Variations of Physiological Parameters and *HSP70* and *HSP90* Polymorphisms in Water Buffaloes in Taiwan During Cool and Warm Season

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Abstract: *Background*: This study examined the physiological parameters of water buffaloes in Taiwan in the cool (February) and warm (August) seasons of 2020 and 2021.

Methods: Data was collected for a study in February, August 2020, and 2021. The ambitious temperature, humidity, water buffaloes' rectal temperature (RT), and respiratory rate (RR) were recorded. The plasma expression levels of heat-shock protein (HSP)70 and HSP90 were examined using an ELISA kit. Furthermore, the *HSP70* and *HSP90* fragment genetic sequence variations were analyzed using the PCR method and MEGA6 software.

Results: The results revealed that in the warm season, the rectal temperature (RT), respiratory rate (RR), and heat tolerance coefficient (HTC) were significantly higher compared to the cool season (all $P < 0.05$). Additionally, the temperature-humidity index (THI) had moderate to high correlations with RT (0.518), RR (0.744), and HTC (0.757). Plasma HSP70 expression levels were higher in the warm season than in the cool season ($P < 0.05$). The genetic sequences of *HSP70* and *HSP90* fragments were compared, and five single-nucleotide variation (SNV) sites were identified. However, each genotype showed no significant physiological difference between the cool and warm seasons.

Conclusion: Temperature and humidity changes in Taiwan had a significant correlation with the physical condition of water buffaloes. This information can be valuable in improving the living conditions of these animals, leading to better animal welfare. Additionally, the *HSP70* and *HSP90* gene variations in water buffaloes in Taiwan could be used as a reference for future research on breeding and identifying molecular markers.

Keywords: Water buffalo, rectal temperature, respiratory rate, temperature–humidity index, heat tolerance coefficient, heat shock protein.

INTRODUCTION

Global warming and climate change have increasingly affected livestock production and related industries; specifically, extreme temperatures, floods, typhoons, and other events associated with global warming and climate change result in damage to livestock facilities, livestock deaths, and decreased reproductive and growth rates [1-3]. Water buffaloes are found in 129 countries and makeup about 11.1% of the world's cattle population [4]. In developing countries, water buffaloes are valuable economic assets that provide milk and meat [5]. Nevertheless, they are affected by events associated with climate change. For example, heat stress causes physiological changes in water buffaloes, including rapid increases in body temperature, pulse rate, and respiratory rate (RR), in addition to signs of discomfort. The intensification of such heat stress can impair water buffaloes' growth, reproduction, and productivity, ultimately affecting herd health. Heat stress in water buffaloes can be estimated using the temperature–humidity index (THI; [6]). By monitoring the THI, farmers can determine the comfort levels of their animals and adjust their livestock management practices or environmental conditions, thus substantially improving the welfare of the animals [6,7]. Somparn *et al*. [8] used the THI to examine heat stress responses in cattle and water buffaloes in Thailand. They determined that a THI of ≤74 can generally be considered to indicate no safety concerns for healthy animals; a THI of 75–78 can be regarded as indicating cautionary conditions, under which water buffaloes experience reduced weight gain; a THI of 79–83 can be considered to indicate dangerous conditions, under which water buffaloes experience a substantial decrease in weight; and a THI of >84, especially if accompanied by inadequate management, can be considered to lead to fatalities among animals. Overall, in water buffaloes, the adverse effects of heat stress, including impaired reproductive performance, begin to appear at a THI of ≥75 [7,9].

