different or similar. Recently published papers have shown some variations based on mitochondria or microsatellite DNA markers or SNPs. The examples include the little variations in Tanzania cattle reported by Gwakisa et al. (1994) and Msalya et al. (2017); variations in Ethiopian cattle described by Edea et al. (2012); and diversity among a few African cattle published recently by Kim et al. (2017).

Table 1 Single nucleotide polymorphisms at *HSP 90* in Nigerian zebu cattle breeds

Position	SNP variants	Type of SNP	Amino acid change	Breed
116	T→G	Transversion	Threonine > Histidine	Red Bororo
220	G→C	Transversion	Arginine > Serine	Sokoto Gudali
346	G→A	Transition	Serine > Leucine	Ambala
390	G→A	Transition	Aspartate > Tyrosine	White Fulani

Fig. 2 Section sequences photograph approximately 450 bp in exon 3 of HSP 90 in Nigerian zebu cattle DNA samples indicating detected SNPs. 116 T > G in RB; 220 G > C in SG; 346 G > A in AM; 390 G > A in WF



In Nigeria, a few authors have shown the capability of the local animals to withstand HS and feed shortage by looking at the phenotypic performances (Yakubu et al. 2015). We initiated the assessment of the genetic basis of the indigenous zebu cattle of Nigeria with respect to tolerating HS through analysis

of coding region of exon 3 of *HSP 90*. A total of 90 adult animals (bulls) representing four zebu breeds from northern Nigeria were included in this study. We detected four breed-specific SNPs namely T116G, G220C, G346A, and G390A which were specific in the DNA samples of RB, SG, AM, and

Table 2 Genotypes and allele frequencies of the single nucleotide polymorphisms at *HSP 90* in four breeds of Nigerian zebu cattle

Locus	No.	Genotype			Allele frequency		HWE test χ^2	P value
116 TG		TT	TG	GG	T	G		
WF	25	0	0	0	0	0	–	–
AM	23	0	0	0	0	0	–	–
SG	21	0	0	0	0	0	–	–
RB	21	5 (0.24)	14 (0.67)	2 (0.09)	0.57	0.43	2.39	0.12
220 GC		GG	GC	CC	G	C		
WF	25	0	0	0	0	0	–	–
AM	23	0	0	0	0	0	–	–
SG	21	3 (0.14)	8 (0.38)	10 (0.48)	0.33	0.67	0.60	0.44
RB	21	0	0	0	0	0	–	–
346 GA		GG	GA	AA	G	A		
WF	25	0	0	0	0	0	–	–
AM	23	6 (0.26)	5 (0.22)	12 (0.52)	0.37	0.63	7.15	0.007
SG	21	0	0	0	0	0	–	–
RB	21	0	0	0	0	0	–	–
390 GA		GG	GA	AA	G	A		
WF	25	4 (0.16)	10 (0.48)	11 (0.44)	0.35	0.65	1.52	0.22
AM	23	0	0	0	0	0	–	–
SG	21	0	0	0	0	0	–	–
RB	21	0	0	0	0	0	–	–

Table 3 Relationships between SNP genotypes and body parameters in Nigerian zebu cattle breeds

SNP site	SNP genotypes	BT (°C)	RT (°C)	RR (bpm)	HTC
116	TT	39.842 ± 0.10 ^a	39.470 ± 0.05 ^a	105.500 ± 0.20 ^a	5.475 ± 0.01 ^a
	TG	37.910 ± 0.01 ^c	38.100 ± 0.13 ^c	101.645 ± 0.10 ^c	5.104 ± 0.01 ^c
	GG	38.564 ± 0.01 ^b	38.123 ± 0.10 ^b	103.229 ± 0.10 ^b	5.200 ± 0.01 ^b
220	GG	39.854 ± 0.10 ^a	39.950 ± 0.05 ^a	105.810 ± 0.20 ^a	5.750 ± 0.01 ^a
	GC	37.900 ± 0.01 ^c	38.224 ± 0.10 ^c	101.130 ± 0.10 ^c	5.203 ± 0.01 ^c
	CC	38.699 ± 0.01 ^b	38.744 ± 0.04 ^b	103.160 ± 0.02 ^b	5.455 ± 0.01 ^b
390	GG	39.738 ± 0.20 ^a	39.427 ± 0.16 ^a	105.454 ± 0.18 ^a	5.548 ± 0.01 ^a
	GA	37.898 ± 0.10 ^c	38.016 ± 0.10 ^c	101.625 ± 0.20 ^c	5.410 ± 0.01 ^c
	AA	38.556 ± 0.01 ^b	38.170 ± 0.12 ^b	103.110 ± 0.10 ^b	5.505 ± 0.01 ^b

a, b, c Means with different superscripts within the same column were statistically significant ($P < 0.001$)

