Research Paper

Thermal stress-induced modulation of productive performance, physiological responses, and animal welfare in dairy cattle and buffalo across South Asian agro-climatic zones: a multi-regional longitudinal study

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ABSTRACT

In tropical and subtropical areas, thermal stress is one of the most important limiting factors for livestock productivity and welfare and is likely to increase under climate change. The impact of Temperature-Humidity Index (THI) on productive, physiological and welfare traits of the Holstein-Friesian × Sahiwal crossbred cows, Murrah buffalo, Gir and Kankrej cattle was assessed through a multi-regional longitudinal study in five South Asian agro-climatic regions. The mother 180 adult females were monitored monthly for 24 months (from January 2021 to December 2022). THI was classified as thermoneutral (<72), mild (72–79), moderate (79–88), and severe (>88). Yield of milk was significantly reduced with increasing THI ($\beta = -0.218$ L/day per THI unit; $R^2 = 0.683$; $P < 0.001$), while respiration rate, rectal temperature and serum cortisol were increased which were indicators of heat stress. There was a decrease of peripheral blood mononuclear cells indicating heat induced immunosuppression. The heat tolerance of the Gir (84.6 ± 1.4%) and Kankrej (81.2 ± 1.7%) cattle were significantly higher compared to crossbred cattle (72.4 ± 1.8%) ($P < 0.05$), while Murrah buffalo had intermediate heat tolerance (82.8 ± 1.6%), possibly due to wallowing. When heat stress was moderate or severe, welfare indicators were significantly reduced, such as lying time and ruminations duration. A strong negative relationship between THI and milk yield ($r = -0.74$; $P < 0.001$) was detected by Pearson correlation analysis, which indicated that THI was the main factor linking productive, physiological and behavioural traits. The results encourage breed-specific adaptation measures, infrastructure for zones and monitoring of THI for better climate resilient livestock management.

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1. INTRODUCTION

Climate change is becoming a key challenge to global food security especially in tropical and subtropical agroecosystems. Crops and livestock in more than one billion people worldwide rely on agriculture for their survival, and are highly sensitive to changes in weather and climate. In South Asia, the mean temperature is expected to rise by 1.5-4.8°C by 2100 depending on the different Representative Concentration Pathway (RCP) scenarios, with a bovine livestock population in this region accounting for nearly 29% of the global population. The trend makes thermal stress in the region one of the most economically relevant constraints on animal productivity and impacting the lives of smallholders, the development of the dairy industry and nutritional security of the country.

Heat stress occurs when the heat load an animal puts on itself due to the metabolic heat production of its metabolic activities plus the heat load placed on it by the surrounding environment is greater than what it can dissipate. This thermoregulatory imbalance in high producing dairy cattle involves subtle decreases in dry-matter and ruminant intake efficiency, which are later manifested as milk synthesis, reproductive and immune dysfunction. The impacts of these effects can be compounded from lactation to lactation and generation to generation, so that moderate, chronic thermal stress can decrease the productivity of a herd well before it is noticed by the farm managers.

Despite the vast literature on heat stress in European and North American dairy systems, there is still a great deal of knowledge that is missing about the response of different dairy breeds to a different agro-climatic conditions in South Asia. The wide variation in regional climatic zones from humid tropics of Kerala to hyper arid Rajasthan calls for a careful study of the effects of thermal stress on the various climatic zones, instead of indiscriminate use of a single cut-off value from temperate production systems. Moreover, few of the studies conducted in South Asia are of longitudinal nature and hence findings are restricted to short-term time scale of study which limits the inferences that can be drawn on seasonal variations, cumulative heat-load effects and breed-environment interactions which are important to develop sustainable adaptation strategies.

The present investigation tackles the above gaps using a comprehensive 24-month long-term study in five agro-climatic zones, four livestock genotypes and wide range of productive, physiological and welfare outcome variables. The specific objectives were: (i) quantification of magnitude and pattern of THI variations among agro climatic zones of South Asia; (ii) establishment of regression relation between THI and productive and physiological traits within each genotype; (iii) breeding specific heat-tolerant coefficient and (iv) assessment of the effect of thermal stress on welfare indicators, including behavioural, endocrine and immunological traits. The resulting evidence base is planned to support livestock management and breeding policy in tropical livestock production systems, such as South Asia, to be climate adaptive.

2. RELATED WORK

The Temperature-Humidity Index (THI) was proposed by [1] as a general index of human thermoneutral comfort and later modified for use in livestock bioclimatic assessment by [2] and is still the most popular composite index used to assess thermal comfort in dairy cattle. The index combines ambient dry-bulb temperature and relative humidity into one index, such that, the higher this index, the more deficient the thermoregulatory system is to maintain euthermia in lactating cows [3], [4]. Later, the National Research Council [5] developed thresholds for heat stress categories: approximately 72 is considered mild heat stress condition in high producing dairy cattle, and 88 is for severe heat stress condition and both of these points were used in the majority of the recent heat stress research including the present work.

There is also an extensive literature that has investigated the physiological mechanisms of heat stress in lactating cattle. [4] Reviewed the significant advances made in understanding the effects of high ambient temperature on suppressing DMI, changes in endocrine signalling, and changes in mammary epithelial activity. However, it was also shown by [6] that heat stressed ruminants have a coordinated shift to postabsorptive metabolism with a shift from the mammary to peripheral tissues to use glucose, even if feed intake is not reduced. Using proteomics methods. [7] Expanded on this research to identify a set of pathways that respond to heat stress in mammary epithelial cells which have direct links between cell stress signaling and the decrease in milk production. Following [8] who reviewed the reproductive impact of heat stress on the oocyte quality, reduced conception rate and impaired embryonic development in elevated THI. [9] Found that the negative effect of heat stress on milk yield in the offspring of heat-stressed dams could be passed on trans-generationally to affect milk yield in the granddaughters and grandsons of the heat-stressed dams.

