Research Topics

Behavior and heat stress

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Behavior and heat stress

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Editorial: Behavior and heat stress

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Editorial on the Research Topic
Behavior and heat stress

Relating the environment to the quality of life of animals becomes essential when the goal is to achieve welfare. Climatic variations, highlighted mainly by the increase in temperature in recent years, are directly associated with the search for thermal balance for several species to avoid situations of heat stress, which can result in cellular and molecular dysfunctions, thermoregulatory and behavioral disorders, and consequently reduce the health of companion, sports, working, and production animals.

This Research Topic is composed of eight original articles and three reviews, which present arguments through the advancement of tools and technologies for measuring animal temperature; physiological, biochemical, and behavioral changes; handling practices and facilities; and economic advantages, which aim to validate the environmental impact on the thermal comfort zone of these individuals.

These changes can be observed in companion animals, such as dogs and cats, that share the same environment as humans. Palestrini et al. demonstrate through their data that the behavior of these animals is altered with the variation of the ambient temperature. Even without showing an increase in aggressive behavior, both species become more active with milder temperatures and sleep more when there is a greater variation, both upward and downward.

Production animals are constantly studied in this line of research because of their dependence on the control of meteorological variables to achieve high performance. As described by Ragab et al. in their study of rabbits, genetic factors can affect an individual's susceptibility to environmental conditions.

To demonstrate this interaction with the environment, Govindasamy et al. working with different genotypes of pigs, found alterations in physiological, behavioral, and biochemical parameters through measurements of temperature (rectal and skin surface), respiration rate, heart rate, behavior, and hormones measured by blood in a specific environment, and different pig breeds may respond differently to seasonal changes. Gómez-Prado et al. in turn, pointed out the main thermoregulation mechanisms used for this heat exchange between pigs and the environment, addressing concepts for situations not only of hyperthermia but also of hypothermia and highlighting the measurement of temperature using infrared thermography.

Non-invasive technologies are increasingly being used as alternatives for monitoring the body temperature of animals, given the need to validate their use between species, and in comparison with internal body temperatures. For example, Verdegaal et al. studied the applicability of monitoring skin surface temperature as a thermoregulatory response metric in endurance horses during field exercises, as well as the relationship between skin surface temperature and core temperature as measured by gastrointestinal temperature. They found that skin surface temperature monitoring does not provide a reliable proxy for the thermoregulatory response in horses, due to many factors that can modulate skin surface temperature without directly affecting core temperature, and revealed important inter-individual differences in skin surface and core temperature. Mota-Rojas et al. who also discuss the use of infrared thermography, highlight the great usefulness of the tool in measuring the body temperature of newborn ruminants, relating moments of hyperthermia with the development of muscle and adipose cells in these animals and the action of metabolic routes for the utilization of these energy reserves for the activation of thermoregulation pathways. Similarly, Napolitano et al. showed that birth weight is a factor that alters the thermoregulation of water buffalo calves during the first days of life, but that colostrum ingestion can be a compensatory action for better development in such situations, making the thermoregulatory mechanism more efficient.

By studying dairy cows, Shu et al. sought to improve the technique for measuring ocular temperature under heat stress, which is often found in high-performance herds in dairy production. Their study results suggested that infrared thermography can provide a non-invasive and practical approach for assessing heat stress in dairy cows. In addition, the results provided useful recommendations regarding the optimal direction of image acquisition, highlighting the regions of the eye that most effectively reflect thermoregulatory responses in these instances.

In relation to thermoregulation and behavior, the facilities, and methods of handling livestock in production systems are crucial points to discuss. Studying dairy cows, Santos et al. traced the thermoregulatory profile of these animals in tropical environments, mainly discussing environmental aspects and installations that can alter the thermal equilibrium point in pasture production systems. Rodríguez-González et al. in their study, addressed transport management as a possible stress trigger for water buffalos and sought to relate different temperature measurements to represent these values for meat-producing animals. Finally, Maia et al. showed economic advantages when using a shade design to offer confined beef cattle a better thermoregulatory condition, relating their thermal comfort zone within the production system and resulting in increased production rates.

Author contributions

The editorial board of this Research Topic was composed by the CT as main editor. MP, CÇ, FH, and PS as guest editors working in the organization and review of articles. The editorial article was written by the FH and revised by CT, MP, CÇ, and PS. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Evaluation of the Best Region for Measuring Eye Temperature in Dairy Cows Exposed to Heat Stress

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Eye temperature (ET) has long been used for predicting or indicating heat stress in dairy cows. However, the region of interest (ROI) and temperature parameter of the eye have not been standardized and various options were adopted by previous studies. The aim of this study was to determine the best ROI for measuring ET as the predictor of heat stress in dairy cows in consideration of repeatability and validity. The ET of 40 lactating Holstein dairy cows was measured using infrared thermography. The mean and maximum temperature of five ROIs—medial canthus (MC), lateral canthus, eyeball, whole eye (WE), and lacrimal sac (LS)—were manually captured. The results show that the ET of left eyes was slightly higher than that of right eyes. The ET taken in MC, WE, and LS within 2 min had a moderate to substantial repeatability. The maximum temperature obtained at the LS had the highest correlation coefficients with respiration rate and core body temperature (all $p < 0.001$). Therefore, the maximum temperature of LS should be considered by future studies that want to use ET as the predictor or indicator of heat stress in dairy cows.

Keywords: dairy cows, heat stress, eye temperature, region of interest, infrared thermography

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INTRODUCTION

Due to the advantages of non-invasive and non-contact measurement, infrared thermography (IRT) has been welcomed for measuring the welfare indicators and health status of livestock (1–3). Dairy cows are extremely vulnerable to heat stress due to their limited heat dissipation capacity and enormous heat production (4). However, the measurement of gold standard animal-based indicators of heat stress, i.e., respiration rate (RR) and core body temperature (CBT), is both time-consuming and labor-intensive (5). Therefore, body surface temperature measured using IRT has been used for predicting or indicating heat stress in dairy cows for a few years, among which the eye temperature (ET) has been considered most commonly due to its high correlation and agreement with CBT (6–8).

However, the region of interest (ROI) and temperature parameter of the eye have not been standardized and various options were adopted by previous studies. To name but a few, the mean or maximum temperature of the eyeball (EB) (9), the orbita plus surrounding (10), the entire eye (11), and the periocular and lacrimal caruncle (12) have been used for indicating heat stress in cattle.

To determine the best region to measure ET, two key parameters are of importance, i.e., repeatability and validity. The former focuses on whether measurements on the same subject under identical conditions over a short period of time are repeatable, whereas the latter focuses on the ability of a method to measure what it is intended to measure. For revealing heat stress of animals, validity is always expressed using the correlation with gold standard indicators (i.e., RR and CBT).

Some studies have used different methods to measure the repeatability of temperature readings of different ROIs of the cattle eye. Montanholi et al. (13) used the maximum temperature of the entire eye as the representative of ET, and correlation coefficients between two infrared images consecutively taken within 10 s were calculated to express repeatability. Byrne et al. (14) took 30 consecutive infrared images of the eye, the hoof, and the udder respectively, and repeatability was determined based on the level of precision that could be achieved by capturing 30 image replicates. Gloster et al. (15) also used the entire eye to obtain ET and the repeatability of thermal imaging was assessed by testing whether the difference in ET of two images taken within 10 min significantly differed from zero. However, none of the abovementioned studies was conducted under a heat stress condition, and only one ROI was adopted in each study. Therefore, it is interesting to know whether the temperature readings of different ROIs of the eye are repeatable across a heat stress event. As for the validity of ET in revealing heat stress in dairy cows, lots of studies have demonstrated a mild to strong correlation of ET with RR and CBT (10, 16, 17). Bleul et al. (17) reported that the maximum temperature of the entire eye, rather than the medial canthus (MC), had the highest correlation coefficient with the rectal temperature in 30 cows. However, a comprehensive evaluation of ROIs is still required.

The aim of this study was to determine the best ROI for measuring ET as the predictor of heat stress in dairy cows in consideration of repeatability and validity. Our hypothesis was that different ROIs would differ in their performance in reflecting heat stress in dairy cows.

MATERIALS AND METHODS

The experimental protocols were approved by the Experimental Animal Care and Committee of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (approval number IAS2021-220).

Experimental Location and Animals

The study was conducted at an organic intensive dairy farm in Shandong, China (coordinates: 34°50′37′′N, 115°26′11′′E; altitude: 52 m) from June to August in 2021. A total of 40 high-producing (daily milk yield: 40.0 ± 5.9 kg/day), primiparous and multiparous (parity: 2.6 ± 1.1), and mid-lactating (days in milk: 149.9 ± 18.2 days) Holstein dairy cows were randomly selected from a herd reared in a free-stall barn (15 m × 90 m). The barn was covered by a double-pitched roof and was oriented along the north-south longitudinal axis, and therefore, most of the solar radiation was prevented from reaching the cows inside the barn. The barn was equipped with a total of 20 fans (1.1 m in diameter; capacity: 25,000 m^3/h each) and 40 sprinklers (1.5 L/min each; 1 min on and 4 min off) fixed 2.5 m above the ground. Fans were installed at the lying zone at an interval of 6 m and along the feeding line at an interval of 12 m, while sprinklers were installed along the feeding line at an interval of 2 m. Fans and sprinklers were operated normally during the entire study. Cows were milked three times per day at 08:30, 16:30, and 00:00 h. Cows were fed a total mixed ration three times per day after milking and had free access to clean water.

Experimental Design

Physiological measurements were conducted twice on each test day (09:30–11:00 and 14:00–15:30 h). A veterinarian checked the health condition of the cows daily, and no cows were excluded due to health issues. The cows were resting quietly in a lying or standing posture during the measurements. Gold standard animal-based indicators (i.e., RR and CBT) were recorded by timing 15 flank movements (and converting to breaths/min) and using data loggers (DS1922L, Maxim Integrated, San Jose, CA, USA) attached to modified vaginal controlled internal drug releases (Pfizer Animal Health, New York, NY, USA), respectively.

The ET was recorded using a portable infrared camera (VarioCAM HR, InfraTec, Dresden, Germany) right after RR measurement. The camera had a spectral range from 7.5 to 14 μm, a temperature measuring range from −40 to 2,000°C, an accuracy of ± 2%, and a resolution of 640 × 480 pixels. The infrared images were taken twice within 2 min from each cow per measurement to evaluate the repeatability of measurements. All images were taken from the cows' side to capture eye regions with an angle of ∼90° and a distance of ∼1 m from the cows. To prevent the cows' body temperature from rising due to the stress caused by prolonged handling, we only measured the temperature of the eye that was on the side of the cow (i.e., left or right) that was close to the approaching thermographer for each cow per measurement.

