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RESEARCH ARTICLE

Seasonal dynamics of Ocular Infrared Thermography (OST) in heifers under tropical conditions: Links with cortisol and physiological stress

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Abstract: Heat stress presents a significant challenge for livestock production in tropical climates, creating a need for reliable non-invasive indicators to monitor animal welfare. This study assessed the seasonal variability of ocular surface infrared thermography (IRT), which has potential to assess an animal's stress response, as well as its association with serum cortisol and physiological parameters. A total of 36 heifers [Sahiwal (SW) = 18; Karan Fries (KF) = 18] were randomly assigned each season, with six animals observed twice per season. OST was documented at three ocular locations-the inner canthus (EYETi), central ocular surface (EYETc), and outer canthus (EYETo) were obtained via IRT, and rectal temperature and respiratory rate were recorded, and serum cortisol was obtained simultaneously using ELISA. Among all the ocular regions, the EYETi region demonstrated the most consistent and responsive thermal variation across seasons, with higher mean temperatures of 33.53 ± 0.30°C in winter to 35.96 ± 0.14°C in spring, and reaching a peak at 40.02 ± 0.17°C during summer in KF. These values corresponded with seasonal increases in RT (from 100.68/ °F to 102.72°F) and serum cortisol levels, which were highest during summer, particularly in Karan Fries, indicating heightened heat sensitivity in them. Ocular surface temperature correlated positively with established stress indicators, including RT (r/ =/ 0.89, p/ </ 0.001), respiration rate RR (r/ =/ 0.92, p/ </ 0.001), and serum cortisol level (r/ =/ 0.88, p/ </ 0.001) suggesting that infrared thermography of the inner ocular region can serve as a non-invasive, reliable indicator of physiological stress in growing animals.

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Introduction

Heat stress poses a significant challenge to animal agriculture, threatening both animal welfare and economic viability (Narayan et al. 2023; Phillips et al. 2024). The increasing frequency and intensity of high ambient temperatures, driven by global climate change (Banerjee et al. 2024), further exacerbate this issue across livestock production systems. Heat stress adversely affects productivity, reproductive efficiency, immune competence, and survival, thereby compromising the sustainability of dairy and meat industries (Hussain et al. 2023). Consequently, the development of accurate and reliable tools for early detection and monitoring of heat stress is of paramount importance. Traditionally, heat stress is assessed using physiological indicators such as rectal temperature, respiration rate, and behavioural responses, including panting scores. Although rectal temperature is considered the gold standard, its invasive nature may induce additional stress and influence physiological measurements. Similarly, panting scores, while non-invasive, are subjective and prone to observer variability, limiting their reliability (Idrish et al. 2021). These limitations necessitate the adoption of objective, non-invasive, and real-time monitoring techniques. Infrared thermography (IRT) has emerged as a promising alternative, offering rapid, non-contact measurement of surface temperature with strong correlations to physiological stress indicators (Johnson et al. 2011). IRT enables continuous monitoring without disturbing the animal, thereby minimizing handling-induced bias and improving welfare (McCafferty, 2007; Polat et al. 2010; Schaefer et al. 2012). Among the various anatomical sites, the ocular region particularly the lacrimal caruncle has been identified as a highly sensitive thermal window due to its rich vascularization and sympathetic innervation. This region exhibits rapid temperature changes that closely reflect alterations in core body temperature and systemic stress responses (Nogami et al. 2014; Comin et al. 2023). Compared to other body regions, ocular temperature is less influenced by hair coat, skin thickness, and external contaminants, making it a more consistent and precise indicator of thermal stress. The ocular surface represents a dynamic interface between internal physiological status and

external environmental conditions (Cholkar et al. 2013). Measurement of ocular surface temperature (OST) using IRT provides a unique opportunity to assess both local ocular responses and systemic thermoregulatory changes. However, limited information is available regarding regional variations within the ocular surface and their consistency across different environmental conditions and breeds. Therefore, the present study was undertaken to (i) evaluate ocular surface temperature across different anatomical regions of the eye (medial, lateral, and central) using infrared thermography, (ii) identify the most reliable ocular thermal window for heat stress assessment, and (iii) assess seasonal and breed-related variations in ocular thermal responses in native and crossbred heifers.

Materials and Methods

Experimental site

The present investigation was carried out at the Climate Resilient Livestock Research Centre (CRLRC), ICAR-National Dairy Research Institute, Haryana, India, within an arid to semi-arid geographic zone characterized by a latitude and longitude of 29° 41' N and 76° 59' E, at an elevation of 243 m above mean sea level. The climatic conditions in this locale exhibit an annual average maximum and minimum ambient temperature spanning from 0 °C to 45 °C. The relative humidity fluctuates between 30% and 80%, and the annual precipitation in the area ranges from 600 to 750 mm.

Meteorological data and Temperature-Humidity Index (THI)

The data on weather parameters such as relative humidity (RH%), and dry and wet bulb temperature (°C) was obtained without interruption from the meteorological station at CSSRI, which is located around 4 Km from the study area. Dry and wet bulb temperatures were then used for the calculation of THI. The calculation of THI followed the formula proposed by McDowell (1972):

$$THI = 0.72 (Tdb + Twb) + 40.6$$

Where T_{db} = Dry bulb temperature (°C) and T_{wb} = Wet bulb temperature in (°C).