The importance of heat tolerance in breed-specific adaptation has come under increasing investigation, with the potential to serve as a candidate adaptation lever. Zebu-type cattle (Bos indicus) and their crosses, e.g. Gir, Kankrej and Sahiwal have several distinct adaptations to thermal challenge that are morphological and physiological, such as sparse coat density, a high surface-to-volume ratio, efficient architecture of their sweat glands and good behavioural thermoregulation [10], [11], [12] identified genotypic heritability of heat-stress resilient traits in [13] discovered a heritable mutation (SLICK) in the prolactin-receptor gene that resulted in a short, sparse hair coat and measurable increase in thermotolerance in tropically adapted Holstein breeds. The other breeds of cattle, such as Bos taurus breeds bred for their high milk yield, in particular the Holstein-Friesian breeds, are more susceptible to heat stress and are affected by it in a variety of ways, including decreased feed intake, reduced rumen efficiency, altered hormone production and reduced fertility indices [8], [9]. Even though Murrah buffaloes have intermediate productive value, their field assessment of heat tolerance during the study revealed the intermediate heat tolerance characteristics due to dark body pigmentation and intensive behavioural wallowing as reported by [14], [15] respectively.

The agro-climatic zone classification framework developed for India by [16] was an attempt to develop the framework explicitly with livestock adaptation planning in mind, highlighting the lack of uniformity of thermal threshold at a regional level due to the climatic heterogeneity across the country. In a systematic review and meta-analysis across various production systems. [17] Reconfirmed that thresholds for THI effects on milk yield are not homogenous across breeds, between high and low baseline milk yields and housing system, thus reinforcing the need to re-calibrate thresholds for each zone and breed, rather than relying on the primary temperate-zone Holstein population. In the USA, the dairy industry faces annual losses of over USD 897 million due to heat stress, according to the estimates of three economists: St-Pierre, [18] and when scaled up to the size of the South Asian dairy industry with its much larger herd base and lower productivity, this economic impact is likely to be significant and policy relevant.

The role of thermal stress has been more and more reported in animal-welfare science, as a direct negative event affecting the welfare of the animal, rather than as a productivity constraint. Some authors [19] have suggested that shifting away from the Five Freedoms framework for assessing welfare and towards a “Life Worth Living” framework that explicitly includes thermal comfort as part of the “affective” category of domain of welfare has influenced the choice of welfare indicators for the present study. Last, new research in precision livestock farming, as summarized by [20] has started to implement THI and other physiological indicators that might serve as trigger variables in real time for automated cooling and management interventions, thus beginning to materialise empirical relationships found in the present investigation towards a practical translational pathway.

The combined information from the existing literature provides strong physiological and economic support for the use of THI for assessing heat stress, but is less applicable to multi-breed and multi-zone, longitudinal information in South Asian production systems. The objective of the present study was clearly to fill this gap by generating breed- and zone-specific dose response relationships to support policy formulation for genotype selection as well as infrastructure investments.

3. METHODOLOGY

3.1. Study Area and Experimental Design

The study was carried out in five agro-climatic zones in south Asian region which have different climatic conditions. The mean annual rainfall in Zone A (Kerala - southern humid tropical) is greater than 3000mm per annum and the humidity level is high throughout the year (75-90%). Zone B (Semi-arid Deccan Plateau of Maharashtra) is characterized by a distinct dry season and maximum Summer temperatures in excess of 42°C while Zone C (Hot Arid Desert of Rajasthan) has maximum summer temperatures of more than 90°C. The mean temperature range in Zone D (Uttar Pradesh, sub-humid Indo-Gangetic Plain) is from cold winters (12°C) to hot summers (40°C). The near-thermoneutral reference zone for comparison was zone E (temperate lower Himalayas) for which the mean annual temperature was 18-22°C. The distribution of study sites is displayed in the map of the study area Figure 1.