Ambient temperature (Ta) and relative humidity (RH) were measured using a Kestrel 5400 heat stress tracker (Nielsen-Kellerman, Boothwyn, PA, USA). Temperature and humidity index (THI) was then calculated according to the following equation recommended by the National Research Council (18):

$$\mathbf{THI} = (\mathbf{1.8} \times \mathbf{Ta} + \mathbf{32}) - (\mathbf{0.55} - \mathbf{0.005} \times \mathbf{RH}) \times (\mathbf{1.8} \times \mathbf{Ta} - \mathbf{26})$$

Infrared Image Processing

Infrared images were processed using IRBIS 3 Standard software (YSHY, Beijing, China). All images were calibrated by setting the emissivity to 0.98 and inputting the corresponding Ta record of each measurement. MC, lateral canthus (LC), EB, whole eye (WE), and lacrimal sac (LS) were manually located using appropriate circles to obtain the mean and maximum temperatures of the areas (**Figure 1**). Two replicated images that did not capture all five ROIs at the same time were

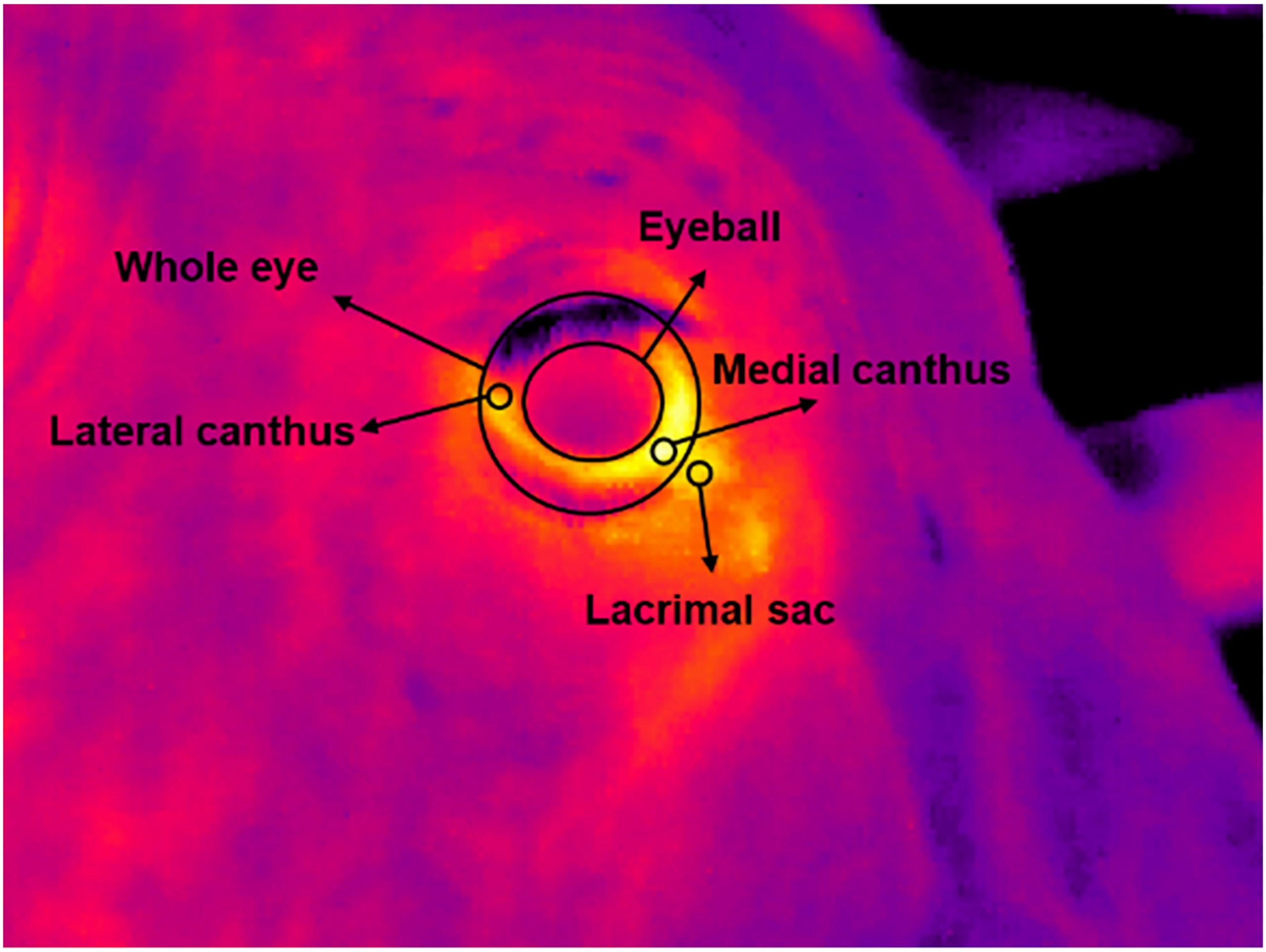

FIGURE 1 | Five regions of interest of eyes for temperature measurement.

manually eliminated. Consequently, 736 infrared images from 318 measurements were finally used for the following analyses.

Statistical Analysis

All statistical analyses were performed using R version 4.1.0 (https://www.R-project.org/). Mean and maximum ET were analyzed separately by ROI and side, unless otherwise stated. To determine the temperature differences among ROIs and side (left and right), mean and maximum ET were analyzed separately using generalized linear mixed models with the "nlme" package including fixed effects of ROI, side, interaction between ROI and side, posture, random intercept effect of cow, and covariates of parity, days in milk, and THI. The repeatability of replicated ET taken within a 2-min interval was assessed by using the concordance correlation coefficient (CCC) for longitudinal repeated measures using the ccclon function included in the "cccrm" package with the index of observation ordered by time as the longitudinal unit for each cow. The correlations between ET and gold standard animal-based indicators were performed using the cor function. Significance was declared at $p < 0.05$.

RESULTS

Descriptive Statistics

The descriptive statistics of meteorological and physiological variables are shown in **Table 1**. Among ROIs, the maximum temperature of WE (37.93°C) and MC (37.92°C) provided the highest temperature values, ∼0.9°C lower than CBT (38.80°C). As expected, MC was a good representative of WE in terms of maximum temperature with nearly 80% of the images having maximum temperature located in MC. The coefficients of variation of all temperature variables (CBT and ET) lay between 1 and 2%, among which the mean temperature of EB and WE had the highest value of 1.79 and 1.86%, respectively. Besides, the coefficients of variation of maximum temperature in all five ROIs were lower than those of mean temperature.

Temperature Differences Among ROIs and Sides

For mean temperature, no significant difference between the left and right eyes was found for ET measured at all five

TABLE 1 | Number of observations (N), mean, standard deviation (SD), coefficient of variation (CV), minimum (Min), and maximum (Max) values of meteorological and physiological variables.

Variable	*N*	Mean	SD	CV (%)	Min	Max
THI	318	80.55	3.01	3.73	70.98	86.09
RR	318	70.70	19.93	28.19	27.17	145.16
CBT	168	38.80	0.45	1.17	38.00	40.10
MCmean	318	37.60	0.46	1.21	35.81	38.64
MCmax	318	37.92	0.41	1.08	36.74	38.96
LCmean	318	37.07	0.49	1.32	35.55	38.32
LCmax	318	37.43	0.45	1.19	36.13	38.63
EBmean	318	36.11	0.65	1.79	33.54	37.60
EBmax	318	37.34	0.50	1.34	35.96	38.50
WEmean	318	36.29	0.67	1.86	33.46	38.11
WEmax	318	37.93	0.41	1.07	36.86	38.97
LSmean	318	37.16	0.55	1.47	34.92	38.58
LSmax	318	37.42	0.51	1.36	35.72	38.81

THI, temperature and humidity index; RR, respiration rate (breaths/min); CBT, core body temperature (°C); MC, medial canthus (°C); LC, lateral canthus (°C); EB, eyeball (°C); WE, whole eye (°C); LS, lacrimal sac (°C). Eye temperatures (°C) were summarized using the average measures of two replicated infrared images.

ROIs ($P > 0.05$; **Figure 2A**). For maximum temperature, the EB temperature of left eyes (37.31°C) was significantly lower compared with that of right eyes (37.49°C) ($p = 0.0007$; **Figure 2B**). In general, a slightly higher ET (mean or maximum) was found in left eyes than right eyes except for EB (**Figure 2**).

Imaging Repeatability

Considering left eyes and right eyes separately, five ROIs all yielded higher CCCs in their mean temperatures compared with maximum temperatures (**Table 2**). When comparing two eyes, left eyes always had CCCs higher than or equal to those of right eyes in both mean and maximum temperatures (**Table 2**). In general, MC, WE, and LS had CCCs higher than 0.90 for all combinations of temperature parameter (mean or maximum) and side (left or right) (**Table 2**), indicating that ET taken in these ROIs within 2 min had a moderate to substantial repeatability.

Correlations Between ET, RR, and CBT

The mean or maximum ET obtained at all ROIs on each or both sides were all significantly correlated with RR and CBT (all $p < 0.001$; **Table 3**). When taking left eyes and right eyes separately, the maximum temperature had higher or equal correlation coefficients with RR and CBT compared with the mean temperature in most ROIs of the eye except for LC in which the mean temperature was correlated more with RR (**Table 3**). The highest correlation coefficients of RR and CBT were both yielded from the maximum temperature obtained at the LS of left eyes (0.60 and 0.52, respectively; **Table 3**).

When combining both eyes into analysis, the maximum temperature had better correlation coefficients with RR and CBT compared with the mean temperature in most ROIs of the eye except for the LC in which the mean temperature still correlated more with RR (**Table 3**). Besides, the correlation coefficients were pooled to some extent, with the results lower than those from left eyes but higher than those from right eyes (**Table 3**). As expected, the maximum temperature obtained at the LS of both eyes still correlated most with RR and CBT ($r = 0.55$ and 0.51, respectively; **Table 3**).

DISCUSSION

According to the revised heat stress categories proposed by Collier et al. (19), the cows were exposed to mild to severe heat stress. Since this study lasted 3 months in the summer, the effects of long-term heat stress were well captured. In the present study, five ROIs of the eye were all determined using images taken from the side of the cows' faces. Some studies also captured ET using the front area of the cattle face (20, 21). However, front images were not considered in the present study since a minor variation in the angle of the infrared camera to the target could result in a very different temperature value. To the best of our knowledge, this is the first study to comprehensively compare the temperatures among various ROIs of the cow's eye.

The temperature of the left and right eyes of cows is rarely compared. Most of the previous studies did not distinguish between the left and right eyes when obtaining ET. Our results show that the left eye seemed to have a higher temperature, better repeatability, and better correlation with gold standard animal-based indicators. Besides, the combined dataset of both left and right eyes provided a pooled correlation with RR and CBT, which was better than right eyes solely and worse than left eyes solely. Byrne et al. (14) also found that left eyes were more repeatable than right eyes, where 70.20% of the total variation could be explained by cow. However, left eyes were found 0.18 and 0.53°C lower than right eyes in the maximum and mean temperature, respectively, which is opposite to our results. A higher temperature in the left eye than the right eye was also found by the study of Church et al. (22) where 79 Holstein dairy cows were measured ET using IRT. The cows were raised in a barn oriented along the north-south longitudinal axis, and an adjustable perforated awning was used to provide three treatment

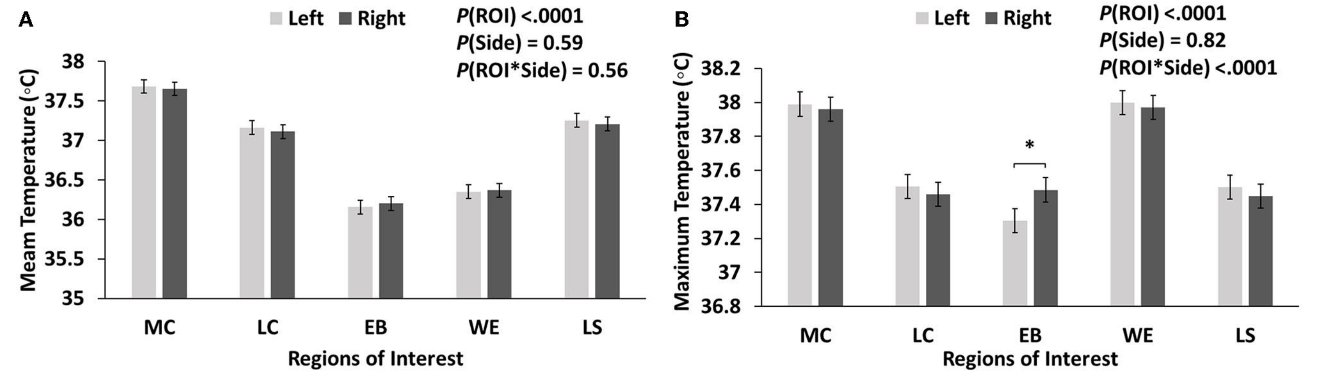

FIGURE 2 | Mean **(A)** and maximum temperature **(B)** of left and right eyes obtained at five regions of interest (ROIs). Data are graphed using the least square mean ± standard error of the interaction (ROI by side). MC, medial canthus; LC, lateral canthus; EB, eyeball; WE, whole eye; LS, lacrimal sac.