Heat-shock proteins (HSPs) are highly conserved chaperone molecules and are classified into several families based on their molecular size and amino acid

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sequences. Among them, HSP70 is a cellular indicator of heat stress in water buffaloes [10], and HSP90 regulates the cell cycle, signal transduction, stress response, protein folding and degradation, and protein transport. When an animal's body temperature becomes excessively high, heat stress stimuli activate heat-shock transcription factor-1, which enhances the expression of HSPs while reducing the synthesis of other proteins. This activation also induces HSP-regulated immune and endocrine systems, altering animals' physiological states to help them adapt to high-temperature environments [11]. Bhat *et al*. [12] analyzed an *HSP70* sequence in Tharparkar dairy cattle. They observed that animals with the TT genotype could effectively maintain their rectal temperature (RT) at 38.3 °C during the warm season, indicating that they exhibited considerable heat tolerance. Water buffaloes in Taiwan are of the swamp type and were introduced from Guangdong and Fujian in the 16th century for agricultural purposes. They were initially used as working animals, with their population in Taiwan peaking at 310,000. Nevertheless, this population has been steadily declining because of agricultural mechanization.

Considering the changes mentioned above in the population of water buffaloes in Taiwan and the effects of climate change, the present study investigated the variations in the physiological parameters of water buffaloes between cool and warm seasons. It analyzed the diversity in *HSP70* and *HSP90*. The study findings can help improve water buffalo farming management in response to climate change.

MATERIALS AND METHODS

Experimental Animals

This study selected 13 male and 13 female water buffaloes (26 in total) aged 1 to 2 years from a farm in Hualien, Taiwan, for examination in an experiment conducted in February and August of 2020 and 2021. The water buffaloes were raised in a semiopen housing system, with an average exercise field area of 1620 m^2 and a shed area of 230 m^2. During the experiment, the buffaloes were provided with 1.5–2 kg of concentrate feed daily, and they had ad libitum access to compacted hay and drinking water. The exercise field had a water-filled trench for the buffaloes to soak in. The use, care, and experimental procedures for the animals in this study adhered to approved practical guidelines stipulated by an institutional animal care and use committee. The approval numbers were HUAIACUC10906 and HUAIACUC11001, issued by Hualien district, Eastern region branch, Taiwan Livestock Research Institute, Ministry of Agriculture.

Analytical Items and Methods

Analysis of Temperature and Humidity in Hualien

This study collected and analyzed temperature and humidity records for the 2020–2021 period from the Hualien Observatory Station of the Central Weather Bureau (cwb.gov.tw https://e-service.cwb.gov.tw/HistoryDataQuery/index.jsp).

Measurement of Ambient Temperature and Humidity at the Experimental Site

A temperature and humidity data collector (THD-8, Jiude Electronics, Taichung, Taiwan) continuously collected ambient temperature and humidity data in the practical barn and exercise field for five consecutive days. Based on the collected data, the THI was calculated using the formula proposed by Kendall and Webster [6]:

$$(1.8 \times Tdb + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times Tdb - 26)$$

Tdb represents dry-bulb temperature (°C)

and *RH* represents relative humidity (%).

Measurement of RT and RR

The water buffaloes' RT and RR were measured daily at 13:30-14:30 for five consecutive days in February and August of 2020 and 2021. Moreover, their heat tolerance coefficient (HTC) was calculated using the following formula: (*R*R/23) + (*R*T/38.33). An HTC value of 2 indicated maximum adaptability, and an HTC value of >2 indicated a lower adaptability state [13,14].

Analysis of Plasma Protein Expression

Blood collection tubes containing ethylenediamin-etetraacetic acid were used to collect blood samples (8–10 mL) from the jugular veins of each animal in February and August. The collected samples were centrifugated at 1,500 g for 15 min (Kubota 5800, Japan). Subsequently, the upper plasma layer was composed for the analysis of HSP70 and HSP90 alpha (HSP90AA1) expression by using bovine HSP70 and HSP90 ELISA kits (Cusabio Technology, Houston, TX 77054, USA) following the manufacturer's instructions.

Extraction of DNA

Blood samples (3–5 mL) were collected from all animals included in the study. Genomic DNA was extracted from the samples. It was then purified using a DNA extraction kit (QIAamp® DNA Mini Kit, Qiagen GmbH, Germany) and stored at −20 °C.