Ethics approval

Experimental approval for conducting the present investigations was obtained from the Institutional Animal Ethics Committee according to the Committee for Control and Supervision of Experiments on Animals rules, laid down by the Government of India, No. 51-IAEC-24-17 dated 24.03.2024.

Animal Management

The heifers were accommodated in a semi-controlled, specially designed animal shed featuring a custom-made gabled roofing, optimal ventilation, and adjustable side curtains to regulate airflow and microclimate. The shed had concrete flooring with a gentle slope for efficient drainage and was maintained under strict hygienic conditions. Animals were provided ad libitum access to fresh drinking water. Prophylactic measures against endo- and ectoparasitic infestations were regularly implemented to ensure the continuous health and welfare of the animals throughout the study period.

Experimental Design

A total of 36 heifers [Sahiwal (SW) = 18; Karan Fries (KF) = 18], aged 12-15 months and averaging 290/ ±/ 30/ kg, were randomly assigned each season, with six animals observed twice per season. The study spanned three seasons-winter, spring, and summer-to capture seasonal thermal stress responses. Twelve observations per session were recorded, all conducted between 13:00 and 14:00 hr.

Parameter in this study:

In this study, three key physiological and biochemical parameters were measured to assess heat stress in cattle. Rectal temperature (RT) was recorded using a digital veterinary thermometer to estimate core body temperature. Ocular surface temperature (OST) was evaluated using infrared thermography across three eye regions: the inner canthus (EYEi), central ocular surface (EYEc), and outer canthus (EYETo), providing a non-invasive thermal profile. Additionally, serum cortisol concentrations were determined via ELISA to serve as a biochemical indicator of systemic stress.

Infrared Thermography - Ocular Surface Temperature (OST, °C) Mapping

A Fluke Tix 580 (Fluke Corp., Everett, WA, USA), an infrared thermal camera with 1280 × 960 quality images having a calibration range of 25.0 °C to 105.0 °C was used for recording body surface temperatures (muzzle, eye, forehead and flank). Before the experiment, the thermal camera was calibrated, and the emissivity was set at 0.95. This camera features a 240$^{\circ\%}$ rotating screen with a 640 × 480 display, a sensitivity of 0.1 °C, and an imaging range of around 1–20 m. Using an uncooled microbolometer with a 120 × 120 focal plane arrays, the camera scanned the picture. The thermal images were captured following the methods given by Sathesan et al. (2024). A schematic graphical presentation has been given in Fig.1, where the left panel shows the anatomical landmarks that are employed for thermographic evaluation: the inner canthus, the central ocular area, and the outside canthus. The right panel displays infrared thermographic images of the eye area in Sahiwal (top) and Karan Fries (bottom) cattle. The

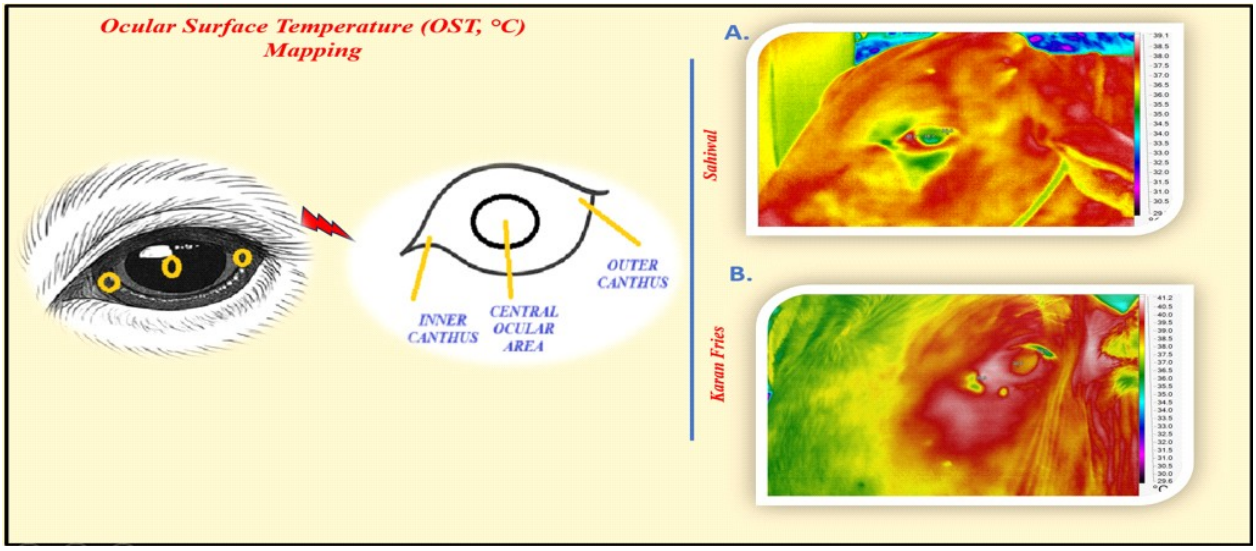


Fig.1 Schematic representation of Ocular Surface Temperature (OST, °C) Mapping via Infrared Thermography. The schematic diagram (left) shows anatomical regions of interest: inner canthus, central ocular area, and outer canthus. The right panels represent infrared thermal images of Sahiwal (A) and Karan Fries (B)

photos were then analysed using the Fluke ® Systems Inc. Smart View Classic 4.4 program (https://assets.fluke.com/SmartView/TUpdates/Setup.exe). A high-contrast colour palette was used, and the "marker" tool was manually selected to determine the average temperatures (°C) of the OSTs of animals. All thermal images of the experimental animals were taken between 1:00 P.M. and 3:00 P.M. A fixed distance of 1 meter was maintained between the camera and the cows during each capture to ensure uniformity in the imaging process. The same operator captured all the thermal images of the animals throughout the study, thus minimizing variability due to operator differences.