TABLE 2 | Concordance correlation coefficients (95% confidence interval) of replicated infrared imaging measures (mean and maximum temperatures) taken from the left and right eyes, respectively.

Side	Parameter	MC	LC	EB	WE	LS
Left	Mean	0.96 (0.94, 0.97)	0.90 (0.86, 0.93)	0.94 (0.92, 0.96)	0.97 (0.95, 0.98)	0.95 (0.93, 0.97)
	Maximum	0.94 (0.92, 0.96)	0.85 (0.78, 0.90)	0.87 (0.81, 0.91)	0.94 (0.92, 0.96)	0.94 (0.91, 0.96)
Right	Mean	0.95 (0.92, 0.97)	0.85 (0.79, 0.89)	0.92 (0.90, 0.94)	0.95 (0.93, 0.97)	0.95 (0.92, 0.97)
	Maximum	0.92 (0.89, 0.95)	0.85 (0.79, 0.89)	0.72 (0.58, 0.82)	0.93 (0.90, 0.95)	0.92 (0.88, 0.95)

MC, medial canthus; LC, lateral canthus; EB, eyeball; WE, whole eye; LS, lacrimal sac.

conditions: direct sunlight (left eyes), indirect sunlight (right eyes), and shaded (both eyes). Even under the shaded condition, a 0.14°C higher temperature was found in left eyes than in right eyes. Rogers (23) reported that both acute and chronic stress of an animal are processed by the right hemisphere of the brain. Since the right hemisphere of the brain is linked to the left eye (24), it may explain why our results show that the temperature of the left eye was better at indicating heat stress in dairy cows. Due to the fact that rumen is on the left side of the cow's body, Brcko et al. (11) measured ET only from the cows' right side to prevent the impact of digestive processes. However, our results might indicate a negligible effect of rumination and digestion on the temperature of the left eye since the left eye and the rumen are anatomically far apart. It is also worth noting that the wind direction along the feeding line was from left to right for cows that were eating in the present study. Thus, we speculate that the better performance of the left eye might also be partly attributed to the direct exposure to fans. Indeed, evaporative cooling including sprinklers and fans together has shown a significant effect on reducing the body surface temperature of cows by promoting local cutaneous evaporation (25, 26). However, further studies are required in which the direction of the air flow relative to the cows is strictly controlled.

As expected, the mean temperature of the ROIs always had better repeatability compared with the maximum temperature, which is consistent with the study of George et al. (27). However, maximum temperature provided better correlations with RR and CBT in most ROIs, reconfirming that maximum temperature could better reflect cows' thermoregulation under heat stress (28). Also, Uddin et al. (29) reported that the maximum temperature of the eye was more correlated with stress- and productivity-related parameters than the mean temperature. Among five candidate ROIs of the eye, LS was determined to be the best area for ET measurement using IRT in dairy cows due to the consistently good results in repeatability and correlations with RR and CBT. Besides, MC and WE had similar results when using maximum temperature since nearly 80% of the images had a maximum temperature located in the MC area. It is well known that the lacrimal caruncle and the small area around the medial posterior palpebral border of the lower eyelid have abundant capillaries of the maxillary and infraorbital arteries, which are innervated by the sympathetic system (8, 30). When the animals are exposed to heat stress, vasodilation leads to an increased surface temperature of these areas to promote heat dissipation (4). On the other hand, EB was found to have a relatively lower CCC for replicated infrared imaging measures and a lower correlation with RR and CBT compared with other ROIs. This may be due to inaccurate temperature readings caused by lacrimal secretions during heat stress. Vasodilation increases blood flow, thereby promoting the secretion of lacrimal gland fluid (31). Although poor quality images with obvious tears or dirt obscuring the ROIs have been manually eliminated, some invisible secretions might cover the surface of the EB and affect IRT by reflecting most infrared light, resulting in the lower temperature value, which was actually the temperature of lacrimal secretions. The higher coefficient of variation (1.79%) and the lowest minimum temperature

TABLE 3 | Correlation coefficients of mean and maximum eye temperature (°C, left and/or right) with respiration rate (RR, breaths/min) and core body temperature (CBT, °C).

Variable	Side	Parameter	MC	LC	EB	WE	LS
RR	Left eye	Mean	0.48	0.48	0.36	0.37	0.58
		Maximum	0.49	0.45	0.39	0.49	0.60
	Right eye	Mean	0.44	0.41	0.33	0.35	0.50
		Maximum	0.44	0.39	0.35	0.44	0.51
	Two eyes	Mean	0.46	0.45	0.35	0.36	0.54
		Maximum	0.47	0.42	0.37	0.46	0.55
CBT	Left eye	Mean	0.38	0.37	0.25	0.25	0.48
		Maximum	0.43	0.39	0.34	0.42	0.52
	Right eye	Mean	0.48	0.36	0.31	0.38	0.50
		Maximum	0.48	0.38	0.38	0.48	0.50
	Two eyes	Mean	0.43	0.37	0.28	0.32	0.49
		Maximum	0.45	0.38	0.34	0.45	0.51

MC, medial canthus; LC, lateral canthus; EB, eyeball; WE, whole eye; LS, lacrimal sac. All $p < 0.001$.

(33.54°C) of the mean temperature of EB also supported the presence of secretions to have biased the actual temperature of EB downwards.

One of the limitations of the present study is that the size of ROIs was not constrained among infrared images. This could have an impact on the result of mean temperature. However, since the distance between the camera and the cows could not be fixed to 1 m completely, it was difficult to use fixed pixel sizes for ROIs. Besides, the maximum temperature was less likely to be influenced by slightly changed pixel sizes and its result should remain robust under our study design. On the other hand, the average temperature of the left and right eyes was used in previous studies (27). However, to prevent the cows' body temperature from rising due to the stress and anxiety caused by prolonged handling, we only measured the temperature of one eye from each cow per measurement. Thus, the average temperature of the left and right eyes was not able to be evaluated in this study. Furthermore, only the infrared images with all five ROIs available were selected for analysis; however, the actual situation is likely to be less favorable. For example, partially closed eyes and eyes covered by secretions or dirt are very common in the field, and will result in difficulty to obtain ROIs. Thus, we suggest that the maximum temperature of LS, MC, or WE should be used as representative ET when available.

CONCLUSION

Collectively, this study demonstrates that the most commonly used ROIs (WE and MC) in the previous studies provided acceptable performance in reflecting the thermoregulatory response of dairy cows exposed to heat stress, whereas LS was the best area to obtain ET. More interestingly, left eyes were found to have a higher correlation with gold standard animal-based indicators (i.e., RR and CBT). Further studies are required to evaluate this phenomenon in which the direction of the airflow relative to the cows is strictly controlled.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the Experimental Animal Care and Committee of Institute of Animal Science, Chinese Academy of Agricultural Sciences (approval number IAS2021-220).

AUTHOR CONTRIBUTIONS

HS and JB: experiment design. HS, YL, TF, MX, FS, and XC: experiment execution. HS: data processing and analysis and writing—original draft. JB: writing—review and editing. WW and LG: resources and supervision. All authors approved the final manuscript.

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Is Continuous Monitoring of Skin Surface Temperature a Reliable Proxy to Assess the Thermoregulatory Response in Endurance Horses During Field Exercise?

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Hyperthermia is a performance and welfare issue for exercising horses. The thermoregulatory stressors associated with exercise have typically been estimated by responses in the laboratory. However, monitoring surface skin temperature (T_{sk}) coincident with core temperature (T_c) has not previously been investigated in horses exercising in the field. We investigated the suitability of monitoring surface T_{sk} as a metric of the thermoregulatory response, and simultaneously investigated its relationship with T_c using gastrointestinal (GI) temperature. We evaluated T_{sk} in 13 endurance horses competing during four endurance rides over 40 km ($n = 1$) or a total of 80 km ($n = 12$) distance. Following each 40-km loop, the horses were rested for 60 min. T_{sk} and T_c were continuously recorded every 15 s by an infrared thermistor sensor located in a modified belt and by telemetric GI pill, respectively, and expressed as mean ± SD. The net area under the curve (AUC) was calculated to estimate the thermoregulatory response to the thermal load of T_{sk} over time (°C × minutes) using the trapezoidal method. The relationship between T_{sk} and T_c was assessed using scatterplots, paired *t*-test or generalized linear model ANOVA (delta T_{sk}) ($n = 8$). Ambient temperature ranged from 6.7°C to 18.4°C. No relationship was found between T_{sk} and T_c profiles during exercise and recovery periods, and no significant difference between delta T_{sk} results was detected when comparing exercise and rest. However, time to maximum T_{sk} (67 min) was significantly reduced compared to T_c (139 min) ($p = 0.0004$) with a significantly lesser maximum T_{sk} (30.3°C) than T_c (39°C) ($p = 0.0002$) during exercise. Net AUC T_{sk} was 1,164 ± 1,448 and −305 ± 388°C × minutes during periods of exercise and recovery, respectively. We conclude that T_{sk} monitoring does not provide a reliable proxy for the thermoregulatory response and horse welfare, most probably because many factors can modulate T_{sk} without directly affecting T_c. Those factors, such as weather conditions, applicable to all field studies can influence the results of

T_{sk} in endurance horses. The study also reveals important inter-individual differences in T_{sk} and T_c time profiles, emphasizing the importance of an individualized model of temperature monitoring.

Keywords: skin surface temperature, thermoregulation, metabolic heat, endurance, exercise, performance, competition, hyperthermia

INTRODUCTION

In the face of climate change, hyperthermia and heat stress have become increasingly challenging issues for a wide array of equine sports disciplines, especially during field competitions (1, 2). An increase in core body temperature (T_c) leading to hyperthermia may cause widespread cytotoxicity as a direct effect of heat, while indirect effects related to decreased cardiac output cause neural and intestinal ischemia. If unchecked, these systemic inflammation processes eventually lead to exertional heat illness (EHI) (3–5). The clinical manifestations of EHI include neurological signs, varying from irritability, depression, ataxia, collapse, and may further progress to exertional heat stroke (EHS) with multi-organ dysfunction and death (3–5). In human athletes, EHS is among the top three causes of sudden death, and, in summer, it is the number one cause of athlete death in the USA (3). Similarly, both EHI and EHS are problematic conditions in equine athletes (6). The prevalence of metabolic disorders in, for example, endurance horses, triggered by thermoregulatory-induced physiological feedback failure and exhaustion, ranges from 4.2% to 15% (7–14). Recently, the prevalence of EHI in racehorses has been reported: two studies from Japan state prevalence of 0.09% during summer with a clear increase over the past few years (15, 16). Moreover, a study in Eastern Australia focused on selected EHI cases post-exercise at the racetrack and suggested an EHI incidence of up to 9.5% during hot summer months (17). The latter study used the four severity levels of EHI reported by Brownlow et al. (4) and concluded that 96% of horses could be categorized as Level 1. This suggests that a low level and thus discrete EHI cases may have been overlooked in the past.