WF, respectively. Two of the SNPs were transversions and two were transitions. Although these SNPs were non-synonymous, all of them led to a change in amino acids which is indicative of biological change in protein product (Lamb et al. 2007a). The authors showed a positional base change at G2033C in *HSP A1A 90* gene leading to amino acid change from glycine to alanine and subsequently affected milk yield and composition in Chinese Holstein cattle. Earlier on, we measured the four body (health/thermo-tolerance) parameters related to temperature and heat resistance in animals including BT, RT, and RR followed by calculation of HTC. Lower HTC index is suggestive of better adaptability and superior heat tolerance ability under thermal and stressful environmental condition while elevated HTC index is indicative of poor adaptability and poor ability to cope under thermal environmental stress. The observed health/thermo-tolerance differences in WF, RB, AM, and SG breeds could be due to genetic differences among our animals. This confirms the earlier report of Christine et al. (2016) who reported that thermal conduction and convection were influenced by coat color, thickness, and thinness of the skin. Thin-skinned animals have better thermal convection advantage over thick-skinned counterparts (Christine et al. 2016). Also genetic difference due to coat color markedly affect heat absorption and dissipation especially where white coat color confers additional genetic advantage over black coat and other colored animals (Blackshaw and Blackshaw 1994; Muralidhar et al. 2004). The values of these parameters were related to the detected SNPs and we showed that heterozygous animals had lower value for each of these parameters. We assumed that the

heterozygous animals can be better tolerant of HS than the homozygous animals in accordance with results of an earlier work of Kumar et al. (2015). These authors showed that some SNP genotypes in *HSP 90* evidently lowered the RR, RT, and HTC values in cattle. Moreover, we associate these results to the genetic variations among the study animals and recommend that more analyses be carried out in large population to evaluate the variations among these animals. We also propose carrying out an association of some genotypes (mainly the heterozygous) to heat tolerance in our animals as argued by the farmers and researchers.

Various findings regarding the bovine *HSP 90* SNPs are found in previous research reports. For example, in a study by Rosenkrans et al. (2010), 11 SNPs were obtained in DNA samples of crossbred Brahman cows. The SNPs included 1 deletion at position 895, 7 transitions (G1013A, G1045A, C1069T, A1096G, G1117A, T1134C, and T1204C), and 3 transversions (A1125C, G1128 T, and C1154G). Among the SNPs detected by Rosenkrans and his team, the deletion at 895 and transversion at 1125 and 1128 were found in the animals. Furthermore, the deletion of C at 895 had the greatest effect on the average Julian calving date. Only 8% of cows with the C deletion calved with average of 109 calving days, which was approximately 35 days longer than cows without the deletion. In large zebu of Asia, *HSP 90* SNP variants have been detected in the coding region of exon 3 including (A1209G) in Sahiwal breed (Kumar et al. 2015). Moreover, the genetic variability in cattle at *HSP 90* as we suspect in our study has been reported elsewhere. For instance, Kerekoppa et al. (2015) discovered SNPs variability at *HSP 90* in Deoni

