

EXPRESSION OF TWO HEAT TOLERANT GENES AND THEIR RELATIONSHIP WITH THERMOREGULATORY TRAITS IN SOME INDIGENOUS CATTLE IN MUBI

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Abstract

Eighty (80) clinically healthy lactating cow breeds (Adamawa Gudali, Bokoloji, Bunaji, and Rahaji), 20 per breed were selected, and blood samples were collected from 16 cows (4 per breed) between February and April 2024. Gene expression was analyzed using real-time PCR (qPCR), evaluating the ΔC_t values for Heat Shock Protein (HSP90AA1) and prolactin receptor gene (PLRL) genes. Thermoregulatory parameters: rectal temperature (RT), respiration rate (RR), pulse rate (PR), and heat tolerance coefficient (HTC), were measured at 8:00 am and 2:30 pm throughout the study period. Significant ($P < 0.05$) positive correlations were found between PLRL and RR ($r = 0.48$), HTC ($r = 0.42$), and PR ($r = 0.79$), while a negative correlation existed between PLRL and PR ($r = -0.38$). HSP90AA1 showed positive relationships with RR ($r = 0.61$), PR ($r = 0.51$), and HTC ($r = 0.59$). A significant negative association was observed between HSP90AA1 and PR ($r = -0.52$), and between HSP90AA1 and HTC. Overall, the study highlighted complex but significant interactions between stress-related gene expressions and thermoregulatory traits in indigenous cattle breeds, indicating their potential as genetic markers for heat stress adaptation in tropical environments. It is recommended that, Adamawa Gudali breed should be used for improvement of heat tolerance because of its high adaptation during the hot period of Mubi, compared to Red Bororo, Sokoto Gudali and Bunaji breeds

Keywords: Heat Tolerant Genes; Thermoregulatory traits; Indigenous; Cattle.

INTRODUCTION

Gene expression is the generation of a functional gene product from the information encoded by a gene, through the processes of transcription and translation. Nucleotides (genetic code) found in DNA is revealed by gene expression, and the characteristics of the expression and the properties of the expression give rise to the organism's phenotype (Bernabucci *et al.*, 2014). Measurement of expression is mostly done by discovering the final gene product which in most cases is protein; however, it is often easier to detect one of the precursors, typically messenger ribonucleic acids (mRNA) which infer gene-expression levels from

these measurements (Alekseev *et al.*, 2009). Gene expression profiling is one of the useful tools for identifying and predicting critical genes and pathways in animal metabolism and functions (Kapila *et al.*, 2013). It is necessary to quantify the level at which a particular gene is expressed within a cell, tissue or organism which in turn provides a lot of valuable information about the function of a particular animal (Alekseev *et al.*, 2009).

The polymorphism of various candidate genes has been investigated in the past years, especially in terms of the association of their allelic variants with certain milk production traits (milk yield, protein and fat

yields, protein and fat percentages) (Ilie *et al.*, 2021). A previous study of the bovine prolactin (PRL) gene was conducted (Grădinaru *et al.*, 2022) revealing its position on the 23rd chromosome, with a spanning of 9.4 kb, containing five exons and four introns. PRL plays an important role in the initiation and maintenance of lactation. Various milk components, including proteins, lactose, lipids, and other important constituents, are synthesized because of its action at the level of mammary alveoli (Grădinaru *et al.*, 2022). Milk synthesis is also supported by PRL because of dry matter intake and the increasing of feed intake (Lacasse *et al.*, 2016). Moreover, PRL has an indirect intervention at the level of milk protein gene expression (Sonmez and Ozdemir, 2017).