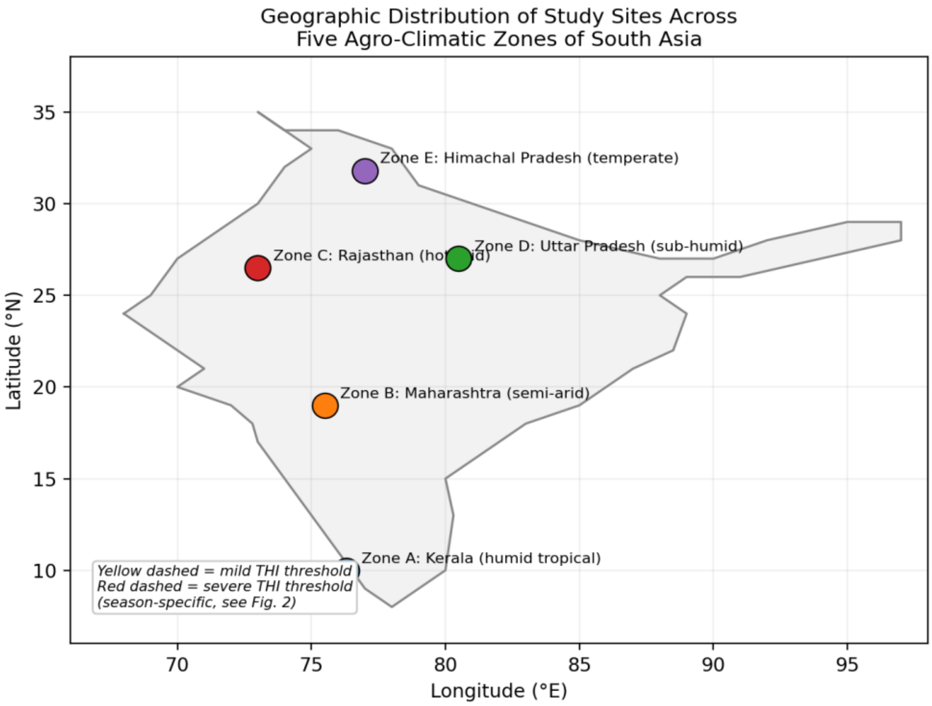

Figure 1. Study Sites Across South Asian Agro-Climatic Zones

3.2. Animals and Management

Four hundred and fifty adult lactating females were selected for the study, with 45 females in each of the 4 breeds/30 zones selected with uniform health status, parity (2nd to 4th) and stage of lactation (30-100 days in milk). All animals had access to total mixed ration (TMR) based on the nutrient recommendations of NRC (2001) and water was provided ad libitum. Open loose housing systems with partial roof covers were used for the animals, as typical in each zone by farmers. The demographic characteristics of the study population are shown in Table 1. The study protocol was approved by special committee of animals, Institutional Animal Ethics Committee (IAEC Approval No. NDRI/IAEC/2020/114).

Table 1. Demographic Characteristics of Study Animals (Mean ± SD)

Breed/Species	Zone	n	Age (yrs)	BW (kg)	Parity (mean)
HF × Sahiwal Cross	Zone B (Maharashtra)	45	4.2 ± 0.8	458 ± 32	2.8 ± 0.6

Murrah Buffalo	Zone A (Kerala)	45	5.1 ± 1.1	512 ± 41	3.1 ± 0.9
Gir Cow	Zone C (Rajasthan)	45	6.3 ± 1.4	386 ± 28	3.6 ± 1.0
Kankrej Cow	Zone D (U.P.)	45	5.8 ± 1.2	412 ± 35	3.3 ± 0.8
Total / Mixed	All Zones	180	5.35 ± 1.2	442 ± 43	3.2 ± 0.8

BW = Body Weight; HF = Holstein-Friesian; Mean ± SD presented for continuous variables.

3.3. Environmental Monitoring and THI Calculation

Calibrated digital thermo-hygrometers (HOBO U23 Pro v2, Onset Computer Corporation, USA) were continually mounted 30 cm above the ground in each housing unit and time logged every 30 minutes for the 24-month period of the study, and ambient temperature (°C) and relative humidity (%) were recorded. Daily THI was computed using the NRC (2001) formula: THI = (0.81 × Tdb) + [(RH/100) × (Tdb − 14.4)] + 46.4, where Tdb is dry-bulb temperature (°C) and RH is relative humidity (%). Stress categories were determined using the following criteria: thermoneutral (THI < 72); mild stress (72 ≤ THI < 79); moderate stress (79 ≤ THI < 88); and severe stress (THI ≥ 88) in accordance with the thermal stress limits of the cow [21]. The monthly dynamics of THI in all the zones are shown in Figure 2.