Analysis of Target Gene Sequences

Specific DNA primers were designed by the Primer3 software (https://primer3.org/) to study the gene fragment sequences of *HSP70* and *HSP90* in the water buffaloes, as reported in Table **1**; these sequences were then aligned for analysis. A polymerase chain reaction (PCR) was performed to amplify the target genes.

Execution of PCR

A PCR was conducted using 100 ng of genomic DNA as a template. The reaction mixture (total volume: 25 μL) contained Fast-Run™ 2X Taq Master mix (Protech, Taiwan), 0.5 μL of forward and reversed primers (10 pmol each), and sterile water. The PCR profile used was 35 cycles at 94°C for 30 s, a primer-specific annealing temperature (see Table **1**) for 30 s, and an extension period of 70 s at 72°C with an initial denaturation for 5 min at 94°C and a final extension at 72°C for 10 min. The PCR products were analyzed through electrophoresis on 1.5% agarose gel at 100 V and visualized using ethidium bromide staining and a gel-imaging system. Further sequencing was performed if fragments of the expected length for the target genes were detected. PCR products were then purified with the Gene-Spin™ 1-4-3 DNA extraction kit (Protech, Taiwan).

Alignment of Target Sequences

The BLAST function on the NCBI website was used to determine whether the sequences matched the gene fragments of interest. All target gene fragments from the 26 water buffaloes were sequenced (Genomics company, New Taipei City, Taiwan), and the sequencing results were aligned using the alignment function of MEGA6 software [15] to identify single-nucleotide variations (SNVs).

Statistical Analysis

All statistical analyses were conducted using SPSS version 22 (IBM Corp, Armonk, NY, USA). The data were expressed as mean ± standard deviation (SD) and analyzed using an independent-sample t-test and Pearson's method correlation coefficient.

RESULTS AND DISCUSSION

Ambient Temperature and Humidity, As Well As RT and RR of Water Buffalo

In Hualien, the average temperature in February (cool season) was 19.5 °C, and the corresponding THI was 66.0; the average temperature in August (warm season) was 28.7 °C, and the corresponding THI was 80.6. Significant differences were observed in temperature, humidity, and THI (all $P < 0.0001$) between cool and warm seasons (Table **2**). The results also indicated significant differences in RT ($P= 0.0004$), RR ($P < 0.0001$), and HTC ($P < 0.0001$) between the two seasons (Table **2**). A Pearson correlation coefficient analysis indicated moderate to high positive correlations between RT (0.518), RR (0.744), HTC(0.757), and THI (Table **3**), suggesting that the water buffaloes physiological parameters increased with temperature and humidity levels. These findings are consistent with those of previous studies [16,17]. Manjari *et al*. [10] compared the RRs, pulse rates, and RTs of 20 Indian Tarai water buffaloes between winter and warm seasons. They observed that all three physiological parameters were significantly higher during the warm season ($P < 0.05$). Shenhe *et al*. [18]

Table 1: Primers for Amplifying and Sequencing *HSP70* and *HSP90* Fragments of Water Buffaloes in Taiwan

Primer Name	5' to 3'	Annealing Temperature (˚C)	PCR Product (bp)	Location
HSP70F1	AAACAGCAGCCTGGAGAGAG	60	942	chromosome 2 HSP70 Exon 1 (Acc. No. NC_037546.1)
HSP70R1	TGGCTGATGTCCTTCTTGTG			
HSP70F2	GGACTTGGGTCTTGCCCTAT	57	302	chromosome 2 HSP70 Exon 2 (Acc. No. NC_037546.1)
HSP70R2	CGCATTTATCATTTTCTTTTTATTCC			
HSP90F1	TCACCCAGGAGGAATATGGAG	63	692 [38]	chromosome 23 *HSP90* Exon7 (Acc. No. NW_001494158.3)
HSP90R1	AGAAGGACCGATTTTCTCACC			
HSP90F2	TCGGTCCTTCTGTTGAATCC	63	669	chromosome 23 HSP90 Exon 8 (Acc. No. NW_001494158.3)
HSP90R2	CCTGCTCTTTGCTCTCACCT			