Various polymorphisms were found in the bovine PRL gene (Mehmannavaz *et al.*, 2009) and 45 SNPs lying within exons have been reported in the Ensembl Variation database (Cunningham *et al.*, 2019). Although some of these are silent point mutations without any change in the encoded amino acid sequence (Wojdak-Maksymiec *et al.*, 2008), studies of bovine PRL genotype associations with milk traits were performed showing strong marker effects due to linkage with other polymorphisms (Boleckova *et al.*, 2012). However, the Animal performance is influenced by its environment and genetic makeup (Nanekarani *et al.*, 2016). A particular genotype (or breed or population of animals) may have different values in various contexts because of interactions between genotype and environment (Rolf, 2015).

MATERIALS AND METHODS

Study Location

The location from which the samples were purposively collected were Mubi cattle market, Mubi abattoir, Fulani herders at Muchala Mountain, and Fulani herders at Lewa Mountain (Vimtim). These areas all fall under Mubi South and North Local Government Areas. Mubi is geo-located at an area lies within the Northern-Guinea Savannah zone of Nigeria, it is located between latitude 9°30'11" north of the equator and longitude 13°30'45' East Greenwich meridian with 579 meters above sea level. The dry season of the area commences in November and ends in March, while the wet season begins from

April and end in late October. The mean annual rainfall is about 1050 mm. The relative humidity is extremely low (20-30%) between January and March but reaches a peak of about 80% in August and September. The maximum temperature can reach 40°C particularly in April while the minimum temperature is about 12°C between December and January (ADSU Metrological Station, 2021).

Data Collected

A total eighty (80) clinically healthy lactating cows comprising (20) cow each from Adamawa Gudali, Bokoloji, Bunaji, and Rahaji in some purposively selected farms in Mubi were used for the experiment. The experiment spanned one season of the year. This is late dry (February- April) 2024.

Gene expression study

5mls of blood samples were collected from 16 cows (4 from each of Adamawa Gudali, Sokoto Gudali, Red Bororo and White Fulani breed) of cows via jugular vein using 5ml syringe. The blood samples were collected in EDTA bottles and stored in ice before being transferred to DNA laboratory at Kaduna for RNA extraction.

cDNA synthesis and real-time PCR (RT-qPCR) involve two main steps: first, high-quality RNA is reverse transcribed into complementary DNA (cDNA) using reverse transcriptase, dNTPs, and primers such as oligo(dT) or random hexamers, typically after a brief denaturation to remove RNA secondary structures. The resulting cDNA is then used as a template in quantitative PCR, where gene-specific primers and a fluorescent dye or probe allow amplification and real-time detection of target sequences through repeated cycles of denaturation, annealing, and extension. This method ensures sensitive and accurate measurement of gene expression levels, if controls such as no-RT and no-template reactions are included, primers are carefully designed, and normalization is performed using stable housekeeping genes (Vincenzetti *et al.*, 2011).

Cells or tissues are first lysed in **QIAzol Lysis Reagent**, a monophasic phenol/guanidine solution that denatures proteins and inactivates RNases; the lysate is homogenized and then mixed with chloroform to separate phases, with RNA partitioning to the aqueous

phase while DNA and proteins remain in the interphase/organic phase (Qiagen, 2012). The aqueous phase is collected, ethanol is added to promote binding of all RNA species (including small RNAs, down to ~18 nt) (Qiagen, 2012), and the mixture is applied to a silica-membrane spin column, where RNA binds while contaminants are washed away with appropriate buffers (Qiagen, 2012). Finally, purified, high-quality RNA is eluted in RNase-free water (Qiagen, 2012). For reverse transcription of miRNAs, the kit's recommended RT system (miScript RT) uses a **universal RT primer** strategy to convert small RNAs into cDNA in a single reaction; after first-strand synthesis, the resulting cDNA can be used in SYBR Green- or probe-based qPCR assays specific to miRNAs (Qiagen, 2012).