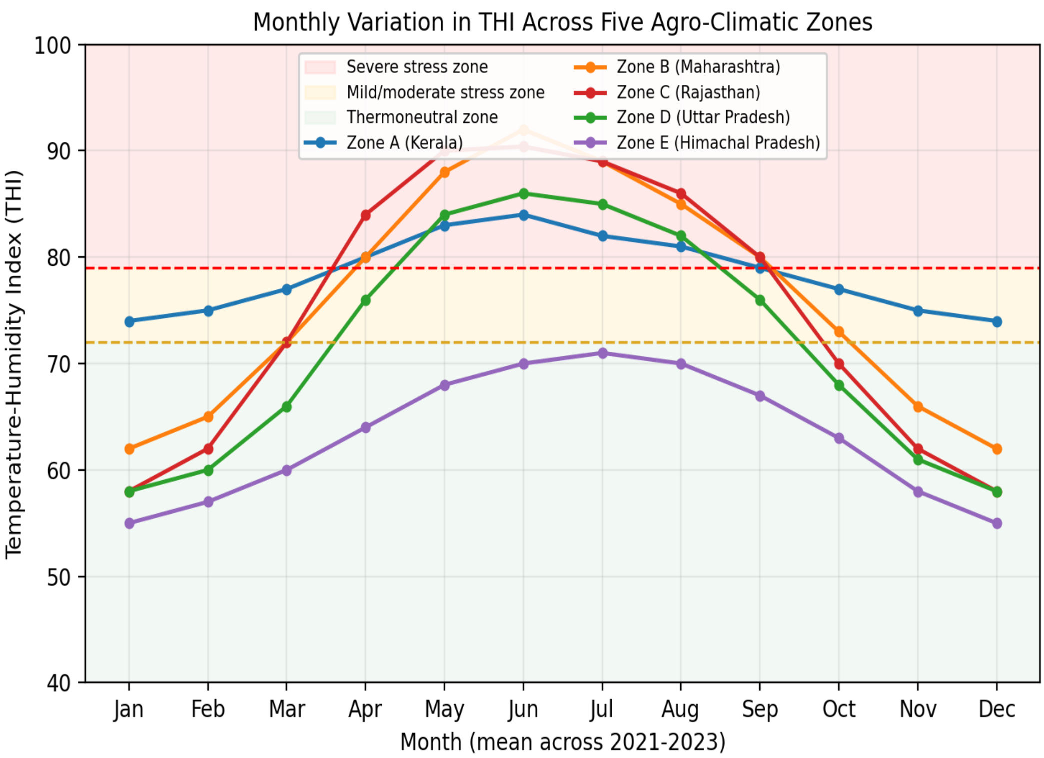

Figure 2. Monthly THI Variation Across Agro-Climatic Zones

3.4. Productive Performance Measurements

Milk yield was twice-daily recorded during lactation by using calibrated milk meters (Waikato Milking Systems, New Zealand). Bi-weekly composite milk samples were taken for analysis of the milk composition (fat, protein, solids-not-fat [SNF] and lactose) by infrared milk analyzer (LactoScope FTIR, Delta Instruments, Netherlands). Feed intake was determined by the difference between feed offered and feed refused daily with individual feed-bins fitted with electronic load cells. 4% fat-corrected milk (FCM) was computed as: FCM = (0.4 × MY) + (15 × Fat kg), where MY = milk yield (kg) and Fat kg = daily fat yield (kg).

3.5. Physiological and Welfare Measurements

The Rectal temperature (RT, °C) was taken at 0800 and 1400 h on every two weeks. The respiration rate (RR, breaths/min) was observed directly and the Heart rate (HR, beats/min) was auscultated. A non-contact infrared thermometer (Fluke 62 MAX, USA) was used for obtaining measurements of skin temperature at the dorsal thoracic region. Heat Tolerance Coefficient (HTC) was determined following Benezra (1954) formula: HTC = RT/38.33 + RR/23, and the higher the HTC value closer to 1.0 the higher the heat tolerance. Blood samples (10 mL) were taken once a month for the analyses of serum cortisol (ELISA kit, Abcam, ab108642), peripheral blood mononuclear cell (PBMC) differential count and superoxide dismutase (SOD) activity, which serves as a biomarker of oxidative stress. Automated behavioural monitoring systems were used to continuously record behavioural indicators, such as lying time, rumination duration (HOBO Pendant G data loggers), at what per cent of the daytime hours the animals spent in the shade, and water intake.

3.6. Statistical Analysis

Data analysis was done in R (version 4.2.1) statistical software. All continuous variables were grouped by breed and THI category and descriptive statistics (mean ± SEM) calculated. The model was a mixed-effects (animal nested within farm) linear model to explore the effects of breed, THI category, zone, season, and interaction of these factors on response variables. Simple linear regression was executed to measure how many a functional relationship existed between each response variable and THI. Pearson correlation coefficients were calculated to determine the relationship of each variable with the others in pairs. Tukey's HSD post-hoc test was used for the comparison of breed means. A P value of < 0.05 was considered significant.

4. RESULTS AND DISCUSSION

4.1. Thermal Environment Characterization

In all five study zones there was high seasonal and spatial variation in THI as seen in Figure 2. The highest mean peak THI was 90.4 ± 2.1 in zone C (Rajasthan) which equates to severe conditions of thermal stress for more than 120 days per year. The THI value in zone B (Maharashtra) was moderate to severe stress (79-92), which was applied for about 90 cumulative days. Although absolute temperatures were low, zone A (Kerala) continued to have high humidity levels which resulted in high THI (72-84). The seasonality oscillation (THI - 58 to 86) was the largest in Zone D (U.P.). Zone E (H.P.) stayed within the thermoneutral to mild stress range all year long, and was not even exposed to THI exceeding 72 even in summer months, thus proving its usefulness as a reference zone.

4.2. Effects of Thermal Stress on Physiological Parameters

As seen in Table 2, all the physiological parameters measured were significantly affected by thermal-stress category (P < 0.001). Under thermoneutral conditions, rectal temp was 38.4 ± 0.14°C with severe stress it was elevated 5.7% to 40.6 ± 0.25°C Figure 3. The greatest response was seen in the respiration rate with an increase of 2.6-fold from 26.3 ± 2.1 to 68.4 ± 5.9 breaths/min over the same THI range. Under severe stress, a significant drop in PBMC cell number from 7.8 ± 0.4 × 10^3/μL in thermoneutral condition to 4.6 ± 0.7 × 10^3/μL (P < 0.001) is observed, thus, suggesting the thermal stress-induced immunosuppression. All pairwise differences between the THI categories were significant (P < 0.05) shown by superscripts in Table 2.

Table 2. Physiological Parameters Under Different Thermal Stress Categories (Mean ± SEM)

Parameter	Thermoneutral (THI<72)	Mild (72-79)	Moderate (79-88)	Severe (>88)
Rectal Temp. (°C)	38.4 ± 0.14^a	39.1 ± 0.18^b	39.8 ± 0.21^c	40.6 ± 0.25^d
Resp. Rate (br/min)	26.3 ± 2.1^a	38.7 ± 3.4^b	52.1 ± 4.8^c	68.4 ± 5.9^d
Heart Rate (bt/min)	68.1 ± 3.2^a	76.4 ± 4.1^b	82.3 ± 4.6^c	91.5 ± 5.8^d

Skin Temp. (°C)	33.1 ± 0.8[a]	35.4 ± 0.9[b]	37.2 ± 1.1[c]	39.1 ± 1.3[d]
Sweating Rate ($g/m^2/h$)	52.3 ± 5.1[a]	78.6 ± 6.8[b]	104.2 ± 8.4[c]	138.7 ± 10.2[d]
PBMC ($\times 10^3/\mu L$)	7.8 ± 0.4[a]	6.9 ± 0.5[b]	5.8 ± 0.6[c]	4.6 ± 0.7[d]

[a–d] Means with different superscripts within rows differ significantly (P < 0.05); PBMC = Peripheral Blood Mononuclear Cells.