Environmental conditions are the dominant risk factor in heat stress events, and EHI cases are expected to further increase in prevalence due to global warming (1, 4, 15–17). That worrying reality drives the ongoing efforts of research groups worldwide to develop reliable approaches to monitor and safeguard thermoregulatory wellbeing in horses (2, 18–21). We have previously reported on the continuous monitoring of the thermoregulatory response in endurance and trotter horses using a telemetric gastrointestinal (GI) pill (2). Briefly, the GI temperature pill is a non-invasive method to monitor T_c during exercise in the field. The GI pill was administered the night before the endurance competition to allow recording of a large temperature data set to establish T_c profiles of exercising horses. The data were telemetrically transferred to a device located in a belt under the saddle girth and supported continuous real-time monitoring. It was demonstrated that the GI telemetry pill is a reliable method to monitor T_c and assess the individual dynamic thermal response in exercising horses in the field. The study revealed important inter-individual differences in T_c time profiles, despite the horses performing the same exercise protocol. This finding emphasizes the importance of an individualized model of temperature monitoring. It can be concluded that the continuous monitoring approach allows for intervention at the early stages of heat accumulation and the possibility to take prompt and effective preventative measures.

Another elegant approach to monitoring the thermoregulatory response in exercising horses in the field could be continuous monitoring of surface skin temperature (T_{sk}) as a reliable proxy for monitoring thermoregulatory wellbeing. Advantages of using tools, such as infrared thermography (IRT), to assess T_{sk} include the non-invasive nature and easy collection of temperature data (22–24). Notably, most exercise studies use such monitoring methods to assess T_{sk} only pre- or post-exercise, not during exercise. To illustrate, a recent systematic review of T_{sk} studies performed in human endurance athletes has reported that only a few studies involved the continuous monitoring of T_{sk}; only two out of the 45 exercise studies monitored T_{sk} every 30 s, one study every 180 s, and four studies every 5 min (24). Also, these human studies highlight the existence of essential inter-individual differences with respect to the T_{sk} response time profile (24). Important inter-individual T_{sk} differences have been reported in all mammals (23, 25). For example, an equine study comparing T_{sk} in 21 horses at rest revealed significant inter-individual differences (26). Most existing equine thermoregulatory exercise studies ($n = 12$ studies) involving T_{sk} have focused on single point post-exercise measurements using a handheld IRT camera, leaving both the pre-exercise and intra-exercise periods out of their scope (21, 22, 27–37). An overview of all T_{sk} studies on horses at rest and during exercise is presented in **Supplementary Table 1**.

To date, only a few equine studies have focused on continuous monitoring of T_{sk} during exercise and recovery ($n = 7$, **Supplementary Table 1**). All were conducted in laboratory conditions, for example, on a treadmill (37–42). Only one study has recorded T_{sk} continuously during a short bout of field exercise of 4.5-min duration to investigate the effect of pre-exercise cooling in 10 horses (20). The study recorded surface T_{sk} using a microchip (i-Button®) attached to the skin with removable glue and simultaneously monitored rectal temperature (T_{re}) using a

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Abbreviations: GI, Gastrointestinal; HR, heart rate; bpm, beats per minute; B.O.M., Bureau of Meteorology; GPS, Global Positioning System; A.E.R.A., Australian Endurance Riders Association; SAERA, South Australian Endurance Riders Association.

rectal probe; both T_{sk} and T_{re} reduced over time (3°C and 0.3°C, respectively).

From a physiological standpoint, it is essential to appreciate that a time lag exists between exercise-induced metabolic heat (MH) output and T_c evolvement. The T_c is subsequently translated into an additional temperature time-lag evolvement, expressed at several different anatomical locations, such as the rectum, the muscular compartment, and the skin surface, whether or not additionally complicated by environmental factors, such as hot and humid weather (1, 25, 39, 43–48). Most importantly, T_{re} evolvement has been reported to significantly lag behind the T_c both during and after exercise (19, 38, 49), which renders the T_{re} less suitable as a "whistle blower" for thermoregulatory instability. In our previous studies, we demonstrated that GI temperature is a more reliable proxy for the thermoregulatory response and T_c when compared to T_{re}, and that continuously monitoring GI temperature evolvement demonstrated how the equine body copes with exercise, challenging the thermoregulatory system (2, 19). Endurance horses, for example, reached their mean maximum T_c (39.0 ± 0.4°C) during exercise at 75% of completion of exercise, and T_c returned to the baseline within 60 min into recovery (2). However, the mean T_c was still 38.8 ± 0.4°C at a heart rate (HR) of 60 bpm, which currently governs "fit-to-continue" competition decisions (50), thus questioning the use of HR values to make such important decisions. However, contrary to this finding, trotter horses reached a comparable mean maximum T_c (38.8 ± 0.5°C) during recovery. Moreover, in 30% of trotters, T_c was still > 39°C at the end of the recovery period (40 ± 32 min), following exercise in a cool environment, findings that may have post-exercise management implications.

To identify a reliable proxy for thermoregulatory response in the field, a solid correlation must exist between that specific proxy and T_c evolvement, despite the existence of a time lag (24, 47). However, currently, very few equine studies have involved the simultaneous continuous monitoring of T_c (either using carotid artery temperature, or a GI pill, or the T_{re}), together with an additional temperature monitoring device during field exercise (19, 20, 51). On the other hand, with the ongoing development of new wearables and sensors, there are an increasing number of exercise studies investigating continuous T_{sk}, monitoring wearables (20, 37–40, 42, 52) (Supplementary Table 1 in **Supplementary Material**). These devices all provide data output, although the physiological meaning of these data is not always clear.

The MH produced during exercise needs to be dissipated from the horse to the surrounding environment through four main pathways, namely, radiation, conduction, convection, and evaporation, the last being the most essential and pivotal pathway in horses (23, 48, 53–56). Evaporation from the body surface is mainly achieved by increased blood flow, cutaneous vasodilation followed by evaporation of sweat from the skin (70–85% of the MH load) (25, 48, 54, 56–59). Heat loss by evaporation can be enhanced by cooling techniques (35, 40, 43). When focusing on T_{sk} as a temperature monitoring method, it is vital to keep in mind that all these pathways to dissipate MH to the environment may influence the T_{sk} data output.

Monitoring T_{sk} simultaneously with T_c using the GI temperature pill during field exercise has not yet been investigated. The relationship between T_{sk} and T_c is not well understood due to physiological, endocrine, or vasomotor influences on both temperatures (25, 56, 59). Some studies have tried to correlate both T_c and T_{sk} (39–42). The current study aimed to evaluate the usefulness of continuous monitoring of T_{sk} by means of a surface IR sensor device as a proxy for the thermoregulatory response. For this purpose, the T_{sk} relationship with T_c was investigated by simultaneous and continuous telemetric measurements during real-time field competitions under cool weather conditions. Endurance horses were equipped with several non-invasive telemetric monitoring devices—a T_{sk} device positioned in a girth belt, an orally administered GI pill (T_c), a global positioning system (GPS), and an HR monitor.

MATERIALS AND METHODS

Horses

Thirteen mainly Arabian ($n = 10$) endurance horses participated in the study: 7 geldings; 6 mares; age, 9.5 ± 2.8 years; body mass (BM), 479 ± 68 kg; body condition scores varied from 2 to 3 out of 5. Two cross-Arabians and one crossbred (quarter horse—thoroughbred) were also involved (**Table 1**). Coat color included bay ($n = 3$), chestnut ($n = 6$), and gray ($n = 4$), and the color was scored as follows: dark (bay and chestnut, $n = 9$) compared to light (gray) (**Table 1**). Relevant rider and horse performance history and the Bureau of Meteorology (B.O.M.) (60) station output information were recorded (**Table 1**; **Supplementary Material S1**). All the horses were deemed to be fit and healthy based on the veterinary inspection conducted before the competition and following each 40-km loop according to AERA riding rules (50). The horses were sourced on a voluntary basis through the South Australian Endurance Riders Association (S.A.E.R.A.), and all the owners signed a written consent form. The study was approved by the University of Adelaide Animal Ethics Committee (project No. S-2011-224).

Study Design

The horses competed over distances of 40 km ($n = 1$), 80 km ($n = 10$) or 100 km ($n = 2$), with each 40-km loop followed by a 60-min recovery period. The endurance horses exercised at four different locations with altitudes ranging from 4 to 462 meters above sea level. Following each 40-km exercise loop, the sweating response was graded, scoring from 1 to 3 (1: mild wet and white foam areas around head, neck, saddle, and inside hindlimbs, 2: moderate dripping sweat from the body, 3: extensive dripping sweat from the body; **Table 1**). In addition, the horses were immediately cooled down for average an duration of 10 min by pouring buckets of tap water (estimated average, 20°C) over their bodies and subsequently scraping it off. Following each loop, a recovery period of 60 min was allowed during which inspection of the horses for "fitness to continue" was performed, including checking for the presence of an HR below 60 beats per minute (bpm) by independent endurance veterinarians under the regulations of the Australian Endurance Riding Association (50).

TABLE 1 | Study population characteristics and monitoring devices.

Horse number	Sex	Age (y)	Breed	Body mass (kg)	Coat color	Distance (km)	GI Pill Y/N	GPS/HR Y/N	B.O.M. (°C) (T_a), min - max	Sweating score post-exercise, 1 tot 3
1	G	11	Arab	669	Gr	80	Y	Y	13–26	2
2	G	9	Arab	484	B	80	Y	Y	13–26	1
3	M	13	Arab	426	C	80	Y	Y	6–19	2
4	M	7	QH x TB	470	C	80	Y	Y	6–19	1
5	M	11	Arab	450	C	80	Y	Y	6–19	1
6	M	8	Arab	370	Gr	80	Y	Y	6–19	2
7	M	9	Arab	450	C	80	Y	^	3–22	2
8	G	11	Arab	470	Gr	80	Y	Y	3–22	3
9	G	7	QH	490	C	80	–	Y	7–13	3
10	G	10	Arab x TB	484	C	80	–	Y	7–13	3
11	G	5	Arab	458	B	40	–	Y	7–13	1
12	G	7	Arab	525	B	80	–	Y	3–22	1
13	M	15	Arab	480	Gr	80	–	^^	3–22	2

Horses 1-13: 13 endurance horses: G (n = 7), M (n = 6). Arabian, including part-Arabian horses, QH, quarter horse; TB, thoroughbred; x, crossbred; G, gelding; M, mare; Gr, grey; C, chestnut; B, bay. The riders' and horses' performance history includes: age start, indicating age (years) when the horse started competing; horse experience, indicating number of years active in competition (40 km or more); GI pill, gastrointestinal pill; GPS, global positioning system; HR, heart rate monitor (Polar); B.O.M, Bureau of Meteorology; the local station closest to the location of exercise at varying km distances from the actual event (in total, 4 endurance locations, distance ranged from 5.3 to 53 km; -, no; Y, yes; ^indicates HR only; ^^indicates second 40 km only.

Horses were allowed to drink water and eat hay *ad libitum* during the 60-min rest period in a shaded area.