According to Bustin *et al.* (2009) in the MIQE guidelines, PCR amplification efficiency is a critical quality parameter in quantitative PCR and is typically determined from a standard curve generated using serial dilutions of a known template. The slope of the curve is used to calculate efficiency with the formula $E = 10^{(-1/\text{slope})} - 1$, where an ideal efficiency of 100% corresponds to a slope of -3.32, while acceptable values generally range from 90–110% (slopes -3.1 to -3.6). Assay reliability is confirmed when the standard curve shows a high correlation coefficient ($R^2 \geq 0.98$) and a broad linear dynamic range. Importantly, target and reference genes should display similar efficiencies to ensure valid relative quantification, and full reporting of slope, efficiency, linear range, and controls is emphasized to maintain reproducibility and transparency.

In real-time PCR as typically described, the assay uses gene-specific primers (and sometimes a fluorescent probe) to monitor the amplification of a DNA target in real time. After reverse transcription (if starting from RNA), the PCR is carried out over multiple cycles, with each cycle including denaturation, annealing, and extension steps. Fluorescence measurements (via intercalating dyes like SYBR Green or hydrolysis probes) are taken each cycle, allowing determination of the **quantification cycle** (Cq) when the signal crosses a threshold. A standard curve made from serial dilutions of template is used to calculate amplification efficiency and linear dynamic range; assay specificity is checked via melting-curve analysis (for SYBR Green) or probe design; and controls (no template, no RT) are

included to ensure the amplification is genuine and without contamination

Data Analysis/Statistical Analysis

The data of the real-time PCR were analyzed as described by Morammazi *et al.*, (2016). The difference (ΔC_t) between the threshold cycles (C_t) for PLRL gene were used for the evaluation of the gene expression of the four breeds of cattle. Isolation of the selected genes and Quantitative PCR and PCR amplification efficiency were done by the method described by Bustin *et al.* (2009).

Physiological data

Measures of thermoregulatory parameters such as; Rectal temperature (RT), respiration rate (RR), Pulse rate (PR) and Heat tolerance coefficient (HTC) were recorded weekly in the morning at 08:00 am and in the afternoon at 14:30 pm for the study period.

Rectal temperature (RT)

Rectal temperature (RT) was determined by using a digital thermometer. The sensory tip of the digital thermometer was disinfected and inserted into the rectum at the display of $^{\circ}\text{C}$ by a thermometer (which is an indication that the thermometer is set for temperature reading) and then removed by the sound of alarm signal. It was recorded in $^{\circ}\text{C}$.

Respiration rate (RR)

This was determined by counting the number of flank movements per minute. It was recorded in breaths per minute.

Pulse rate

Pulse rate was measured by placing the stethoscope on the femoral arteries of the hind fore for 1 minute. It was recorded in beats/minute. The summary of the collection is shown in Table 1

Table 1: Heat Tolerance Traits, PRLR and HSP90AA1 in Nigeria Indigenous Cow

Traits	Adamawa Gudali	Bunaji	Red Bororo	Sokoto Gudali
RT	36.85 ± 0.96	37.96 ± 0.70	36.91 ± 1.55	37.23 ± 1.19
RR	19.00 ± 3.42	19.00 ± 2.73	17.75 ± 3.11	21.50 ± 3.66
PR	17.00 ± 4.34	22.63 ± 2.11	17.50 ± 4.47	15.88 ± 2.91
HTC	1.79 ± 0.16	1.82 ± 0.12	1.73 ± 0.11	1.91 ± 0.18

Foot note: PRLR=Prolactine Receptor Gene HSP90AA1=Heat Shock Protein RT=Rectal Temperature RR=Respiratory Rate PR=Pulse Rate HTC=Heat Tolerance Coefficient.