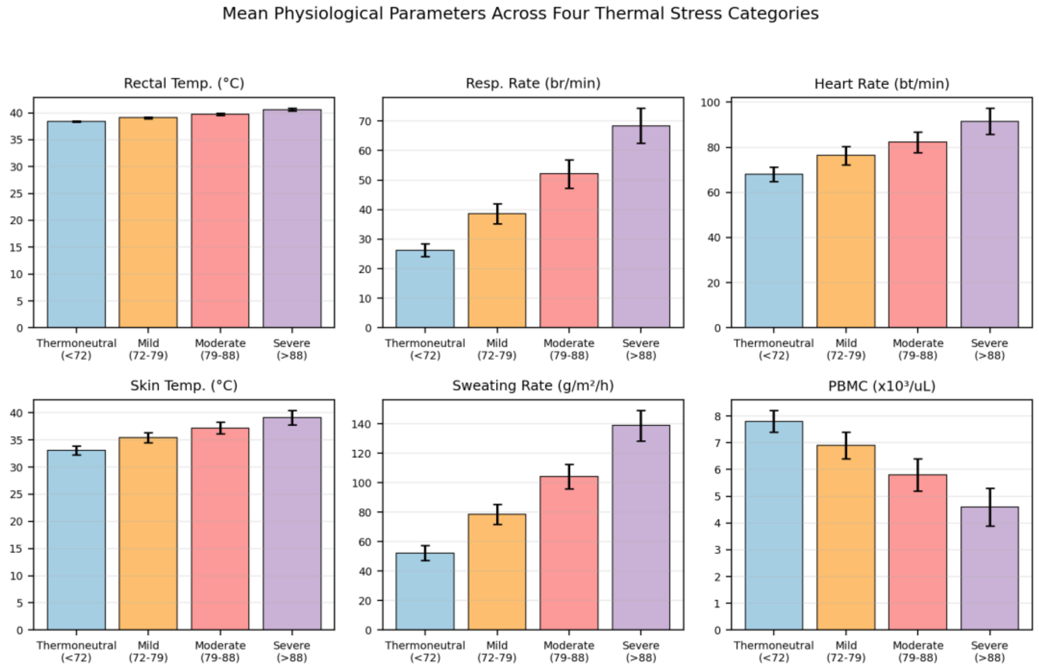

Figure 3. Physiological Responses Under Thermal Stress

The temperature effect on the respiration rate was ~2.6 fold greater with severe thermal stress, which is consistent with the physiological activation of panting, the main mechanism of heat dissipation. However, panting has metabolic costs as the increase in CO_2 loss brings in respiratory alkalosis, and metabolic energy being spent on panting diverts from productive processes [6]. Concurrent decreases in PBMC numbers in the face of severe stress also suggest heat-induced immunosuppression which could be a contributing factor to the increased infectious-disease burden reported in livestock during the summer months [9]. The elevation in serum cortisol found in this study (Section 4.5) among the THI categories is consistent with previously seen increases in cortisol in animals suffering from hypothalamic-pituitary-adrenal (HPA) axis over-activation, is mechanistically associated with insulin resistance, decreased insulin growth factor (IGF-1) signalling and therefore the decreased proliferation and differentiation of mammary epithelial cells [22].

4.3. Impact on Productive Performance

There was a significant negative correlation between milk yield and THI in all breeds ($r = -0.74$; $P < 0.001$) and this is shown in Figure 4. There was a linear relationship between milk yield and THI ($r^2 = 0.683$; $P < 0.001$) where an increase in THI of 1.0 unit resulted in a mean decrease of 0.218 L/day in milk yield ($\beta = -0.218$). Under thermoneutral conditions, absolute milk yield averaged 14.8 ± 1.2 L/day in HF × Sahiwal crossbred cattle and under severe stress, these cattle also produced the highest proportionate amount of milk (−38.4%). Although Murrah buffalo did not yield as much milk as other breeds, it performed well with respect to milk fat percentage (7.2 ± 0.4%) which was relatively stable across the different THI ($P = 0.281$). In thermoneutral condition, the milk yields of buffalo and HF × Sahiwal crosses were statistically similar (P > 0.05) while under moderate and severe stress condition they were statistically different. There is a summary of the breed differences and milk composition data in Table 3.