Statistical analysis

Data were analysed using IBM SPSS Statistics Version 22.0 (SPSS Inc., Chicago, IL, USA). A General Linear Model (GLM) was used to assess the main effects of breed (SW vs. KF), season (SPRING, HD, and HH), and their interaction on (EYEi, EYEc, EYETo) physiological responses, hormone levels, and body surface temperatures. Results were expressed as mean ± standard error (SE), and significance was determined at $P < 0.05$. Pearson's bivariate correlation analysis was conducted, with correlation coefficients (r) evaluated at a two-tailed significance level of $P < 0.01$ and $P < 0.05$. The magnitude and direction of the correlations were classified into five-part quintiles according to Landis and Koch (1977): $0 \leq r < 0.2$ very weak; $0.2 \leq r < 0.4$ weak; $0.4 \leq r < 0.6$ moderate; $0.6 \leq r < 0.8$ strong, and $0.8 \leq r < 1$ very strong. Linear regression analysis was used to examine relationships between physiological responses, cortisol levels, and ocular surface temperatures.

Blood sampling and hormonal quantification

Blood samples were collected weekly from all the cows using sterile serum vacutainer tubes (Vacuette®-clot activator, Greiner Bio-One GmbH, Austria) via jugular vein puncture, minimizing animal disturbance. The blood samples were immediately transferred to the laboratory and allowed to clot at ambient temperature for 30 minutes, and centrifuged at 3,000 rpm for 15 minutes. The separated serum was stored at –20°C in labelled cryovials until further analysis. Cortisol levels were determined by a competitive Bovine ELISA kit (BT Lab, Cat. No: EA0021Bo) with a detection limit of 1–400 ng/mL, and a sensitivity of 0.52 ng/mL. All reagents, standard solutions were prepared as instructed, and serum samples were brought to room temperature (~30!) before use. The assay was carried out according to the manufacturer's recommended protocol and the optical density was measured at 450 nm using a microplate reader (MultiSkan GO, Thermo Scientific). Intra-assay and inter-assay coefficients of variation (CV) were determined for intra-assay CV to 8 % and inter-assay CV to 10 %.

Results and Discussion

Metrological variation and physiological responses:

Seasonal variation in Temperature-Humidity Index (THI) has been illustrated (Fig.2) with winter showing the lowest value (50.14), spring (60.95), and summer reaching the highest level (81.64), reflecting severe heat stress conditions. The effects of season, breed, and their interaction have been measured. (Table no.1) Presented physiological parameters, including respiration rate (RR), rectal temperature (RT), and serum cortisol levels, which were found to be statistically significant ($p < 0.001$) across the

groups, with varying patterns observed between the two breeds: KF and SW. A highly significant ($p < 0.001$) seasonal effect was observed in the respiration rate ($p < 0.001$), with a sharp increasing trend from winter to summer. For the KF breed, RR increased from 20.17 ± 0.42 breaths/min in winter to 24.50 ± 0.35 in spring, and peaked at 33.83 ± 0.37 during summer. Similarly, SW breed showed a parallel trend with RR values rising from 17.75 ± 0.50 (winter) to 24.75 ± 0.41 (spring) and 31.75 ± 0.47 (summer). The interaction between breed and season was also significant ($p = 0.00$), suggesting breed-specific seasonal variation. Notably, KF maintained consistently higher RR values than SW across all seasons. Rectal temperature was also significantly influenced by season and breed (both $p < 0.001$), and their interaction was statistically significant, though smaller ($p = 0.04$). In the KF breed, RT increased from 100.98 ± 0.05°F in winter to 101.37 ± 0.06 in spring, and 102.72 ± 0.10 in summer. SW breed followed a similar pattern with RT values of 100.68 ± 0.09 (winter), 101.11 ± 0.08 (spring), and 101.98 ± 0.08 (summer). While both breeds experienced significant seasonal increases, KF consistently showed slightly higher RT than SW in all seasons.

Serum cortisol levels

Cortisol levels demonstrated a marked seasonal variation ($p < 0.001$) and were significantly influenced by breed ($p < 0.001$) and the breed × season interaction ($p = 0.00$). For the KF breed, cortisol levels rose from 16.34 ± 0.26 ng/ml in winter to 22.30 ± 0.20 in spring, reaching 32.68 ± 0.52 ng/ml in summer. Interestingly, the SW breed exhibited a different seasonal pattern, with cortisol levels highest in winter (22.95 ± 0.17), decreasing in spring (17.44 ± 0.34), and then increasing again in summer (27.83 ± 0.20). This crossover interaction suggests a breed-specific stress response pattern to seasonal changes.

Ocular thermogram analysis

Infrared thermographic measurements of ocular regions, including inner canthus, outer canthus, and central ocular /conjunctival surface temperatures, were significantly affected by season, breed, and their interaction ($p < 0.001$ for all parameters).