Note: HTC were calculated based on heat tolerance index developed by Benezra (1954). The formula is based on both respiration rate and rectal temperature. HTC: $RR/23 + RT/38.33$

RESULTS AND DISCUSSION

Relationship between the Expressions of Heat Stock Protein (HSP90AA1) and Heat Tolerance Traits in Bunaji during the Hot Period in Mubi

The association between the expressions of heat stock protein (HSP90AA1) with heat tolerance traits in Bunaji during the hot period in Mubi is presented in Table 2. A Significantly ($P < 0.05$) positive relation exists between HSP90AA1 and respiration rate (RR) ($r = 0.61$) and pulse rate (PR) ($r = 0.51$), but there exist a significantly ($P < 0.05$) negative relationship between the expression of HSP90AA1 with heat tolerance coefficient (HTC). The association between Pulse rate is negatively related ($r = -0.52$) with all other heat tolerance traits studied. This implies that, significant increase in the expression of PSP90AA1 in Bunaji, Red Bororo, and Sokoto Gudali with

respiration rate will result in significant increase in pulse rate and heat tolerance coefficients. Likewise, significant increase in pulse rate may result in significant increase in HTC which affirms with findings of Thatcher *et al* (2010) who stated that, HSP90AA1 regulation during heat stress result from fast induction of HSP90 protein translation to protect cell from heat stress which is also in conformity with the report of (Collier *et al*, 2008). The result obtained from this study also conforms with previous findings that HSP90AA1 can be regulated owing to climatic condition and physiological characteristic in cattle breeds be it the *Bos taurus* or zebu the cattle breeds (Kumar *et al* 2015). This author's report also agrees with the result from this work where the higher expression of HSP90AA1 recorded in the month of March can be associated with RR, PR and climatic condition, even when HSP90AA1 expression was not significantly different between Bunaji, Red Bororo and Sokoto Gudali breed. When considering the month and breed interaction it is evidence that both breeds are well adapted to tropical climate (Cooke *et al* 2020).

Table 2. Relationship between the Expression of Heat shock proteins (HSP90AA1) With Heat Tolerance Traits in Bunaji Breed of Nigerian Indigenous Cattle

	HSP90AA1	RT	RR	PR
RT	-0.44*			
RR	0.61**	0.20		
PR	0.51*	0.36*	-0.50*	
HTC	-0.65**	0.34*	0.99***	-0.43*

RT=Rectal Temperature, RR=Respiratory Rate, PR=Pulse Rate, HTC= Heat Tolerance Coefficient

Relationship between the Expression of Heat Shock Protein and Heat Tolerance Traits in Adamawa Gudali during the Hot Period in Mubi

The association between the expression of heat shock protein and heat tolerance traits in Adamawa Gudali during the hot period in Mubi is shown in Table 3. Significantly ($P < 0.05$) positive relationships exist between heat shock protein (HSP90AA1) and respiration rate ($r = 0.38$) and heat tolerance coefficient (0.14), while a negative association was observed between the expression of HSP90AA1 and pulse rate ($r = -0.36$). Traits that are positively related suggest that a significant increase in one parameter will result in significant increase on the other related parameter or vice versa. The high positive values observed among the thermoregulatory traits (RR and HTC) suggested that these traits are influenced by the same gene (pleiotropy) as stated by

(Fayeye, 2014 and Abbaya *et al.*, 2022) while the negative correlation observe between the expression of HSP90AA1 with pulse rate depicted that there is no mutual relationship between pulse rate and the expression of HSP90AA1 in Adamawa Gudali. Traits that are positively related suggest that a significant increase in one parameter will result in significant increase on the other related parameter or vice versa. The high positive values observed among the thermoregulatory traits (RR and HTC) suggested that these traits are influenced by the same gene (pleiotropy) as stated by (Fayeye, 2014 and Abbaya *et al.*, 2022) while the negative correlation observe between the expression of HSP90AA1 with pulse rate depicted that there is no mutual relationship between pulse rate and the expression of HSP90AA1 in Adamawa Gudali.

Table 3: Relationship between the Expression of Heat Shock Protein (HSP90AA1) With Heat tolerant traits in Adamawa Gudali Breed of Nigerian Indigenous Cattle.