Simultaneous Continuous Monitoring of Skin Temperature (T_{SK}) (°C) and Core GI Temperature (T_C) (°C)

The T_{sk} (°C) was continuously recorded using an infrared (IR) sensor measuring 78 × 53 mm located in the Sensor Electronics Module (**Figure 1B**) (SEM, EQ02 Equivital data Logger®, Hidalgo, UK), with a 0–60°C temperature range, an emissivity of 1 and ± 0.3°C accuracy according to the manufacturer's specifications. The SEM device was located ventrally in a pocket of a modified Equivital Sensor Belt® fitted around the saddle girth (**Figure 1C**). The GI temperature (T_c) (°C) was continuously telemetrically recorded using the ingestible GI pill ($n = 8$) (**Figure 1A**) as previously described (T_c data are to be found in the **Supplementary Material S1**). The T_{sk} and T_c data were recorded every 15 s and uploaded and processed in the Equivital Software Manager®.

Monitoring of Traveled Distance, Speed, and Heart Rate Over Time

For each horse, the distance traveled and speed achieved were recorded telemetrically using GPS monitoring equipment (Garmin Forerunner 910XT GPS Watch®; Garmin Ltd., Schaffhausen, Switzerland) attached to the gullet of the saddle (**Figure 1C**). In addition, the HR was recorded by the Garmin Watch using Polar electrodes (Polar Electro®, Kempele, Finland) (**Figure 1C**) (61). The GPS and HR data were recorded every second and uploaded from the Garmin watch to the Garmin Connect and processed in the Garmin Training Centre.[1]

Ambient Environment

Horses exercised under varying degrees of solar radiation during the Australian winter months (June–August). On each data collection day, the ambient temperature (T_a, °C) and relative humidity (RH, %) were recorded continuously every 30 s in a shaded section of the rest area using a data logger device (OnsetHOBO Pro V2 logger temp/RH U23-00®, Onset Computer Corporation, Bourne, Maine, USA). In addition, T_a data were obtained from the nearest B.O.M. weather station, presented in **Table 1**. The estimated wet bulb globe temperature (WBGT) was derived from a WBGT chart (B.O.M).

Data Processing

Recordings of each exercise period of 40 km and each recovery period following that exercise loop were processed. The net AUC (the baseline set at rest T_{sk} and T_c) was calculated using the trapezoidal method of T_{sk} (and T_c) over time expressed as °C × minutes. The net AUC was summated to present the cumulative T_{sk} – time distribution (62). The net AUC T_{sk} provided an estimate of the dynamic thermal response to the thermal load on the skin. This thermal load on the skin during exercise and the recovery included the T_c and T_a together with solar radiation.

Statistical Analysis

All data are presented as mean ± SD (range). Comparison and correlation analyses were performed using IBM SPSS Statistics 26.0 software or GraphPad Prism version 9.3.0 for MacOS,

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[1] https://connect.garmin.com/

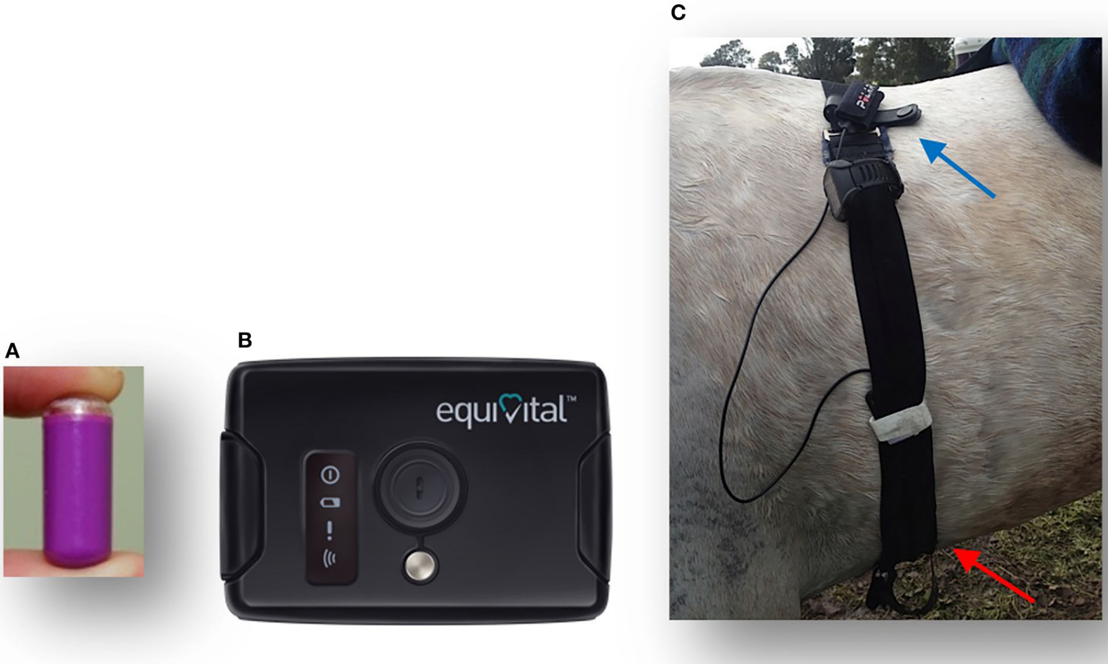

FIGURE 1 | T_{sk} and T_c monitoring equipment: Jonah gastrointestinal temperature pill **(A)**; an external receiver Equivital® Sensor Electronics Module (SEM) with an infrared sensor to measure T_{sk} **(B)**; a modified belt for use on horses with the GPS Garmin®Watch and Polar® electrodes (identified by the blue pointer), with the red pointer, indicating the SEM device, including the T_{sk} thermistor position **(C)**.

GraphPad Software, San Diego, California USA[2]. Different approaches were taken to evaluate the potential of the T_{sk} data as a reliable proxy to assess the thermoregulatory response. The relationship between T_{sk} and T_c was assessed using scatterplots (8 horses each performing two subsequent 40-km loops). In addition, maximum T_{sk} and T_c and the time to reach maximum T_{sk} and T_c were compared using the paired *t*-test. Delta T_{sk} during exercise and recovery periods was compared. The association between T_{sk} and T_c at different points in time and the association with HR or coat color were analyzed using a general linear model ANOVA (when no significant effects of horse identity and treatment interaction were indicated and subsequently removed using backward model selection). Statistical significance was set at $\alpha < 0.05$.

RESULTS

All the horses completed their exercise trials without any adverse occurences. The Equivital belt became dislodged in Horse 1 at the end of the first 40-km loop, causing T_{sk} and T_c data loss. As a result, additional modifications were applied to the belt for the subsequent recordings by fitting sturdy straps sandwiched into the belt to stabilize the girth position (**Figure 1C**). During recovery after the first loop, T_{sk} was not recorded in Horses 1 and 2 due to the owners' premature removal of the belt. The sweating response varied from 1 to 3 out of a score of 3 for all the horses (**Table 1**). The T_c was recorded in 8 horses over 80 km (previously published, **Supplementary Material S1**) (2).

Environmental Field Conditions

The T_a and RH were successfully recorded between 5.00 a.m. and 3.00 p.m. on all occasions. The T_a was relatively cool with a mean minimum of 6.7 ±.4°C and mean maximum of 18.4 ± 2.9°C (B.O.M.) (**Table 1**). More specifically, the T_a on the four separate days of endurance exercise showed a minimum value of 13.4, 6.3, 2.8, and 6.6°C, respectively, and a maximum value of 26.3, 19.0, 22.0, and 18.8°C, respectively (HOBO data). The minimum RH ranged from 47.1% to 61.7% to a maximum of 84.8–100% value. Overall mean calculated values were 15.3°C (T_a) and 75.6% (RH), respectively, while the approximate WBGT was <20°C. In summary, all the endurance horses competed in a cool environment.

Speed and Heart Rate Data

All the horses executed their endurance competition at a mean speed of 14.0 ± 1.4 km h^{-1} over the first 40 km ($n = 11$) and 14.2 ± 2.1 km h^{-1} over the second 40-km ($n = 11$) loop, with a mean HR of 114 ± 13 bpm. An overview of recorded

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[2] www.graphpad.com

speeds and HR data for individual horses can be found in **Supplementary Material S1**.

Individual T_{sk} and T_c Recordings During Endurance Exercise Over Time

An overview of the simultaneously recorded individual T_{sk} (°C) and T_c (°C) time profiles is provided for all the horses in **Figure 2**. All individual T_{sk} parameters, their respective descriptive analysis, and specific T_{sk} points in time during the 40-km endurance loops are presented in **Table 2**.

Overall T_{sk} Profiles and Comparison to T_c

The overall T_{sk} profiles during endurance exercise and recovery and their associated parameters are presented in **Table 3**, showing a mean time to maximum T_{sk} of 88 ± 51 min ($n = 13$). The mean maximum T_{sk} during exercise was 32.4 ± 4.3°C, and the mean minimum T_{sk} during recovery was 17.3 ± 3.1°C ($n = 13$). The mean overall response of T_{sk} was 1,164 ± 1,448°C × minutes for each 40-km exercise period. During recovery, the T_{sk} response was −305 ± 388°C × minutes (**Table 3**). The T_{sk} and T_c profiles over time were compared in the 8 horses, and no relationship was found (**Figure 2**).

Different T_{sk} and T_c points in time were compared to assess associations. Interestingly, the only significant correlation found was between the T_{sk} (°C) at the end-of-exercise period and the T_c (°C) at the end-of-recovery period ($F_{1,14} = 5.519$, $p = 0.034$). More precisely, a higher T_{sk} at the end-of-exercise period was associated with a lower T_c at the end-of-recovery period. The additional analyses revealed no significant correlations between T_{sk} (°C) and T_c (°C), including no correlation between T_{sk} at the start-of-exercise period (baseline T_{sk}) and the maximum T_c ($F_{1,14} = 0.127$, $p = 0.727$). The study could not identify a significant effect of time to maximum T_{sk} (67 min) during exercise on the maximum T_c (39°C) ($F_{1,14} = 0.001$, $p = 0.978$, $n = 8$). On all occasions, peak T_c values (39°C) were significantly greater than peak T_{sk} values (32°C) ($p = 0.0002$) (**Figure 3A**). In addition, in all cases, there was a significantly shorter time to maximum T_{sk} (88 min) compared to the time to maximum T_c (139 min) ($p = 0.0004$) (**Figure 3B**).

Delta T_{sk} data were compared, and, when comparing exercise and recovery periods, no significant difference could be found between the two delta T_{sk} (°C) (mean 13°C and 11°C, respectively) ($p = 0.41$) (**Figure 3A**). The delta T_{sk} was greater when compared to delta T_c on all occasions (**Figure 3A**). Also, delta T_{sk} and T_c points in time were compared to assess associations. A correlation between the delta T_{sk} during cooling in the first 10 min of the recovery period and the T_c at the end of the recovery period was not identified ($F_{1,13} = 1.348$, $p = 0.266$).

Additionally, there were no significant effects of coat color on the maximum T_c ($F_{1,14} = 0.010$, $p = 0.920$) nor on the maximum T_{sk} ($F_{1,22} = 0.015$, $p = 0.904$). Furthermore, coat color was not associated either with delta T_{sk} during exercise (12.5 ± 6.6) ($F_{1,22} = 1.098$, $p = 0.306$) or delta T_{sk} during recovery (13.0 ± 5.1) ($F_{1,21} = 1.246$, $p = 0.277$).

Evaluation of Heart Rate in Relation to T_{sk}

Additional analysis to evaluate the relationship between the T_{sk} and HR < 60 bpm revealed no significant correlation between the T_{sk} at the end of exercise and the duration to HR < 60 bpm ($F_{1,13} = 4.019$, $p = 0.066$). In conclusion, the study did not identify an association between HR recovery and the recorded T_{sk} during endurance exercise and recovery.