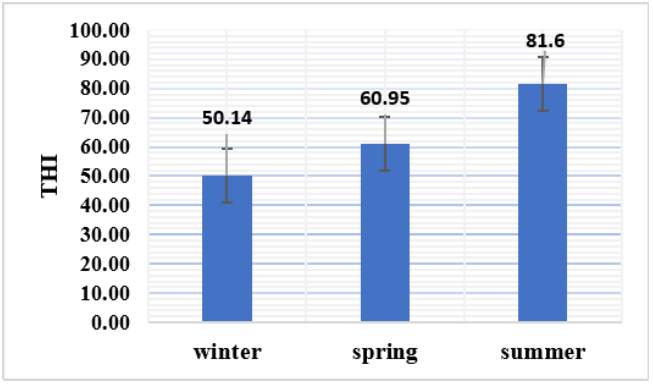


Fig. 2. Seasonal variation in Temperature-Humidity Index (THI) during the experimental period.

Representative thermal images have been shown in fig.3, highlighting the temperature gradients observed in the eye region of both breeds. Notably, KF animals exhibited relatively higher ocular surface temperature (OST, °C), particularly in the inner canthus area, compared to SW. The box plot compares overall OST in KF and SW cattle across three eye regions: inner canthus (EYE_{Ti}), outer canthus (EYE_{To}), and central surface (EYE_{Tc}). Overall, KF exhibited higher temperatures, especially at the inner canthus (~36–40°C). The radar plot illustrates (Fig.4) a clear trend of increasing ocular surface temperatures (EYE_{Ti}, EYE_{To}, EYE_{Tc}) with rising THI. Distinct seasonal trends were found, as THI escalates, particularly in KF heifers, all ocular temperature values increase correspondingly. In contrast, SW heifers show lower temperature profiles across all ocular regions for the same THI levels, indicating a breed-specific thermal response. Among ocular regions, the inner canthus (EYE_{Ti}) maintains the highest temperature values, followed by central (EYE_{Tc}) and outer canthus (EYE_{To}), consistently aligning with the THI variation were observed, with higher temperatures were recorded during summer in both breeds. However, breed-specific differences in temperature distribution and interaction effects were evident.

Table 1: Mean Values of Rectal temperature and respiration rate and Serum Cortisol in Karan Fries and Sahiwal

Parameters/Seasons		Winter (THI=50.14)	Spring (THI=60.95)	Summer (THI=81.64)	Breed	season	Breed*season
RR (Breath/min)	KF	20.17^{cX}±0.42	24.50^{bX}±0.35	33.83^{aX}±0.37	<0.001	<0.001	0.00
	SW	17.75^{cY}±0.50	24.75^{bY}±0.41	31.75^{aY}±0.47	<0.001	<0.001	0.00
RT(ºF)	KF	100.98^{cX}±0.08	101.37^{bX}±0.09	102.72^{aX} ±0.10	<0.001	<0.001	0.04
	SW	100.68^{X}±0.09	101.11^{bY}±0.08	101.98^{aY}±0.08	<0.001	<0.001	0.17
Cortisol(ng/ml)	KF	16.34^{cX}±0.26	22.30^{bX}±0.20	32.68^{aX}±0.52	<0.001	<0.001	0.00
	SW	22.95^{cY}±0.17	17.44^{bY}±0.34	27.83^{aY}±0.20	<0.001	<0.001	0.00

Superscript a,b within row indicates significant difference between seasons(P<0.05), and Superscript X, Y within column indicate significant difference between breeds (P<0.05).

Inner Canthus Temperature (EYE_{Ti})

Ocular surface temperature presented in Table 2 shows that Seasonal variation had a significant ($p < 0.001$) impact on inner canthus temperature (EYETi). In the KF breed, temperature increased significantly from 33.53 ± 0.3°C in winter to 35.97 ± 0.14°C in spring, reaching a peak at 40.02 ± 0.17°C in summer. Similarly, in the SW breed, temperatures followed a similar ascending pattern: 34.01 ± 0.10 °C (winter), 34.90 ± 0.12 °C (spring), and 37.95 ± 0.10 °C (summer). The interaction effect (breed × season) was highly significant ($p = 0.00$), and variations in superscripts (X vs Y) indicate breed-specific thermal responses across seasons, with SW generally exhibiting slightly higher inner canthus temperature than KF in winter and spring. For the outer canthus, both seasonal and breed effects were significant ($p < 0.001$ and $p < 0.05$, respectively), and their interaction effect was also highly significant ($p = 0.00$). KF showed a progressive increase from 28.48 ± 0.35°C in winter to 33.17 ± 0.35°C in spring, and 37.47 ± 0.31°C in summer. SW demonstrated slightly higher values in winter (34.01±0.10 °C) but lower in summer compared to KF (37.95 ± 0.10°C). The statistical interaction implies a breed-specific difference in heat dissipation through the outer canthus.

Central Ocular Temperature (EYE_{Tc})

Central ocular surface temperature also exhibited significant differences ($p < 0.001$) across seasons, breeds, and their interaction. In KF, the temperature increased markedly from 29.00 ± 0.32°C (winter) to 33.54 ± 0.60°C (spring) and further to 37.82 ± 0.27°C (summer). SW also showed a similar increasing trend with lower overall values: 29.96 ± 0.15°C (winter), 31.44 ± 0.23°C (spring), and 34.66 ± 0.12°C (summer).