	HSP90AA1	RT	RR	PR
RT	0.36*			
RR	0.38*	0.33*		
PR	-0.36*	-0.52*	-0.76**	
HTC	0.41*	0.47*	0.99***	-0.79**

RT=Rectal Temperature, RR=Respiratory Rate, PR= Pulse Rate, HTC=Heat Tolerance coefficient

Relationship between the Expression of Heat Shock Protein (HSP90AA1) and Heat Tolerance Traits in Red Bororo breed of Nigerian Indigenous Cattle

The association between the expression of heat stock protein and heat tolerance traits in Red Bororo breed during the hot period of Mubi is shown in table 4. Significant ($P < 0.05$) positive relation exist between heat shock protein (HSP90AA1) and respiration rate ($r = 0.47$) and heat tolerance coefficient ($r = 0.59$), while observed association between the expression of HSP90AA1 and pulse rate is ($r = 0.31$). The high positive relationships observed among the thermoregulatory traits (RR and HTC) suggested that these traits are influenced by the same gene (pleiotropy) (Fayeye, 2014; Abbaya *et al.* 2022) while the negative correlation observe between the expression of HSP90AA1 with pulse rate depicted that there is no

mutual relationship between pulse rate and the expression of HSP90AA1 in Red Bororo. The Red Bororo breed is known for its adaptation to the harsh tropical environment of Nigeria. Their ability to tolerate heat is likely linked to both genetic factors, including HSP90AA1 polymorphisms, and other physiological and behavioral adaptations (Abbaya *et al.*, 2024) Research on various cattle breeds (e.g., Chinese Holstein, Sahiwal, Nelore, Caracu) has demonstrated the link between HSP90AA1 gene polymorphism and heat tolerance traits like rectal temperature, respiration rate, and heat tolerance coefficient. For instance, one study found that cows with a specific HSP90AA1 allele had higher expression of the protein and were more heat tolerant. (Badri *et al.*, 2018) Another study identified SNPs in the HSP90AA1 gene that were significantly associated with rectal temperature and respiration rate in Holstein cows.

Table 4: Relationship between the Expression of Heat Shock Protein (HSP90AA1) With Heat Tolerance traits in Red Bororo breed of Nigerian Indigenous Cattle

	HSP90AA1	Rectal T	RR	PR	HTC
Rectal T	0.01				
RR	0.47*	-0.75**			
PR	0.31*	0.79**	-0.45*		
HTC	0.59**	-0.56**	0.97***	-0.27*	

Rectal T=Rectal Temperature, RR=Respiratory Rate, PR=Pulse Rate, HTC=Heat Tolerance Coefficient

Relationship between the Expression Heat Shock Protein (HSP90AA1) With Heat Tolerance Traits in Sokoto Gudali Breed of Nigerian Indigenous Cattle

The association between the expression of heat shock protein and heat tolerance traits in Sokoto Gudali breed during the hot period in Mubi is shown in Table 5. Significant positive ($P < 0.05$: $r = 0.14$ to 0.32) correlation exist between heat shock protein (HSP90AA1) with respiration rate (0.09) RR and heat tolerance coefficient (0.14) HTC, while Significant negative ($P < 0.05$: $r = -0.19$ – 0.52) correlation was observe between the expression of HSP90AA1 with pulse rate PR (-0.52). Traits that are positively correlated suggested that a significant increase in one parameter will result in significant increase on the other correlated parameter or vice versa. The high

positive correlation observes among the thermoregulatory traits (RR and HTC) suggested that these traits are influenced by the same gene (pleiotrophy) (Fayeye, 2014; Abbaya *et al.* 2022) while the negative correlation observe between the expression of HSP90AA1 with pulse rate depicted that there is no mutual relationship between pulse rate and the expression of HSP90AA1 in Sokoto Gudali. Studies have shown that certain HSP90AA1 gene polymorphisms are associated with variations in thermoregulatory parameters (like body temperature, respiratory rate, etc.) in Sokoto Gudali cattle. Animals with specific SNP genotypes may exhibit better heat tolerance compared to those with other genotypes (Onasanya *et al.*, 2020).