DISCUSSION

This is the first study to simultaneously monitor both T_{sk} and T_c continuously by means of several telemetric temperature recording devices on exercising horses in the field. The thermal sensors functioned correctly throughout the study, hence, there was little to no data loss. Consistent with our previous field study (2), the current study confirmed a substantial inter-individual variability in the T_{sk} time profiles despite execution of the same exercise protocol. Similar findings have been reported in human athlete studies and underline the physiological complexity of the mammalian thermoregulatory response, which is governed by a plethora of individually intrinsic variables (63–66).

With respect to T_{sk} and T_c monitoring in the current study, there was a lack of correlation between continuous monitoring of T_c and T_{sk}. Nevertheless, the association of a higher single point T_{sk} at the end-of-exercise period with a lower T_c at the end-of-recovery period is an interesting finding in the current study.

Up until now, a limited number of studies, almost all of which were treadmill based, monitored the T_{sk} continuously in the horses during the exercise and compared the T_{sk} to T_c (38–40, 42, 52). Only one recent field study has been performed, monitoring T_{sk} and T_{re} (20). The treadmill studies confirmed the lack of correlation between T_{sk} and T_c. For example, two submaximal exercise studies using arterial blood temperature compared the effect of different environments on thorax surface T_{sk} and showed T_{sk} was different from T_c (38, 42). Two high-intensity studies reported tail surface T_{sk} responses to cooling methods and acclimation, respectively, and both studies showed T_{sk} recordings were different from the T_c recordings (40, 41). Apart from those studies, only one laboratory-based high-intensity exercise study, comparing exercise in four horses in a cool vs. hot environment, suggested that the tail T_{sk} evolvement pattern seemed to follow the T_c pattern (using arterial blood temperature), although a statistical correlation was not investigated (39). A recent laboratory equine exercise study using the implantation of microchips, measuring muscle temperature (defined as “outer shell temperature” in that study) which may be extrapolated to field exercise in the future (67). There was a good correlation between central venous temperature (CVT) evolvement and outer shell temperature during a short bout of exercise (8–11.5 min) until CVT reached 41°C, although the outer shell temperature was reported to lag behind CVT during the recovery phase. While most of these experimental studies did not reveal a statistical correlation between T_{sk} and T_c, efforts to further investigate T_{sk} continue as the technique could easily be employed in the field. Consequently, wearable thermo-sensor techniques are being upgraded at an ever-increasing pace.

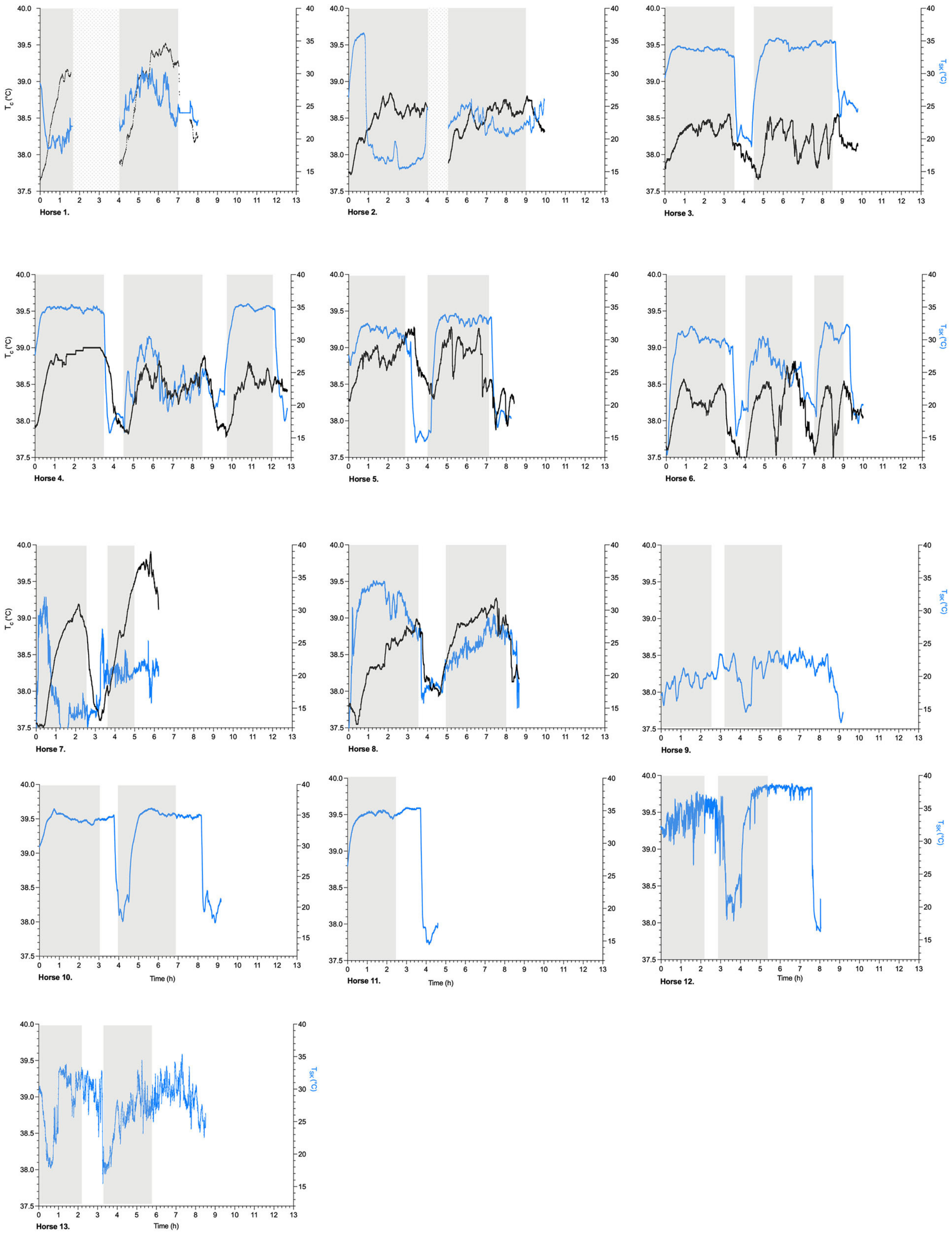

FIGURE 2 | Scatterplots of T_{sk}, skin temperature (°C, identified as blue) and T_c, gastrointestinal temperature (°C, identified as black) (left y-axis) per subsequent exercise loop of 40 km (gray blocks) (h, hours, x-axis) in endurance horses, Horses 1–13; dotted blocks identify blocks of no data recording; after each exercise loop, the horses were cooled down by pouring buckets of water over their bodies, followed by scraping the water off the bodies for a period of ∼10 min.

TABLE 2 | T_{sk} (°C) parameters during 40-km exercise (or extra 20 km in 2 horses exercising over a total of 100 km) and recovery.

Horse	Distance 40 km – 1st or 2nd	Net AUC T_{sk} (°C x min) exercise 40 km	Net AUC T_{sk} (°C x min) recovery 40 km	Base T_{sk} (°C) at start of exercise	Mean ± SD T_{sk} (°C) exercise	Min - max T_{sk} (°C) 40 km exercise	Delta T_{sk} (°C) exercise	T_{sk} (°C) at start of recovery (end exercise)	Delta T_{sk} (°C) first 10 min recovery	Delta T_{sk} (°C) recovery	Delta T_{sk} (°C) end exercise to end recovery/min	Mean ± SD T_{sk} (°C) recovery	Min –max T_{sk} (°C) recovery	Time to max T_{sk} exercise (min)	Time to min T_{sk} in recovery (min)	T_{sk} (°C) at end of recovery	T_{sk} (°C) at end of recovery
1	1st	−743.7	–	28.5	20.7 ± 2.4	17.8–28.4	10.6	21.9	–	–	–	–	–	0	–	23.2	23.2
1	2nd	587.3	−0.78	23.2	26.4 ± 2.6	21.2–31	10.8	24	−0.3	3.7	0.06	23.7 ± 1.1	22.1–25.8	74	21	26.4	22.8
2	1st	−1,328.6	–	26.4	20.9 ± 7.2	15.3–36.2	20.9	24.2	−1	6.6	–	23.3 ± 1.7	–	45	–	24.7	24.3
2	2nd	−515.3	54.9	24.3	22.6 ± 1.4	20.4–26.1	5.7	22.3	−1	5.1	0.09	23.3 ± 1.3	21–26.1	76	20	26	25.9
3	1st	838.1	−624.6	29.4	33.5 ± 0.8	29.4–34.2	4.8	32.5	−9.3	13.9	0.23	21.8 ± 3.5	18.8–32.7	39	29	22.1	22.5
3	2nd	2,820.5	−597.3	22.5	33.9 ± 2.0	22.5–35.4	12.9	34.8	−6.4	11.5	0.19	26.4 ± 2.7	23.4–34.9	72	23	24.6	24.4
4	1st	1,382.4	−85.5	27.7	21.0 ± 1.3	27.8–35.4	7.7	34.2	−12.3	18.4	0.31	21.8 ± 3.5	18.8–32.7	110	28	18.2	18.2
4	2nd	1,422.9	169.2	18.2	24.0 ± 3.2	15.8–30.5	14.7	24.7	−0.6	7.5	0.13	22.1 ± 1.8	19.3–26.5	79	16	26.8	26.8
4	*Extra 20*	*(1,051.1)*	*(−468.1)*	*26.9*	*34.4 ± 1.6*	*26.9–35.5*	*8.5*	*34.7*	*−8.6*	*17.2*	*0.29*	*22.8 ± 4.7*	*17.6–34.7*	–	*27*	*19.5*	*19.6*
5	1st	570.8	−635.5	27.6	30.7 ± 1.5	26.1–32.5	6.4	28.3	−10	15.4	0.26	16.7 ± 3.5	14.3–29.7	56	26	16.2	16.1
5	2nd	2965,.8	−997.3	16.1	31.9 ± 3.8	15.8–34	18.2	33.5	−12.9	16.9	0.28	19.2 ± 3.3	16.6–33.4	79	16	18.2	18.2
6	1st	2,736.5	−408.7	13.7	29.0 ± 4.0	12.4–32	19.6	29.7	−0.8	14.5	0.24	22.8 ± 5.3	15.3–29.5	74	12	19.3	19.5
6	2nd	995.4	251.0	19.5	26.2 ± 2.8	19–30.5	11.5	24.6	0.8	6.5	0.11	23.2 ± 2.2	20.1–26.6	55	19	20.1	20.1
6	*Extra 20*	*(809.8)*	*(−427.0)*	*20.1*	*29.0 ± 4.1*	*18.1–32.7*	*14.5*	*31*	*1.2*	*15.1*	*0.25*	*23.8 ± 5.9*	*17.2–32.3*	*36*	*46*	*20.1*	*20.1*
7	1st	960.6	−3	11	17.7 ± 6.3	10–32	22	13.3	−0.7	15	0.25	16.4 ± 3.9	12.1–27.1	26	10	18.9	19.2
7	2nd	80.4	97.5	19.2	20.4 ± 1.0	18.2–23.9	5.7	19.7	0.8	9.5	0.16	20.8 ± 1.2	15.8–25.3	64	18	20.2	20.2
8	1st	4,170.6	−547.4	10	30.4 ± 4.1	10–34.5	24.5	25.9	1	11.4	0.19	19.3 ± 3.1	15.8–34.5	75	10	20.3	20.2
8	2nd	884.2	110.8	20.2	24.8 ± 2.1	19.2–29.4	10.2	26.7	−1	12.2	0.20	23.8 ± 3.1	15–27.2	150	19	18.3	18.8
9	1st	535.8	133.2	18.8	19.8 ± 1.7	15.6–23.5	7.8	23.4	−2.8	8.9	0.15	18.0 ± 2.6	14.6–23.4	179	16	22.6	22.6
9	2nd	−68.3	−172.4	22.6	22.3 ± 0.9	18.7–24.4	4.7	23.4	−3.2	10.4	0.17	17.6 ± 2.8	13–32.3	141	20	14.3	14.5
10	1st	934	−628.4	29.8	34.2 ± 1.1	29.9–36	6	34.7	0.3	17	0.28	23.9 ± 5.9	17.7–35	44	30	26.1	26.2
10	2nd	1,825.3	−852.4	26.2	34.7 ± 1.5	26.2–36.1	10	34.8	−15.3	17	0.28	20.4 ± 2.3	17.5–32.3	63	26	21.2	20.9
11	1st	1,691.6	−944.0	26.5	34.3 ± 1.4	26.6–35.5	9	35.4	−15.9	21	0.35	18.5 ± 5.7	14.5–35.4	179	27	17.7	17.7
12	1st	4,748.6	−597.2	32.3	30.9 ± 7.6	9.5–37.5	29	35.3	−10.4	19	0.32	22.8 ± 3.5	17.9–35	152	26	28.4	28.7
12	2nd	1,356.4	−524.7	28.7	37.4 ± 1.7	28.7–38.7	10	38.1	−19.4	22.2	0.37	19.6 ± 4.9	16.3–38.2	90	29	16.8	16.8
13	1st	−204.5	−169.3	29.5	24.2 ± 4.4	17.9–33.8	16	25.5	4.6	17	0.28	22.6 ± 4.6	15.5–32.8	84	18	26.8	26.4
13	2nd	453.9	−45.1	26.4	28.6 ± 2.5	21.6–35.2	14	27.9	2.6	11	0.18	27.1 ± 2.2	22.6–33.4	196	22	24.1	26.2