Correlation and regression analysis

The correlation matrix (Table 3) shows a strong positive relationship between THI and all ocular temperature parameters: EYEi (0.93), EYETo (0.87), and EYEc (0.89). Among these, EYEi has the highest correlation with both THI and physiological indicators—respiration rate (RR: 0.92) and rectal temperature (RT: 0.89). Cortisol is also positively correlated with THI (0.82) and ocular temperatures, with a strong correlation with EYEi (0.88). RR and RT exhibit strong correlations with THI ($r = 0.98$ and 0.86, respectively). Linear regression analysis (Fig. 6a–d) revealed significant associations between the Temperature–Humidity

Table 2: Mean Values of Ocular Surface Temperature Across Breeds and Seasons in Karan fries and Sahiwal heifers

Parameters/Seasons		Winter (THI=50.14)	Spring (THI=60.95)	Summer (THI=81.64)	Breed	season	Breed*season
Inner Canthus temperature (EYEi)	KF	33.53 ^{bY}±0.30	35.96^{bX}±0.14	40.02^{aX}±0.17	<0.001	<0.001	0.00
	SW	34.01^{bX}±0.10	34.90^{bY}±0.12	37.95^{aY}±0.10	<0.001	<0.001	0.00
Outer Canthus temperature (EYEi)	KF	28.48^{bX}±0.35	33.17^{bX}±0.35	37.47^{aX}±0.31	<0.05	<0.001	0.00
	SW	31.85^{bX}±0.19	33.21^{bX}±0.21	36.05^{aY}±0.15	<0.05	<0.001	0.00
Central ocular temperature (EYEi)	KF	29.09^{bX}±0.32	33.54^{bX}±0.60	37.82^{aX}±0.27	<0.001	<0.001	0.00
	SW	29.96^{bY}±0.15	31.44^{bY}±0.23	34.66^{aX}±0.12	<0.001	<0.001	0.00

Superscript a,b within row indicates significant difference between seasons($P<0.05$), and Superscript X, Y within column indicate significant difference between breeds ($P<0.05$).

Table 3: Correlation matrix of ocular surface temperature, serum cortisol, and physiological parameters

Variable	THI	CORT	EYETi	EYETo	EYETc	RR	RT
THI	1.00						
CORT	0.82**	1.00					
EYETi	0.93**	0.88**	1.00				
EYETo	0.87**	0.81**	0.87**	1.00			
EYETc	0.89**	0.84**	0.93**	0.85**	1.00		
RR	0.98**	0.79**	0.92**	0.82**	0.87**	1.00	
RT	0.86**	0.76**	0.89**	0.76**	0.88**	0.87**	1.00

Values represent Pearson's correlation coefficients (r). ** indicates significance at $p < 0.01$; * indicates $p < 0.05$. THI = Temperature-Humidity Index; CORT = serum cortisol; EYETi = inner ocular temperature (medial canthus); EYETc = central ocular temperature; EYETo = outer ocular temperature; RR = respiratory rate; RT = rectal temperature.