Table 5: Relationship between the Expression of Heat shock protein (HSP90AA1) and Heat Tolerance traits in Sokoto Gudali Breed of Nigerian Indigenous Cattle

	HSP90AA1	Rectal T	RR	PR
Rectal T	0.32*			
RR	0.09	0.52*		
PR	-0.52*	-0.19	-0.19	
HTC	0.14	0.64**	0.99***	-0.20

Rectal T=Rectal Temperature, RR=Respiratory Rate, PR=Pulse Rate, HTC=Heat Tolerance Coefficient

Relationship between PLRL and Heat Tolerance Traits in Adamawa Gudali during the Hot Period in Mubi

The association between the expression of PLRL and heat tolerance traits in Adamawa Gudali is as shown in Table 6. Significantly ($P < 0.05$) positive ($r = 0.42$ to 0.48) association exist between prolactin receptor gene with respiration rate (RR) ($r = 0.48$) and heat tolerance coefficient ($r = 0.42$) (HTC), while a significantly ($P < 0.05$) negative $r = -0.38$ relationship was observe between the expression of PLRL with PR ($r = -0.38$). Reparatation rate was

reported to be the most sensitive cardinal and most useful animal- based physiological indicator of heat stress in livestock (Ganghan *et al.*, 2000; Yaqub *et al.*, 2017). This is because change in RR always precedes change in the other cardinal physiological variables such as RT, sweating and pulse rate (Jian *et al*; 2015; Dalcin *et al.*, 2016; Singh *et al*, 2018. Still, Sailo *et al.*, 2017) reported an increase in RR of 15.74, 18.16 and 29.82 breaths/minutes in Sahival and 15.178, 22.98 and 47.30 breaths/minute in Karan Fries cattle during the winter, spring and summer seasons

respectively. By implication, significant increase in respiration rate will result in significant increase in pulse rate and heat tolerance coefficient. Likewise, significant

increase in respiration rate will equally result in a significant increase in HTC.

Table 6: Relationship between Prolactin Receptor Gene (PLRL) and Heat tolerance traits of Adamawa Gudali Breed of Nigerian Indigenous Cattle

	PLRL	Rectal T	RR	PR
Rectal T	-0.23			
RR	0.48*	0.33*		
PR	-0.38*	-0.52*	-0.76**	
HTC	0.42*	0.47*	0.99***	-0.79**

RT=Rectal Temperature, RR=Respiratory Rate, PR=pulse Rate, HTC=Heat Tolerance Coefficient

Relationship between the Expressions of PLRL and Heat Tolerance Traits in Red Bororo during the Hot Period in Mubi

The association between the expressions of PLRL with heat tolerance traits in Red Bororo during the hot period Mubi is depicted in Table 7. The Relationship between PLRL with heat tolerance traits were not significant ($p > 0.05$). The Relationship between PLRL with heat tolerance traits were not significant ($p > 0.05$) as well. This suggested that PLRL is not related to heat tolerance trait in Red Bororo. It also implies that a significant increase in PLRL will not lead to any significant increase in any heat

tolerance traits. The result obtained in this study is in conformity with the report made by (Brinez *et al.* 2003) who stated that traits in Red Bororo is majorly determined by environment, season, feeding, breed or other genes rather than PLRL. The relationship between PRLR expression and heat tolerance is multifaceted. PRL and PRLR play roles in regulating physiological responses to heat stress, and genetic variations in these genes, along with other genes involved in heat tolerance mechanisms, can influence an organism's ability to withstand elevated temperatures.