Data are presented as mean ± SD. T_{sk}, skin temperature; AUC, area under the curve; min, minutes; min-max, minimum to maximum; italic indicates an extra loop of 20-km exercise (total, 100 km, $n = 2$ horses); delta (°C change), T_{sk} change during exercise, and recovery periods, including the first 10-min recovery period; – indicates no data collected/not available.

TABLE 3 | Overall T_{sk} and T_c variables during exercise and recovery of endurance exercise in a cool environment.

Variables	Endurance 40 km $n = 13$ ($n = 8$*)
Duration (minutes) exercise	198 ± 63
Duration (minutes) recovery	60
T_{sk} (°C) overall	27.8 ± 5.6 (17.71–37.37)
Base T_{sk} (°C) (at start-of-exercise)	23.1 ± 6.1 (10–32.3)
Min T_{sk} (°C) exercise	19.8 ± 6.2 (9.5–29.9)
Max T_{sk} (°C) exercise	32.3 ± 4.3 (23.5–38.7); 31.6 ± 3.5°C*
T_{sk} (°C) exercise	27.2 ± 5.7 (17.7–37.4)
Time to max T_{sk} exercise (minutes)*	88 ± 51 (0–196); 67 ± 34*
Delta T_{sk} (°C) exercise	12.5 ± 6.6 (4.7–29); 12.9 ± 6.4*
Net AUC T_{sk} exercise (°C × minutes)	1,164 ± 1,448 (−1,329 to 4,749); 1,114 ± 1,469*
T_{sk} (°C) at end-of-exercise	28.0 ± 6.1 (13.3–38.1)
Min T_{sk} (°C) recovery	17.3 ± 3.1 (12.1–23.4); 17.7 ± 3.3*
Max T_{sk} (°C) recovery	30.9 ± 4.1 (23.4–38.2)
T_{sk} (°C) recovery	21.5 ± 2.8 (16.4–27.1
Delta T_{sk} (°C) recovery	13.0 ± 5.1 (3.7–22.2)
Delta T_{sk} (°C) first 10 min recovery	−4.7 ± 6.7 (−19.4 to 4.6)
Delta T_{sk} first 10 min recovery/minute (°C/min)	−0.5 ± 0.7 (−1.9 to 0.5)
Number of horses T_{sk} > 39°C**	None
T_{sk} (°C) at end-of-recovery 40 km ($n = 25$)	21.8 ± 3.8 (14.5–28.7)
Number 40 km periods T_{sk} returned to base T_{sk} at the end of 60 min recovery	14/25
Net AUC T_{sk} recovery (°C × minutes)	−305 ± 388 (−997 to 251); −230 ± 392*

*Data are presented as overall mean ± SD. T_{sk}, skin temperature; AUC, area under the curve; T_c, GI pill temperature; max T_c or T_{sk}, maximum T_c or T_{sk}; n, number identified only if different 40-km exercise periods. *Indicates a total of 8 horses (comparison T_{sk} to T_c in 8 horses; total, 16-x-40-km periods); **T_{sk} > than 39°C based on (21).*

Continuous T_{sk} Sensor Recording During Field Exercise

Ongoing efforts to identify a reliable proxy for continuously monitoring the thermoregulatory response in horses during field exercise are not always successful or practical. A more invasive method with thermistors placed in blood and brain was used in three horses during free field exercise and reported a good correlation; however, for obvious reasons, this is not easily applicable in practice (51). Several field studies have investigated less-invasive continuously monitoring approaches, such as the intra-uterine temperature (two mares) or the GI temperature; both approaches (intra-uterine and GI) showed a good correlation with the T_c (18, 19, 68). A recent study has continuously monitored surface T_{sk} using i-Button® and simultaneous T_{re} during two canter bouts of 4.5 min of field exercise at a speed ranging from 6.7 to 7.5 meters per second (20). The T_{sk} was continuously monitored at the level of the rump and shoulder regions in 10 eventing horses using a cross-over study design. The study showed that pre-exercise cooling resulted in a delta T_{sk} ranging from −2.3 to −3.3°C and a reduced median T_{re} of 0.3°C, which peaked at 9 min into recovery, compared to the control group (20). Although the study did not investigate correlations between T_{sk} and T_c, the effect of lower T_{sk} pre-exercise on a reduced T_{re} is of interest, and consistent with previous human sports studies (69, 70).

In brief, the current reliance on continuous T_{sk} sensor recordings during field exercise has been proven to be inconsistent and unreliable as a proxy for the thermoregulatory response. This is consistent with a study comparing the effects of precooling in 10 human athletes to estimate the T_c (using a GI pill) (71).

Comparing T_{sk} With T_c

When comparing the delta T_c with delta T_{sk} in the current study, the delta T_{sk} was greater during endurance exercise as depicted in **Figure 3A**. However, a laboratory-based high-intensity equine exercise study using thermocouples attached to the skin with tape and located in pulmonary artery blood to continuously monitor T_{sk} and T_c in six horses revealed a delta T_{sk} of 2.5°C (42) similar to the delta T_c value in the current study (3°C). Associations between T_{sk} and T_c were not evaluated in that study; however, the difference in the exercise duration may indicate a difference in thermoregulatory activity over time, namely, 200 min of endurance exercise in the current study vs. average of 40 min in the former study evidenced by the end-of-exercise $T_c > 41$°C (42).

Another interesting finding was the significantly higher time to maximum T_c when compared to the time to maximum T_{sk} (**Figure 3B**). This finding suggests that the endurance horses in the current study performing in a cool environment were efficiently thermoregulating during exercise without the development of hyperthermia ($T_c > 39$°C) as has been documented previously (2). On the other hand, a short duration of high-intensity exercise in more challenging environmental conditions may trigger $T_c > 41$°C and, consequently, requires dissipation of excess MH to occur post-exercise (38, 42, 53).

Despite all these ongoing efforts to practically incorporate surface T_{sk} monitoring into thermoregulation and wellbeing monitoring in the field, researchers should always keep in mind the possible factors that challenge a potential correlation between T_{sk} and T_c. In addition, the monitoring device used must be able to correctly function and cope with the practical conditions under which horses exercise and compete. Important factors are environmental variables; among which are weather conditions, and whether or not additional cooling is applied. Furthermore, the type of temperature sensor equipment and the anatomical site at which the T_{sk} equipment is placed have their influence, together with individually intrinsic horse-related factors.

Environmental Factors

Factors Influencing T_{sk} and T_c Evolvement and How They Relate to Each Other

The T_{sk} at any site on the skin surface reflects a balance between heat being delivered to the skin by arterial blood, body, and local skin metabolism, and heat exchange with the environment by convection, radiation, and evaporation. Any factors that interfere with this balance can change the T_{sk}. Many factors that modulate

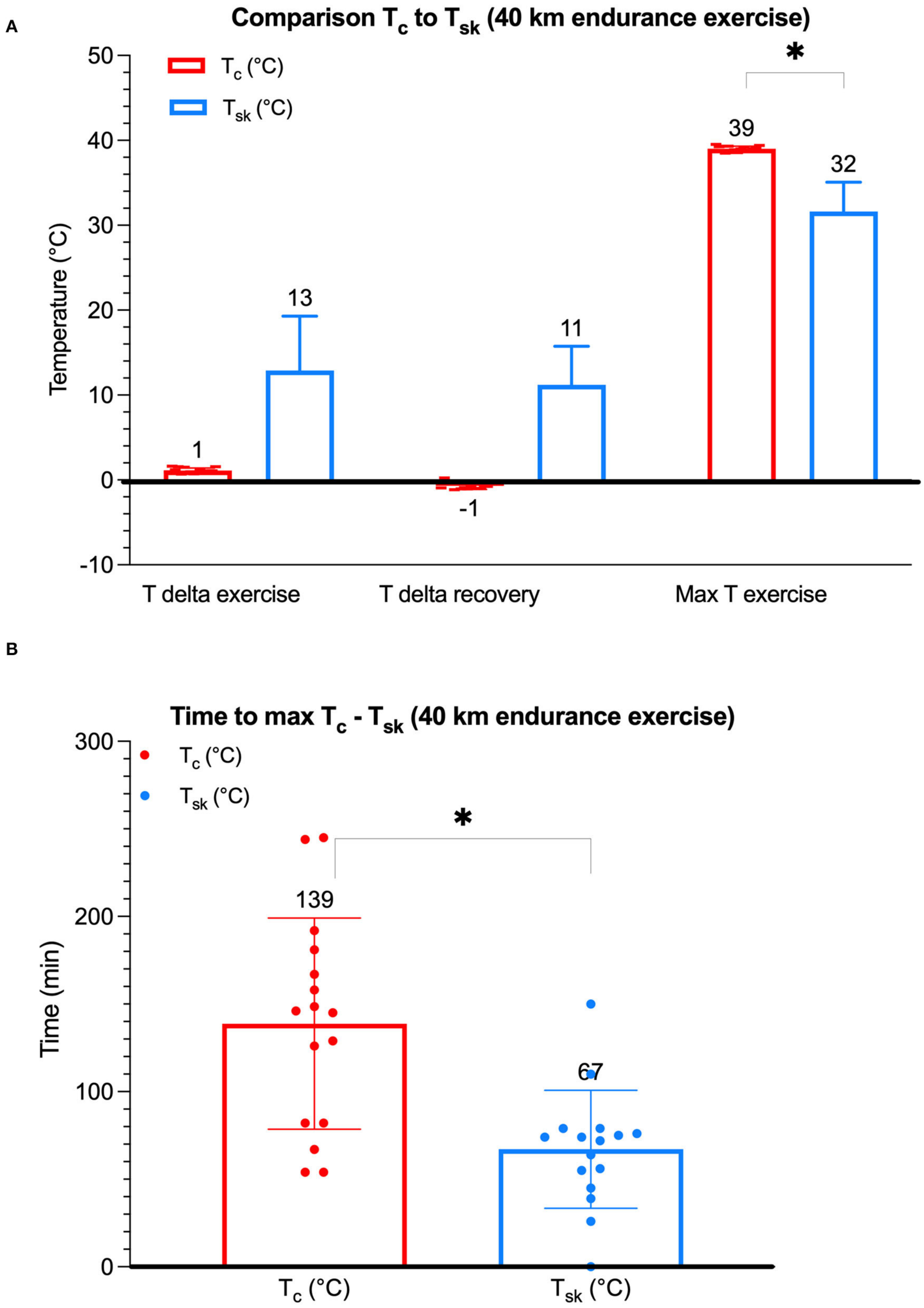

FIGURE 3 | A boxplot diagram depicting T_c and T_{sk}, respectively, in endurance horses. Mean (a horizontal line) ± SD (error bars) of individual maximum T_c (°C, $n = 8$) and maximum T_{sk} (°C, $n = 8$) values. There was no significant association between T_{sk} and T_c; no significant difference between delta T_{sk} exercise and recovery ($p = 0.41$); a significantly greater maximum T_c when compared to maximum T_{sk} ($p = 0.0002$) **(A)**; and a significantly increased time to maximum T_c than T_{sk} ($p = 0.0004$) **(B)**; *indicates a significant difference.