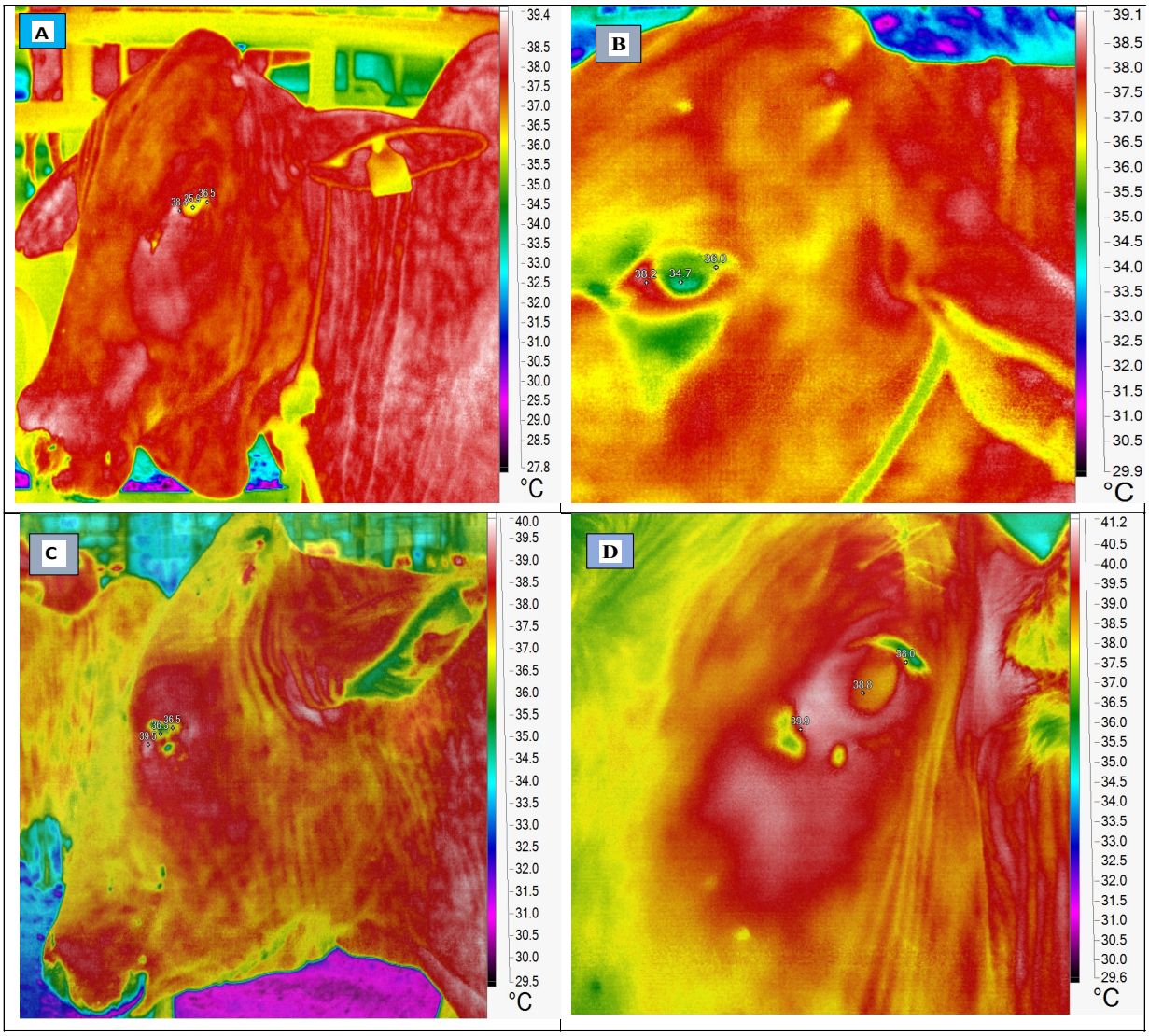


Fig. 3. Infrared thermographic images of Sahiwal and Karan Fries cattle showing ocular surface and facial temperature mapping (B, A) Close-up of the eye and full facial view in Sahiwal. (D, C) Close-up of the eye and full facial view in Karan Fries

Index (THI) and both physiological and ocular surface temperatures (OSTs) in Sahiwal (SW) and Karan Fries (KF) heifers. For cortisol, the regression equations were $y = 0.2011x + 9.82$ ($R^2 = 0.370$) in SW and $y = 0.516x - 9.414$ ($R^2 = 0.970$) in KF, indicating a stronger THI–cortisol relationship in the crossbred animals. For OST, the inner canthus (EYE Ti) showed close associations with THI, with regression equations of $y = 0.1278x + 27.416$ ($R^2 = 0.943$) in SW and $y = 0.2046x + 24.361$ ($R^2 = 0.932$) in KF. Similar positive relationships were observed for the central globe (EYE Tc: SW, $y = 0.2676x + 16.290$, $R^2 = 0.891$; KF, $y = 0.1503x + 22.368$, $R^2 = 0.922$) and the outer canthus (EYE To: SW, $y = 0.1337x + 25.116$, $R^2 = 0.892$; KF, $y = 0.2749x + 15.379$, $R^2 = 0.839$). Among the ocular regions, the inner canthus exhibited the strongest and most consistent association with THI across breeds.

The present study investigated OST mapping using IRT across different seasons, alongside measurements of physiological parameters and cortisol levels in two distinct livestock breeds. Our findings provide valuable insights into the complex interaction between environmental factors, physiological responses, and OST regulation. Notably, aligning with the highest temperatures were recorded during the summer months and the lowest during winter, consistent with previous findings by Stewart et al. (2017) and Idris et al. (2021). As illustrated in the Box plot (Fig. 5), comparative ocular temperature measurements were taken at these anatomical sites EYETi (inner canthus), EYETo (outer canthus), and EYETc (cornea)-across both breeds, Karan Fries (KF) and Sahiwal (SW), reveal consistent patterns. Across all three anatomical sites, KF exhibited higher median temperatures than SW, which may be attributed to higher metabolic activity or

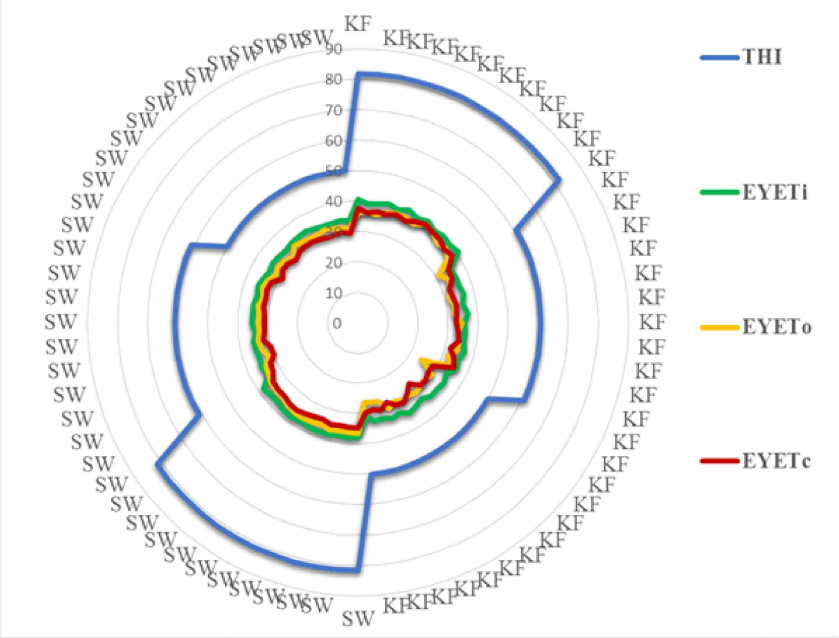


Fig. 4 Radar plot showing the relationship between ocular surface temperatures and Temperature-Humidity Index (THI).