Table 7: Relationship between Prolactin Receptor Gene (PLRL) and Heat Tolerance Traits in Red Bororo Breed of Nigerian Indigenous Cattle

	PLRL	Rectal T	RR	PR
RT	-0.21			
RR	-0.07	-0.75**		
PR	0.01	0.79**	-0.45*	
HTC	-0.13	-0.56**	0.97***	-0.27*

RT= Rectal Temperature, RR=Respiratory Rate, PR=Pulse Rate, HTC= Heat Tolerance Coefficient

Relationship between the Expressions of PLRL and Heat Tolerance Traits in Sokoto Gudali during the Hot Period in Mubi

Table 8. shows the association between the expressions of PLRL with heat tolerance traits in Sokoto Gudali during the hot period of Mubi. Respiration rate (0.16) and heat tolerance coefficient (0.15) did not relate significantly with all other heat tolerance traits studied ($P > 0.05$). But

a significantly positive ($P < 0.05$: $r = 0.30$) correlation exists between PLRL with pulse rate (0.30). Respiration rate (0.16) and heat tolerance coefficient (0.15) did not correlate significantly with all other heat tolerance traits studied ($P > 0.05$). However, significant positive ($P < 0.05$: $r = 0.30$) correlation exists between PLRL and pulse rate (0.30). (PR) reflects the homeostasis of circulation along the general metabolism status (Sejian *et a.*, 2011). This

suggested that PLRL is a candidate gene for heat tolerance traits. This implies that an increase in PR causes an

increase in the blood flow to the surface and thereby facilitating heat loss (cooling) (Marai *et al.*, 2001).

Table 8. Relationship of Prolactin Receptor Gene (PLRL) With Heat tolerance traits in Sokoto Gudali Breed of Nigerian Indigenous Cattle.

	PLRL	RT	RR	PR
RT	0.08			
RR	0.16	0.52*		
PR	0.30*	-0.19	-0.19	
HTC	0.15	0.64**	0.99***	-0.20

RT=Rectal Temperature, RR=respiratory Rate, PR=pulse Rate, HTC= Heat Tolerance Coefficient

Relationship between the Expression of PLRL and Heat Tolerance Traits in Bunaji during the Hot Period of Mubi

Shown in Table 9. are the associations between the expression of PLRL and heat tolerance traits in Bunaji during the hot period of Mubi. Respiration rate (- 0.78) and heat tolerance coefficient (- 0.73) related significantly ($P < 0.05$) negative with all other heat tolerance traits studied. While significantly ($P < 0.05$) positive ($r = 0.79$) relationship existing between prolactin receptor gene and

pulse rate ($r = 0.79$). The positive relationship existing between the PLRL and PR in Bunaji indicates that PR may have caused an increase in the blood flow which enhances heat loss. This confirms with the report of Shaji *et al.* (2016) who reported an increase in PR in Osmanabadi goats exposed to heat stress indicating its role in assessing heat loads in animal. The negative correlation between RR and HTC signifies that as the respiration rate decreases the heat tolerance coefficient decreases or vice- versa

Table 9 Relationship of Prolactin Receptor Gene (PLRL) With Heat tolerance traits in Bunaji Breed of Nigerian Indigenous Cattle

	PLRL	RT	RR	PR
RT	0.14			
RR	-0.78**	0.20		
PR	0.79**	0.36*	-0.50*	
HTC	-0.73**	0.34*	0.99***	-0.43*

RT=Rectal Temperature, RR=Respiratory Rate, PR=Pulse Rate, HTC=Heat Tolerance Coefficient

Conclusion and Recommendation

Findings obtained from this study reveal that there is a significant association between the expressions of HSP90AA1 and PLRL with heat tolerance traits. Hence, there exists an association between heat shock protein (HSP90AA1), prolactin receptor gene (PRLR) with thermoregulation parameter of indigenous breeds of cattle in Nigeria. Adamawa Gudali breed should be used for improvement of heat tolerance because of its high adaptation during the hot period of Mubi, compared to Red Bororo, Sokoto Gudali and Bunaji.

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CONFLICT OF INTERESTS

The authors declared that there are no conflicts of interest concerning this manuscript.

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