Table 2: Mean Ambient Temperature, Ambient Humidity, THI of the Barn, RT, RR, and HTC in Water Buffaloes in the Cool and Warm Seasons

Item \ Season	Cold	Warm
Ambient temperature (˚C)	22.63 ± 3.71[b]	31.87 ± 1.21[a]
Ambient humidity (%)	68.40 ± 12.15[b]	71.26 ± 5.38[a]
THI	70.10 ± 5.47[b]	84.35 ± 1.17[a]
RT (°C)	38.24 ± 0.24[b]	38.51 ± 0.26[a]
RR	20.31 ± 2.76[b]	28.14 ± 3.74[a]
HTC	1.88 ± 0.12[b]	2.23 ± 0.16[a]

[a,b]Means within the same row with different superscripts differ significantly ($P < 0.05$).

Table 3: Correlation Coefficients Between Biological Parameters of Water Buffaloes

	RT	RR	HTC	THI	HSP70	HSP90
RT	1					
RR	0.294*	1				
HTC	0.325*	0.999**	1			
THI	0.518**	0.744**	0.757**	1		
HSP70	0.494**	0.743**	0.751**	0.891**	1	
HSP90	0.056	0.282	0.282	0.352*	0.319*	1

RR: respiratory rate; RT: rectal temperature; HTC: heat tolerance coefficient; THI: temperature– humidity index; HSP: heat-shock protein.
*$P < 0.05$.
**$P < 0.01$.

found a significant correlation between THI, RR (r = 0.84), and RT (r = 0.81) in crossbred buffaloes. The water buffaloes are homeothermic animals that maintain their body temperature through metabolic heat production and heat dissipation [19,20]. In water buffaloes, higher RTs in warm climates indicate inadequate heat dissipation; such insufficient heat dissipation can lead to increased stress levels, elevated pulse rates, respiratory frequency, and discomfort in these animals, which can gradually affect their feed intake and feed conversion efficiency [21,22]. However, healthy water buffaloes maintain normal body temperatures through physiological and behavioral adaptation strategies. Physiologically, they shiver to generate heat during cold stress and sweat to reduce body temperature during heat stress; behaviorally, they seek shade to avoid direct sunlight and wallow in water ponds to regulate body temperature [23]. Vale [7] demonstrated that water buffaloes with a 3-minute shower of cool water twice daily effectively dissipate excess heat.

The results of this study indicated that in the same season, the RTs of the male water buffaloes were significantly higher than those of the female water buffaloes (Table **4**); nevertheless, no significant between-sex differences were observed in RR or HTC. These results are consistent with the findings of Abbaya *et al*. [24]. Factors influencing temperature regulation include sex hormone levels, water balance regulation, physical activity, body weight, body size, muscle mass, body fat content, and behavior. Compared with males, females generally have smaller body sizes and a higher surface-to-volume ratio, causing faster heat dissipation. However, males have more muscle mass than their female counterparts, which increases heat production. The higher RTs of the male can be attributed to their greater muscle mass; specifically, even at rest, muscles contribute to approximately 25% of the body's heat production, resulting in increased body temperatures [25,26]. The HTC analysis results revealed that regardless of the season (i.e., cool or warm season), water buffaloes with an HTC of <2 exhibited significantly lower RRs than did those with an HTC of ≥2; nevertheless, no difference in RT was noted between water buffaloes with an HTC of <2 and those with an HTC of ≥2 (Table **5**). These findings are consistent with previous studies, which reported a weaker correlation between RT and heat tolerance and a strongly positive correlation

Table 4: Mean RTs, RRs, and HTCs of Male and Female Water Buffaloes in Cool and Warm Seasons

Item \ Seasons	Cool		Warm	
	Male (n=13)	Female (n=13)	Male (n=13)	Female (n=13)
RT (˚C)	38.34 ± 0.11[a]	38.15 ± 0.29[b]	38.62 ± 0.29[a]	38.39 ± 0.15[b]
RR.	20.08 ± 2.06	20.55 ± 3.39	28.23 ± 3.45	28.04 ± 4.15
HTC	1.87 ± 0.09	1.88 ± 0.14	2.23 ± 0.15	2.22 ± 0.18

[a,b]Means within the same row in the same season with different superscripts differ significantly ($P < 0.05$).