less efficient heat dissipation. Specifically, for EYETi, KF maintained a higher median temperature (~38°C), while SW displayed a broader range around ~35°C, reflecting more flexible temperature regulation. This trend persisted at the other sites: for EYETo, KF had a median of ~36°C compared to SW's ~34°C; and for EYETc, KF recorded a median of ~37.5°C, whereas SW's median was ~32.5°C. suggesting individual differences in thermoregulation. Church et al. (2014) suggest that such seasonal variation probably reflects the ocular surface thermoregulatory response to changes in ambient temperature. This thermographic study focused on three separate eye areas: inner (medial canthus), centre (cornea), and outer (lateral canthus). Among these areas, the inner eye showed the more pronounced temperature changes across seasons and between breeds. consistent with the finding of Jansson et al. (2021), who reported greater thermal responsiveness of the medial/inner canthus in horses. This increased thermographic variation in the inner eye area is likely due to its rich vascular supply from the angularis oculi vessels and superior lacrimal artery (Dyce et al. 2010; Gloster et al. 2011), which is highly responsive to autonomic nervous system modulation under stress and environmental challenges Phillips et al. (2015). In contrast, the Lateral and central areas exhibited less heat reactivity, possibly due to greater exposure to environmental factors such as angle of incidence, air speed, anatomical variation, and difference in eyelash type and density (Stewart et al. 2008; McManus et al. 2016).

The range of temperatures for KF was wider at around 25 to 40 degrees centigrade. It is quite interesting that we noted a change in the boundaries of the seasonal OST shift. Throughout the study KF breed had bigger OST measurement changes compared to the SW breed, which had consistent measurements regardless

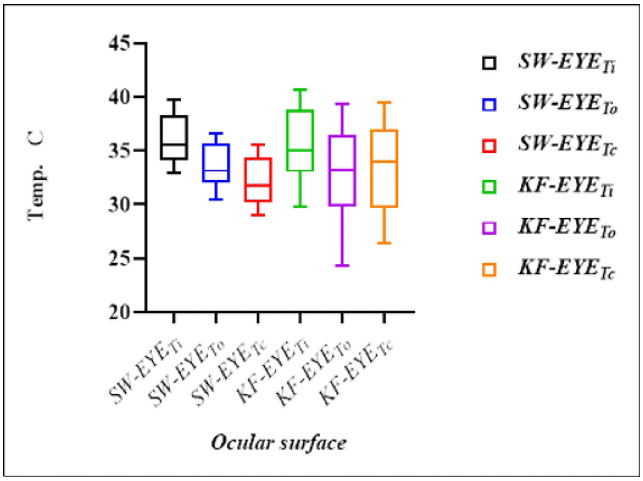


Fig. 5 Comparative ocular surface temperature (OST, °C) at specific anatomical sites.

of the weather. This breed difference stood out the most, especially around the inner eye region. The temperature difference between winter and summer in SW was 3.94°C, while it was only 7.51°C for KF. This observation is consistent with the findings of McManus et al. (2016), suggesting that different breeds of cattle have various mechanisms for thermoregulation. As suggested by Faghihi et.al. (2023), variations in ocular surface morphology, tear film composition, or vascular supply between breeds may underlie the physiological differences.

Ocular surface temperature (OST) showed strong associations with physiological responses, consistent with previous reports (Stewart et al. 2008; Martins et al. 2018). Respiratory rate and core temperature were positively correlated with OST, likely