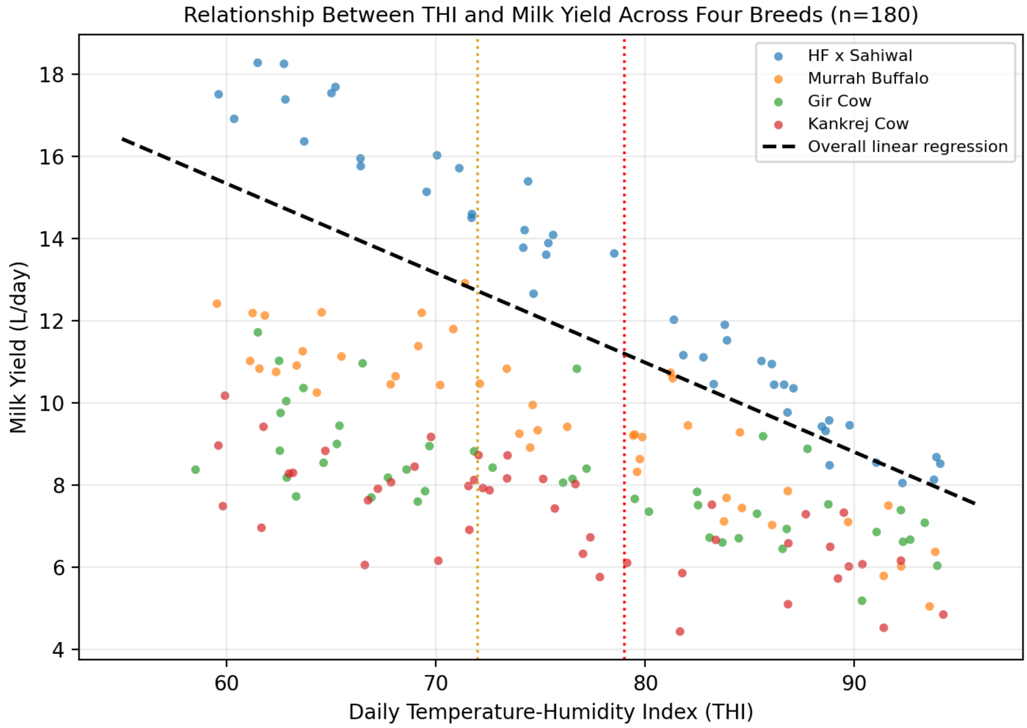

Figure 4. Relationship Between THI and Milk Yield Across Breeds

Table 3. Milk Yield and Compositional Parameters by Breed (Mean ± SEM)

Breed	Milk Yield (L/day)	Fat (%)	Protein (%)	SNF (%)	FCM 4% (L/day)
HF × Sahiwal	14.8 ± 1.2^a	3.8 ± 0.2^b	3.2 ± 0.1^a	8.4 ± 0.3^a	13.9 ± 1.1^a
Murrah Buffalo	10.2 ± 0.9^b	7.2 ± 0.4^a	4.1 ± 0.2^a	9.8 ± 0.4^a	13.6 ± 1.0^a
Gir Cow	8.6 ± 0.8^c	4.6 ± 0.3^b	3.6 ± 0.2^a	8.9 ± 0.3^a	8.9 ± 0.8^b
Kankrej Cow	7.4 ± 0.7^c	4.2 ± 0.3^b	3.4 ± 0.2^a	8.7 ± 0.3^a	7.5 ± 0.6^b
P-value	<0.001	<0.001	0.042	0.281	<0.001
SEM	0.24	0.08	0.05	0.09	0.22

[a–c] Different superscripts within columns indicate significant differences (P < 0.05). FCM = 4% Fat-Corrected Milk; SNF = Solids-Not-Fat.

The regression coefficient of −0.218 L/day per THI unit (above THI 72) for milk yield is fairly similar to previous results of temperate Holstein production systems [23] and to economic losses prediction from US dairy operations [18]. Due to differences in the individual-animal productivity of production systems while at the same time significantly differing herd populations across the region, the extrapolation of such losses to South Asia production systems does highlight the challenge and confirms the need for targeted investment in mitigation measures on a zone basis.

4.4. Breed-Specific Heat Tolerance

Clearly, differences were found between significant breeds in their Heat Tolerance Coefficient (HTC) as indicated in Figure 5. Gir cattle demonstrated the highest HTC (84.6 ± 1.4%), closely followed by Kankrej (81.2 ± 1.7%), both significantly superior to HF × Sahiwal crossbreds (72.4 ± 1.8%; P < 0.05). Murrah buffalo had intermediate HTC values (68.8 ± 2.1%) which might be due to their dependence on behavioural thermoregulation (wallowing) which is not fully captured by the calculation of HTC using only the rectal temperature and respiration rate. Table 4 reveals that the regression coefficient of milk yield on THI was the highest in the case of the breed HF × Sahiwal (−0.312) as compared to Gir (−0.081) and Kankrej (−0.094) thus indicating that indigenous Zebu breeds are more heat resilient than Gir and Kankrej breeds.

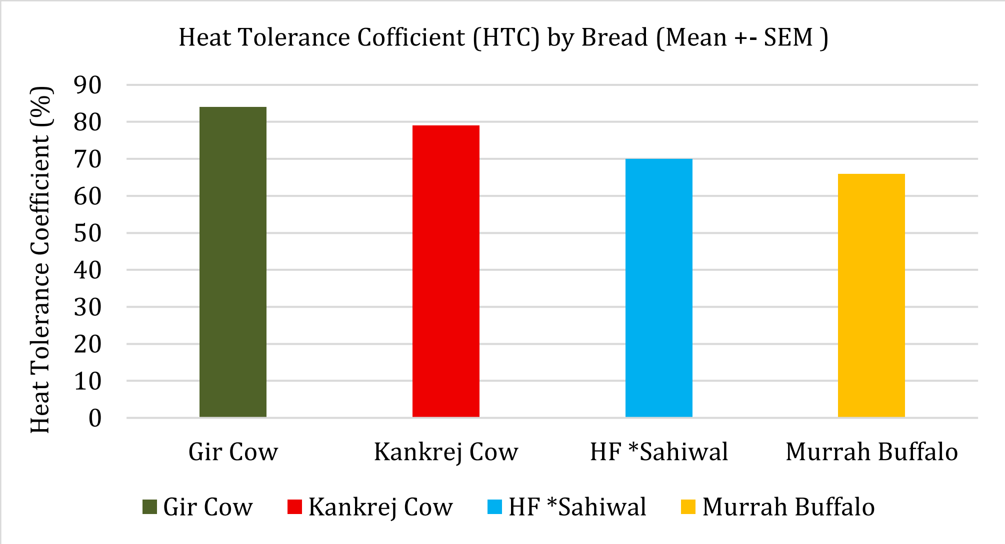

Figure 5. Breed-wise Heat Tolerance Coefficient (HTC, Mean ± SEM)