Table 5: Mean RTs and RRs of Water Buffaloes for Various HTC Values

Item \ Seasons	Cool		Warm	
	HTC<2 (n=23)	HTC≧2 (n=3)	HTC<2 (n=4)	HTC≧2 (n=22)
RT (˚C)	38.23 ± 0.25	38.36 ± 0.07	38.43 ± 0.16	38.52 ± 0.27
RR	19.79 ± 2.47[b]	24.37 ± 0.55[a]	21.28 ± 0.98[b]	29.38 ± 2.44[a]

between RR and heat tolerance [17,27]. Liu *et al*. [28] used principal component analysis to classify cattle populations into heat-tolerant and heat-sensitive types; they revealed that heat-tolerant cattle exhibited significantly lower RTs and RRs than heat-sensitive cattle. The sweat glands in the skin of water buffaloes are only one-sixth of those in the skin of other cattle breeds [21]; therefore, water buffaloes are less heat-tolerant mammals [29]. Water buffaloes efficiently regulate body temperature by sweating and panting for evaporative cooling. Accordingly, for water buffaloes, respiration is a more effective mechanism of heat dissipation than sweating [21,29,30].

Water buffaloes have weaker physiological responses to extreme heat and cold than other cattle breeds [7]. In summer, water buffaloes must be provided with shelters or shower facilities; in winter, they should be provided with appropriate housing systems to protect them from cold winds [21]. Water buffaloes cool themselves by wallowing in mud; alternatively, they can be cooled by being continuously sprayed with water and then allowing the air or wind to dry them off. Water buffaloes cool down rapidly in shaded or dark areas; a possible reason is that their black skin, rich in blood vessels, effectively conducts and dissipates heat. Even without mud baths, water buffaloes can maintain their growth if provided with sufficient shade [21]. In addition, Garcia [31] suggested that the introduction of trees in grazing areas can increase local biodiversity, provide shade and resting places for animals, reduce direct sunlight exposure, and improve the comfort and well-being of animals. In Brazil, water buffaloes raised in silvopastoral and rotational grazing systems were reported to achieve a daily weight gain of up to 0.911 kg [32]. According to the data collected from the Central Weather Bureau from 2015 to 2019, the average THI in Hualien, Taiwan, was 73.3, and it was >75 (75.8–80.1) from April to September. Therefore, in these months, appropriate adjustments should be made to the management and environment of water buffalo herds in Taiwan; such adjustments can include planting trees and installing simple shade structures or sprinkler systems.

Impact of Environmental Changes during Cool and Warm Seasons on Plasma HSP70 and HSP90 Expression Levels

This study determined that HSP70 expression levels in the plasma of the water buffaloes were significantly higher during the warm season than during the cool season (Table **6**). The Pearson correlation coefficient analysis results (Table **3**) revealed a strong positive correlation (0.891) between HSP70 levels and the THI but a weak positive correlation (0.352) between HSP90 levels and the THI. Moreover, HSP70 levels exhibited moderate to high positive correlation with RT (0.494), RR (0.743), and HTC (0.751). A weak correlation (0.319) was observed between HSP70 and HSP90 levels. These findings are consistent with those in the literature. For example, Shenhe *et al*. [18] compared the physiological parameters of crossbred water buffaloes (Nili-Ravi × Murrah) between spring, summer, and winter. They reported that the R.R.s, R.T.s, and plasma HSP70 and HSP90 expression levels were significantly higher in summer compared to spring and winter ($P < 0.05$). During the warm season, they also reached the physiological parameters of crossbred and purebred Mediterranean water