Table 4 The values of thermo-tolerance traits of Nigerian zebu cattle

Breed	BT (°C)	RT (°C)	RR (bpm)	HTC
White Fulani	38.243 ± 0.23 ^c	38.343 ± 0.842 ^b	102.929 ± 0.56 ^c	5.475 ± 0.03 ^c
Ambala	38.633 ± 0.24 ^b	38.544 ± 0.84 ^a	103.533 ± 0.60 ^b	5.507 ± 0.03 ^b
Sokoto Gudali	38.925 ± 0.30 ^a	37.841 ± 0.08 ^c	104.350 ± 0.74 ^a	5.550 ± 0.04 ^a
Red Bororo	38.269 ± 0.17 ^c	38.221 ± 0.60 ^b	102.805 ± 0.41 ^c	5.470 ± 0.02 ^c

a, b, c Means with different superscripts within the same column were statistically significant ($P < 0.05$)

breed and Holstein-Friesian crossbred. In their study, the authors showed that the 7 SNPs in the former (5 transitions and 2 transversions) were quite different from the 5 SNPs (2 indels, 2 transitions, and 1 transversion). Genetic variability based on *HSP 90* has also been shown in other indicine breeds and other livestock including the Thai native cattle (Charoensook et al. 2012), Indian Sahiwal (Sajjanar et al. 2015), and in sheep (Marcos-Carcavilla et al. 2010).

The importance of the molecular chaperon genes including the *HSPs* is overemphasized in literature. An earlier of Wilkerson and Sarge (2009) reported that *HSP 90* gene expression was crucial in embryonic survival and overall pregnancy success. SNP variants such as the positional base change at G2033C in *HSPA1A* (90) gene resulted in an amino acid change from glycine to alanine in the translated products with an evident effect on milk yield and milk content in Chinese Holstein cattle (Lamb et al. 2007a). Rosenkrans et al. (2010) and Lamb et al. (2007b) reported that *HSP 90* gene could be used as bio-markers for thermo-tolerance in animals under thermal assault. Therefore, we suggest further evaluation of the gene in Nigerian cattle and find out the effect of these polymorphisms on the performance of the animals.

Conclusion

We have reported the prevalence of four breed-specific SNPs within the nucleotide sequences of exon 3 of *HSP 90* gene in four Nigerian zebu cattle breeds. We showed that heterozygotic SNP genotypes at various sites within the nucleotide sequences of exon 3 of *HSP 90* gene possibly contributed to the lower BT, RT, RR, and HTC values in our animals. Furthermore, we also indicated that differences existed within body parameters (health/thermo-tolerance) presumably caused by some genetic differences among the animals. In this study, the WF and RB showed significantly lower values ($P < 0.05$) than the AM and SG breeds. Conclusively, our results suggest potential thermo-tolerance, survivability, and ability to cope with stressful thermal environment among the Nigerian zebu cattle breeds. We recommend carrying out further analyses in a large sample or including more populations to confirm these facts and finally plan breeding strategies for thermo-tolerance in Nigerian *Bos indicus*. However, this is only possible with a system of recording keeping in place backed up with sufficient genomic information of the animals. On the other hand, it is important to find out more information regarding the SNPs detected in this study and the possibility of employing them as a molecular bio-marker for screening and selection of large

population of Nigerian cattle breeds in terms of better thermo-tolerance and adaptability to HS.

Acknowledgments We thank the management of slaughter houses in Nigeria for giving us permission to sample from the animals in their custodian.

Authors' contributions Conceptualized and designed the experiments: GOO, COI, AOF, OO, AKT, CS; contributed reagents: AKT, CS, GKT; performed the experiments: GOO, GMM, CS; carried out the analysis: GOO, GMM, CS; did the statistical analyses: GOO; drafted the manuscript: GOO, GMM; structured scientific content: GOO, GMM, CS, TMS, OO, AY; all authors provided editorial suggestions and revisions, and read and approved the final draft: GOO, GMM, AKT, CS, GKT, TMS, JSD, ASA, OO, AOF, MO, AY, COI.

Funding information The Government of India funded the main analyses of this study in a form of visiting scholarships through the Research Fellowship for Developing Countries Scientists (RFD-CS) to GOO and CV Raman International Fellowship for African Researchers for GMM. Both programs are administered by the Department of Science and Technology (DST) and Ministry of External Affairs (MEA) of the Government of India, New Delhi.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Declaration We declare to the Editor of the Biochemical Genetics that this is our original work and has not been submitted for publication elsewhere. Animal welfare was not compromised anytime during this study and all of our research protocols were cleared by the responsible institutions both in Nigeria and India prior to embarking on the various activities reported here.

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