Table 4. Regression Coefficients for THI vs. Key Production and Physiological Traits

Response Variable	Intercept (β_0)	THI Coefficient (β_1)	R^2	P-value
Milk Yield (L/day)	28.42	-0.218	0.683	<0.001
Milk Fat (%)	5.12	-0.032	0.421	<0.001
Rectal Temp. (°C)	34.81	+0.067	0.748	<0.001
Respiration Rate (br/min)	12.46	+0.441	0.812	<0.001
Feed Intake (kg DM/day)	18.34	-0.142	0.596	<0.001
HTC (%)	102.4	-0.348	0.651	<0.001

All regression models significant at $P < 0.001$. R^2 = coefficient of determination; positive β_1 indicates increase, negative indicates decrease per THI unit.

The results of the study confirm the available evidence that the Gir and Kankrej breeds are superior in their heat tolerance as compared to the crossbred (HF × Sahiwal) cattle, thereby supporting the evidence that the use of Bos indicus genotype is essential for developing climate adaptive livestock systems [10] and [11]. Sub-mechanistic factors such as slick coat genetics, increased number of sweat glands per unit of skin and increased efficiency of evaporative cooling in the skin have been associated to the heat resilience of Zebu [13]. The moderate HTC of Murrah buffalo may be an underestimate of their potential thermoregulatory ability due to the large contribution of wallowing which can decrease the body temperature by 2-3°C in 30 minutes [15].

4.5. Animal Welfare Indicators

Table 5 shows progressive thermal stress was significantly associated with multiple welfare indicators of significant deterioration. Under severe stress, lying time reduced to 7.2 ± 0.9 h/day ($P < 0.001$) in comparison to thermoneutral conditions suggesting a decrease in rest and recovery. The rumination time also decreased in parallel (8.2 to 4.3 h/day; $P < 0.001$) and this has implications on rumen functionality and nutrient utilization. The activation of the HPA-axis, as reflected in the level of serum cortisol, was significantly increased in both categories of stress compared to thermoneutral stress, by 4.6-fold in the severe stress category ($P < 0.001$) and 4.0-fold in the thermoneutral stress category ($P < 0.001$), reflecting a significant neuroendocrine perturbation. Shade-seeking behavior rose from 18.3% to 82.6% of day time and aggressive interactions, an indicator of welfare, related to social stress rose 4.6-fold (P < 0.001). There was a significant increase in water intake (38.2 to 84.1L/day) to meet evaporative cooling requirements.

Table 5. Welfare and Behavioral Indicators Across Thermal Stress Categories (Mean ± SEM)

Welfare Indicator	Thermoneutral	Mild Stress	Moderate Stress	Severe Stress
Lying time (h/day)	12.4 ± 0.6^{a}	10.8 ± 0.7^{b}	9.1 ± 0.8^{c}	7.2 ± 0.9^{d}
Rumination (h/day)	8.2 ± 0.5^{a}	7.1 ± 0.6^{b}	5.8 ± 0.5^{c}	4.3 ± 0.6^{d}
Water intake (L/day)	38.2 ± 2.4^{a}	52.6 ± 3.1^{b}	68.4 ± 4.2^{c}	84.1 ± 5.3^{d}
Shade-seeking (%time)	18.3 ± 3.1^{a}	42.7 ± 4.8^{b}	64.3 ± 5.9^{c}	82.6 ± 6.4^{d}
Cortisol (ng/mL)	8.4 ± 0.6^{a}	14.2 ± 0.8^{b}	22.8 ± 1.2^{c}	38.4 ± 2.1^{d}
Aggressive acts/h	1.8 ± 0.4^{a}	3.4 ± 0.6^{b}	5.6 ± 0.8^{c}	8.2 ± 1.0^{d}

$^{a-d}$ Different superscripts within rows indicate significant differences (P < 0.05). HPA = Hypothalamic-Pituitary-Adrenal.

The lying time data showed a decrease in lying time, and increased shade-seeking behaviour, as a direct measure of the negative welfare events in the Five Domains of thermal stress [19]. Increased number of aggressive interactions by just 4.6 times could be caused by competition for thermodynamically preferred resting/cover and shading spots, a secondary welfare and injury risk dimension. The farming design implication of these results is that adequate shaded resting area (4 m^2/cow at least in the conditions simulated for zone C in 2050) is suggested as a priority intervention for farm design using the current data.

4.6. Correlation Analysis

Figure 6 shows that there are complex inter-relationships among thermal, productive, physiological and welfare variables; these relationships are presented in a Pearson correlation matrix. Strong positive correlation was seen between THI and Rectal temperature (r = 0.82), Respiration rate (r = 0.88) and Heart rate (r = 0.76) and strong negative correlation with Milk yield (r = −0.74), Feed intake (r = −0.65) and Heat tolerance coefficient (r = −0.79). There was a positive correlation between milk yield and feed intake (r = 0.55) and HTC (r = 0.71) while milk yield was negatively correlated with the respiration rate (r = −0.72). All correlations were significant at P < 0.001 where the absolute value of r > 0.50.