Table 6: Comparisons of Plasma HSP70 and HSP90 Expression Levels in water Buffaloes in Cool and Warm Seasons

Item \ Season	Cold	Warm
HSP 70(ng/ml)	1.55 ± 0.15[b]	2.44 ± 0.16[a]
HSP 90(ng/ml)	25.70 ± 26.60	69.92 ± 74.29

[a,b]Means within the same row with different superscripts differ significantly ($P < 0.05$).

buffaloes. They observed that the hybrid animals exhibited significantly lower RRs and RTs and substantially higher plasma HSP70 and HSP90 expression levels ($P < 0.05$). Baek *et al*. [33] also examined physiological parameters in Korean Hanwoo cattle. They reported that serum HSP70 and HSP90 expression levels increased in response to short-term heat stress, but the increase in HSP90 expression was nonsignificant. The present study observed a lower correlation coefficient between HSP90 expression levels and the other parameters (Table **3**), possibly owing to large individual variations in HSP90 expression levels. Studies have also indicated that livestock and poultry exhibited increased HSP70 and HSP90 mRNA expression levels in their organs or peripheral blood mononuclear cells as an adaptation mechanism to dry and warm–humid environments; additionally, HSP70 mRNA expression levels were significantly higher during the warm season than they were during winter and were strongly correlated with RR, pulse rate, and RT [33-36].

This study compared plasma HSP70 and HSP90 expression levels between the male and female water buffaloes during the same season (Table **7**). The results revealed that the plasma HSP70 expression levels were significantly higher in the female water buffaloes during the cool season. Under normal conditions, plasma HSP70 expression in female animals is twice that in male animals [37]. Furthermore, this study compared HSP70 and HSP90 expression levels between heat-tolerant and heat-intolerant water buffaloes—grouped based on HTC values—in either season and found no significant difference in these levels between the groups (Table **8**). By contrast, Liu *et al*. [28] reported that plasma HSP70 and HSP90 levels were higher in heat-tolerant water buffaloes than in heat-intolerant water buffaloes. The reason for this discrepancy is unclear. A small group of water buffalo could cause it. Further research is warranted to clarify the differences in HSP70 and HSP90 levels between heat-tolerant and heat-intolerant water buffaloes.

Sequencing Results and Genetic Polymorphism Analysis

Primer Design for Amplification of Target Gene Fragments

This study designed primer sequences based on the target gene sequences of marsh-type water buffaloes published in GenBank. Thus, the study could successfully amplify *HSP70* and *HSP90* fragments (Table **1**).

Table 7: Mean Plasma HSP70 and HSP90 Expression Levels in Male and Female Water Buffalo in Cool and Warm Seasons

Item \ Seasons	Cool		Warm	
	Male (n=13)	Female (n=13)	Male (n=13)	Female (n=13)
HSP 70(ng/ml)	1.47 ± 0.12[b]	1.64 ± 0.15[a]	2.44 ± 0.11	2.44 ± 0.21
HSP 90(ng/ml)	21.06 ± 20.89	30.77 ± 31.98	57.35 ± 45.15	83.63 ± 97.48

[a,b]Means within the same line in the same season with different superscripts differ significantly ($P < 0.05$).

Table 8: Plasma HSP70 and HSP90 Expression Levels in Water Buffaloes for Various HTC Values in Cool and Warm Seasons

Item \ Seasons	Cool		Warm	
	HTC<2 (n=23)	HTC≧2 (n=3)	HTC<2 (n=4)	HTC≧2 (n=22)
HSP 70(ng/ml)	1.56 ± 0.16	1.52 ± 0.14	2.52 ± 0.37	2.43 ± 0.12
HSP 90(ng/ml)	23.47 ± 24.84	40.62 ± 39.26	81.20 ± 82.47	68.22 ± 75.17

Table 9: Genotypic and Allelic Frequencies at Different SNVs of Target Genes in Water Buffaloes in Taiwan