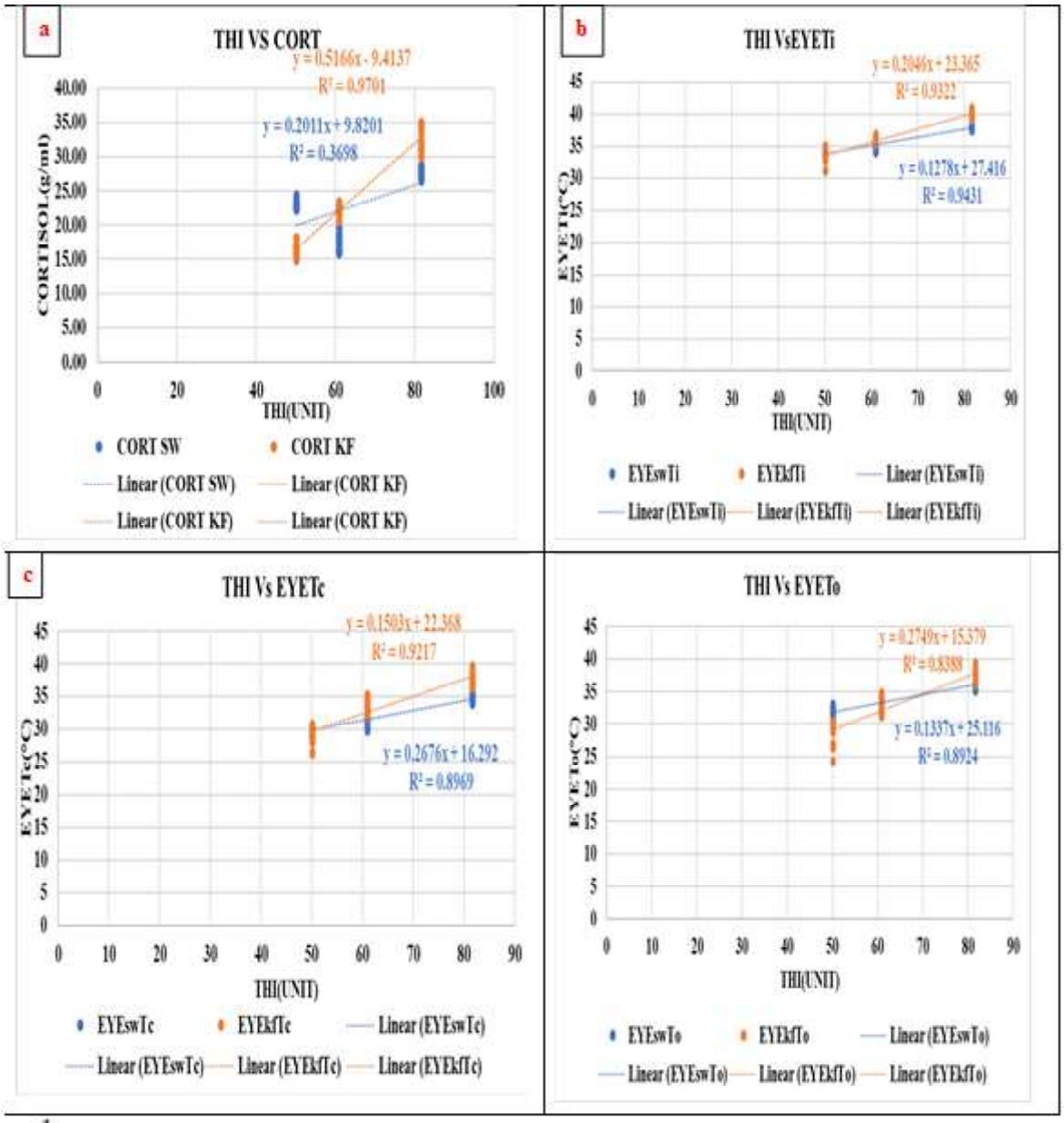


Fig. 6a–d Regression analysis illustrating the relationship between Temperature-Humidity Index (THI) and physiological/ocular thermal parameters in Sahiwal and Karan heifers, (a) THI vs CORT levels; (b) THI vs EYETi (inner canthus); (c) THI vs EYETc (central ocular region); (d) THI vs EYETo (outer canthus).

reflecting changes in blood flow and heat dissipation (Schaefer et al. 2007), with the strongest relationship in the inner ocular region (r = 0.89), followed by central (r = 0.88) and outer regions (r = 0.76), supporting earlier findings (Martins et al. 2018; Shu et al. 2022; Arfuso et al. 2022). Regression analysis further revealed significant associations between THI, cortisol, and ocular temperatures, particularly in Karan Fries heifers, where cortisol showed a strong relationship with THI ($R^2 = 0.97$, $p < 0.001$). Among ocular regions, the inner canthus (EYETi) consistently demonstrated the highest sensitivity to thermal stress, whereas the outer region showed weaker associations. Among ocular regions, the inner canthus (EYETi) consistently demonstrated the highest sensitivity to thermal stress, whereas the outer region showed weaker and less consistent associations. This suggests that the outer canthus temperature is more variable and has a reduced sensitivity to ambient heat. Compared to conventional indicators such as rectal temperature and respiration rate, ocular IRT provides a non-invasive, real-time assessment with reduced handling stress and improved repeatability. Its ability to detect subtle thermal variations enhances sensitivity for early heat stress detection. Further integration of hormonal and infrared thermographic biomarkers highlights breed-specific differences

in heat resilience. Ocular infrared thermography (inner, middle, and outer eye regions) showed strong linear associations with THI in both breeds, with steeper regression slopes in KF, indicating greater sensitivity to thermal stress. Among the regions, inner ocular temperature (EYETi) consistently exhibited the strongest association, supporting its closer reflection of core body temperature. These findings align with previous reports advocating ocular temperature as a reliable non-invasive stress indicator (Arfuso et al. 2022; Bartolomé et al. 2019). Additionally, a clear relationship between cortisol levels and ocular surface temperature was observed, particularly in the inner ocular region during summer, suggesting a strong interaction between environmental stress and physiological response.

Conclusions

The present study reveals that ocular surface temperature (OSTs), particularly at the inner ocular region (lacrimal caruncle), is a reliable and non-invasive indicator of heat stress in livestock when assessed using infrared thermography. The findings emphasize the importance of a standardized imaging protocol to ensure accuracy and comparability. Importantly, early detection of thermal stress using ocular IRT can facilitate timely management interventions. Overall, ocular IRT offers a practical and objective tool for real-time monitoring of animal welfare and production efficiency. Further research is necessary to validate its applicability across diverse species, including lactating animals, and to integrate it with conventional physiological indicators for improved heat stress assessment.

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