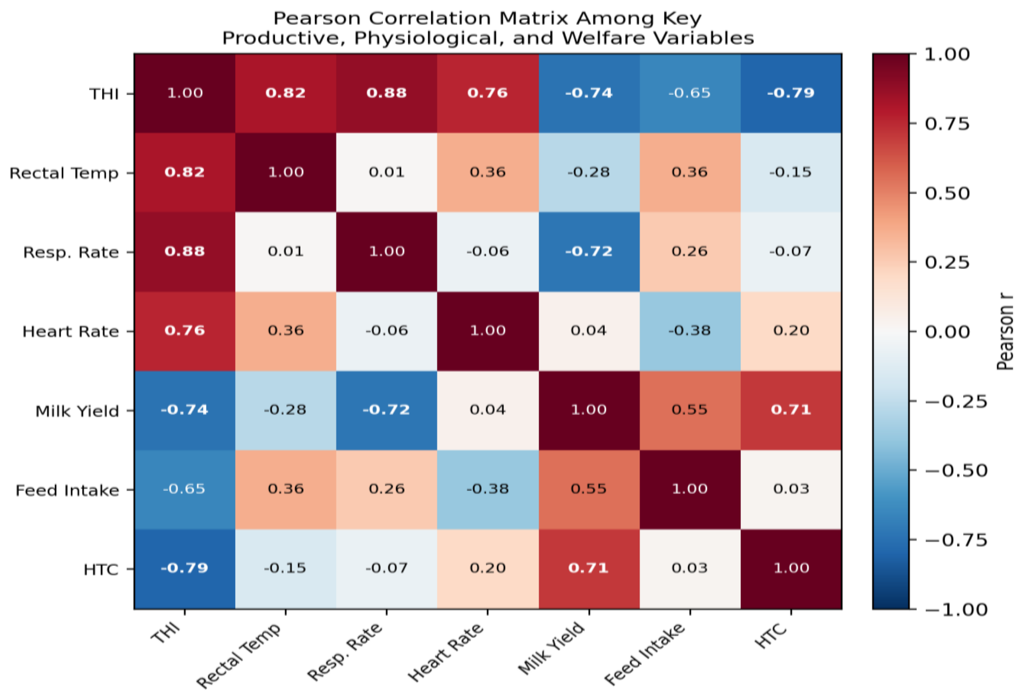

Figure 6. Correlation Matrix of Production, Physiology, and Welfare Traits

The correlation heatmap shows that THI is a key variable in the center of the domain with high correlation with almost all the measured outcome domains, reinforcing that THI is a valuable indicator that can be used as one composite variable in routine and early-warning monitoring systems. The high correlation between respiration rate and milk yield ($r = -0.72$) in particular indicates that this parameter, which can easily be observed, could be used as an indicator of productive impairment in real time, especially in resource-limited farming systems where a milk-recording system is not available [20]. This finding directly contributes to the precision livestock farming translational pathway that has been observed in related work and cited, where low cost, observation-based proxies are incorporated into cooling-trigger systems and systems are automated [20].

5. CONCLUSION

This study offers all-round, longitudinal evidence of the substantial effects of thermal stress on productive performance, animal's state of homeostasis and animal welfare in different livestock systems in South Asia with a quantification of these effects that have been determined to be different depending on the livestock species. The main conclusions are as follows: (1) THI>72 is correlated with gradual significant (excluding a plateau effect) decreases in milk yield, fat percentage, feed consumption and lying time for all genotypes investigated, with dose-response relationships being linear over the range of THI studied, which justifies the use of THI based regression in predictive productivity modelling; (2) There were statistically significant increases in rectal temperature, respiratory rate, cortisol and shade seeking behaviour with increasing THI for all genotypes and dose-response relationships were linear over the range of THI investigated, with no plateau effect noted; (3) Markedly greater changes in milk productivity with increasing THI were observed for the lower milk yield per day genotypes than for the higher milk yield per day genotypes; (4) There were no differences among the genotypes in either lying time or feed consumption in response to THI. Zebu-origin breeds (Gir and Kankrej) have higher heat-tolerance coefficients (HTC > 80%) as compared to HF × Sahiwal crossbreds (HTC = 72.4%) and hence, they can be recommended for breed selection in agro-climatic zones B, C and D based on quantitative parameter.

The critical thermal welfare-risk areas are Zone C (Rajasthan) where THI is above the severe-stress threshold for over 90 cumulative days/year and Zone B (Maharashtra) where THI is above the severe-stress threshold for over 70 cumulative days/year, indicating the need for urgent infrastructure interventions such as evaporative cooling, shade structures and feeding regimen modifications in these zones. The well-established relationship between the THI, physiological measurements, productivity and welfare measurements justifies the development of a multi-parameter precision-livestock-monitoring tools that includes THI as one of the trigger variables for automated farm-management interventions. The relationships should be confirmed in the future using 2050 and 2100 climate projections with coupled climate-livestock modelling systems as well as extending the current breed panel of genotypes to other indigenous breeds and crossbred populations appropriate to other tropical production areas.

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Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Dr. T Rajendran	✓		✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓

C : **C**onceptualization
M : **M**ethodology
I : **I**nvestigation
R : **R**esources
Vi : **Vi**sualization
Su : **Su**pervision

So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

No conflict of interest.

Informed Consent

We have obtained informed consent from all individuals included in this study.

Ethical Approval

Not applicable.

Data Availability

Data availability does not apply to this paper as no new data were created or analyzed in this study.

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