SNV Name and Sequence	Genotype Frequencies			Allele Frequencies		Hardy-Weinberg (p-value)
HSP70 g.24927355 T>C	TT(10)	TC(9)	CC(7)	T	C	0.632
ATCTTTGA[T/C]CTGGGCGG	0.38	0.35	0.27	0.56	0.44	
HSP70 g.24927474 G>A	GG(7)	GA(12)	AA(7)	G	A	1
AGGCCAC[G/A]GCCGGGGA	0.27	0.46	0.27	0.5	0.5	
HSP70 E2 g.24928813 C>T	CC(13)	CT(9)	TT(4)	C	T	0.864
GTTATTT[C/T]TATATGTTA	0.5	0.35	0.15	0.67	0.33	
HSP90 g.17755706 G>A	GG(9)	GA(11)	AA(6)	G	A	0.878
TCTCTGGC[G/A]GCTTTACC	0.35	0.42	0.23	0.56	0.44	
HSP90 g.17756478 T>C	TT(9)	TC(14)	CC(3)	T	C	0.864
GCAGCAAG[T/C]ACTAGCT	0.35	0.54	0.12	0.62	0.38	

Sequencing Results and Genetic Polymorphism Analysis

After sequencing, this study analyzed the sequence diversity of the target gene fragments. The analysis revealed the presence of SNVs at certain loci in *HSP70* and *HSP90* of the water buffaloes: *HSP70* g.24927355 T>C, *HSP70* g.24927424 G>A, *HSP70* E2 g. 24928813 C>T, *HSP90* g.17755706 G>A, and *HSP90* g. 17756478 T>C. Their genotypic and allelic frequencies are presented in Table **9**. All identified SNV loci in this experiment are in Hardy-Weinberg equilibrium, according to the analysis.

Based on the three SNVs loci in *HSP70*, six genotype combinations were derived: TTAACC (27%), TTGACC (12%), CCGGTT (15%), CCGGCT (12%), TCGACC (12%), and TCGATC (23%). Furthermore, based on the two SNVs loci in *HSP90*, six genotype combinations were derived: GGTT (4%), GGCC (12%), GGTC (19%), AATT (23%), GATT (8%), and GATC (35%). However, this study observed no significant differences in the expression of RT, RR, HTI, or plasma HSP70 or HSP90 between the various genotypes, irrespective of season or sex. In a study involving Tharparkar cows, Bhat *et al*. [12] reported a G>T SNV in *HSP70*, which was associated with improved heat tolerance. Similarly, Charoensook *et al*. [38] reported a significant enhancement of heat tolerance associated with the T allele of the *HSP90* g.4338T>C polymorphism. Kumar *et al*. [39] studied Sahiwal cows and identified a significant difference in the heat adaptation coefficient among individuals with different *HSP90* exon3 A1209G polymorphism genotypes. Moreover, Onasanya *et al*. [40] reported that among Nigerian Fulani cattle, individuals with heterozygous genotypes at four SNVs loci in *HSP90* exhibited significantly lower body temperatures, R.T.s, R.R.s, and heat adaptation coefficients than did individuals with homozygous genotypes.

HSPs are crucial molecules involved in animals' response to heat stress. Most HSP genes lack introns, which may contribute to their rapid expression. The HSP40, HSP60, HSP70, and HSP90 family proteins prevent misfolding of denatured proteins, assisting in the refolding of denatured proteins into their native conformations, activating immune and endocrine systems and altering physiological states to help animals adapt to their environments [36]. Crossbreeding with heat-tolerant breeds improves the heat tolerance of buffaloes [16,22,41], but breeding may make them more susceptible to stress instead of enhancing their stress resistance [34,42].

CONCLUSIONS

Our results revealed a close relationship between the physiological parameters of water buffaloes in Taiwan and changes in ambient temperature and humidity. These findings can guide management practices to provide a more comfortable environment for water buffaloes, enhancing animal welfare. Furthermore, *HSP70* and *HSP90* polymorphisms in water buffaloes in Taiwan can serve as references for future research on breeding and relevant molecular markers.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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