T_c evolvement during exercise simultaneously influence T_{sk}, such as a plethora of performance capacity parameters, as well as environmental conditions. Environmental factors can easily and quickly change T_{sk} without directly affecting T_c (1, 47, 59, 72–74). These factors include the T_a, solar radiation, soil radiation, humidity, shade, and wind speed (air movement). For example, a varying T_a ranging from 20 to 30°C was directly related to the onset of skin vasodilation and sweat evaporation (72), while, on the other hand, a low T_a was shown to induce a lower sensitivity (50%) of percutaneous T_{sk} microchips in 52 foals and 30 adult horses to identify fever compared to measuring T_{sk} in a hotter T_a (29°C) (73). That would mean that, in case of fever (also known as an increased T_c set-point), a cool sunless environment renders T_{sk} monitoring using microchips less representative for T_c monitoring. In addition, Holcomb et al. (74) demonstrated that T_{sk} and T_{re} were highest at the peak solar radiation during the day. The T_{sk} sensor in the current study was located ventrally on the chest of the horse covered by the belt and the girth, thus avoiding T_a effects, such as solar radiation.

It is common practice to cool down endurance horses during the recovery period between subsequent exercise loops. Cooling down was also applied in the current study design in a real-life competition context. The goal was to challenge the temperature monitoring devices with real circumstances in which they would be required to function. With respect to cooling down approaches, the mechanism by which the thermoregulatory systems are challenged greatly depends on how the loss of heat counteracts MH production through non-evaporative pathways as well as evaporative methods. The evaporative exchange of heat of the skin with the environment depends on the thermal gradient between T_{sk} by local skin perfusion and its immediate environment, including vapor pressure, airflow, and solar radiation, especially during field exercise (17, 49, 75). At the end-of-exercise period, the cooling of sport horses is standard, especially in endurance and 3-day eventing competitions. However, post-race cooling methods are not standardized in the racing industry. The duration of cooling endurance horses in the field is, on average, 10 min based on each owner's judgment, which could include HR monitoring. The mean end-of-exercise T_{sk} in our study with endurance horses exercising in a cool environment (mean, 15.3°C) was 28°C, and no horses developed a T_{sk} higher than 39°C.

On the other hand, exercise studies in warmer environments documented a post-exercise T_{sk} higher than 39°C. For example, a recent report has revealed that 28 out of 38 horses exercising in a hot, dry environment (mean $T_{a,}$ 38.8°C), and 6 out of 37 horses exercising in a warm, humid environment (mean $T_{a,}$ 31.1°C) showed a post-exercise IRT T_{sk} higher than 39°C. These researchers suggested horses recording T_{sk} higher than 39°C were at risk of developing heat stress and EHI and used this T_{sk} response as an indicator for racehorses requiring cooling (21). The association between T_{sk} and EHI risk could be physiologically explained by a low T_c-to-T_{sk} gradient, therefore decreased capability to transfer MH to the skin, thus compromising the dissipation of MH by evaporation (21, 56).

A similar mean IRT T_{sk} of 40°C was recorded at the end of exercise in a recent study, evaluating cooling methods in racehorses in a warm environment (mean $T_{a,}$ 31.8°C) (35). A T_{sk} higher than 39°C is consistent with earlier laboratory-based studies in a warm T_a (29.1°C and 31.1°C, respectively) (40, 45). In retrospect, scraping off water from the horses during cooling down was not the most optimal approach since Takahashi et al. (35) favored continuous application of cold water without subsequently scraping it off.

T_{sk} Equipment-Related Features and Location

Equipment to Measure T_{sk}

Within the rapidly expanding wearable digital device industry, surface T_{sk} monitoring devices are constantly being upgraded to provide data output. However, in that respect, the critical question remains: how should we interpret those data? Overall, three different types of temperature sensor equipment are reported: thermistors (such as microchips), thermocouples, and IRT devices, with IRT being the most studied device recently in horses (21, 22, 26, 32–37, 76–80). It is essential to understand that those sensors use different physical processes to obtain data, which may result in significant differences in data output. These sensor surface T_{sk} differences due to the type of equipment may show only a minor bias, which may prove to be clinically meaningful (75). For example, a study comparing IRT and thermocouples at single pre-exercise, intra-exercise and post-exercise points in 12 human athletes revealed a poor Bland Altman agreement and low reliability between the different methods (81).

To produce IRT imaging to picture surface T_{sk} of different parts or the whole body, a remote IRT camera positioned at 30-cm proximity to the skin surface has been recently evaluated with varying results (26, 37, 82). For example, a study compared IRT T_{sk} to T_{re} in 40 adult horses and concluded T_{sk} was not an accurate method to determine the T_c (82). The remote position of the IRT held far from the skin has the advantage of not interfering with the local T_{sk} balance, although the remote T_{sk} measurement will be partly affected by the adjacent environment surrounding the skin (22, 23, 75). The temperature sensors that were used in the current study were in direct contact with the skin and covered by a belt. This belt might have interfered with the local thermal conductivity and the local evaporative cooling capacity and thus might have delayed equilibration of the local T_{sk} with the surrounding skin. On the other hand, an adequate and essential sensor-to-skin contact was ensured by the position of the sensor in the belt. Furthermore, the skin surface covered by the sensor was small enough to prevent causing local skin changes (22, 75).

We were unable to calibrate and validate the IR sensor prior to the study; however, studies of different T_{sk} recording methods and comparisons with a certified thermocouple in a thermostatically controlled water bath are extremely rare. One study evaluated sensor systems in human athletes during rest and exercise in a hot environment and revealed a good agreement for employing a telemetric thermistor system when compared to the standard hard-wired thermistor system and a poor agreement for using a thermal camera (83).

In summary, IRT techniques differ widely in human and equine medicine, including positioning of the camera and environmental control measures (22, 24, 75). A consensus guideline has been developed only recently, addressing the multiple data collection methods of the human T_{sk} using IRT (84), while Soroko and Howell (22) described a protocol using IRT in equine medicine.

T_{sk} Equipment Location

The anatomical location of the sensor on the horse to record T_{sk} measurements has been shown to influence T_{sk} results (22, 23, 26, 29, 37, 47). For example, remote IRT was used to evaluate differences between 10 locations on the body during two seasons in the year with the highest T_{sk} recorded at the level of the chest (22.5°C) and shoulders (20.4°C) in horses at rest in a cool T_a (mean, 6.7°C) (26). In another study, the IRT T_{sk} was greatest at the shoulder area when compared to three other T_{sk} locations measured at the start and the end of 20-min exercise (32.3°C and 34.2°C, respectively) in a moderate T_a (mean, 23°C) (37). The results of our study in a cool T_a (mean, 15.3°C) revealed a mean T_{sk} measured at the lower chest area of 23.1°C during an average of 200-min exercise and a mean T_{sk} of 21.8°C during recovery. The different T_{sk} values between the current study and Soroko et al. (37) illustrate the effect of exercise intensity (submaximal vs. maximal) and duration (long vs. short). The differences in T_{sk} over several body areas may relate to the varying networks of blood vessels in those body regions and their vasodilation to exchange thermal heat with the proximal environment (23, 55, 56, 59). It is essential to note that both monitoring methods share some vasomotor or endocrine mechanisms, although they present differences depending on the degree of heat dissipation or retention that the organism needs. Consequently, monitoring of T_{sk} in the current study revealed a physiological response of the local T_{sk} to the changes of T_c during endurance exercise over time, although the responses were not correlated.

While IRT cameras are increasingly used in equine sports medicine, this method involves a single point in time measurement. One exception is the study by Soroko et al. (37), who reported dynamic IRT monitoring every 15 s during treadmill exercise. To be precise, a review of the use of IRT in human endurance athletes reported that 25 of the 45 studies were conducted over the last 5 years (2017–2021), but, up until now, only five real-life field endurance studies have been performed (24). The latter review concluded that further analysis is required to assess whether T_{sk} could be used as a reliable proxy to describe real-time thermoregulation (24). Another important relevant finding is that surface T_{sk} may be low in human athletes with EHI and, hence, provide misleading information (85). A different IRT method approach is measuring eye surface temperature; that study revealed no relationship with T_c in horses (86).

Individual Horse-Related Factors

Horse-related factors include breed, body condition score, age, character (such as nervousness), and skin-related properties, such as sweat rate, skin thickness, blood vessel density, hair coat properties, clipping, and coat color (23, 27, 30, 36, 55, 77, 78, 80, 87). The sweat loss in the current study was subjectively scored from 1 to 3 by E-L.V., and, in retrospect, more accurate sweating scoring based on objective specific phenotypic descriptions would have been a better approach (88). The effect of breed on T_{sk} relates to the ratio of BM to the body surface area—the higher the body surface area in relation to the BM, the higher the heat dissipation (72). The low-surface-area-to-mass ratio of the horse results in greater demands being imposed on the thermoregulatory system during long-term submaximal exercise (30, 49, 53). Our study included mainly Arabian horses, known to have a lower BM and, hence, a higher-surface-area-to-mass ratio.

The hair coat length in the current study was similar (all clipped), which is essential as clipping the winter coat resulted in improved heat dissipation during and after exercise, resulting in decreased T_{sk} and T_{re}, as reported in previous studies (27, 30). One of those previous studies used a thermistor probe to evaluate the effect of coat clipping in three horses on both the surface T_{sk} and T_{re}. That study reported no effect of clipping on post-exercise $T_{sk,}$ while T_{re} was ∼0.2°C higher in unclipped horses (30). Indeed, a longer haircoat length limited the thermal imaging in a study assessing T_{sk} in mares (77). Furthermore, coat colors may be relevant (87); however, the current study of 13 horses revealed that light or dark coat color had no significant effect on T_{sk}, which is consistent with a previous study (73). Individual horse-related character differences may exist, such as nervousness that triggers sympathetic nerve activity associated with vasoconstriction of skin blood vessels. This neurophysiological response may explain varying reduced local T_{sks}, decreased heat loss, and hyperthermia (23, 55, 78, 89).

Modeling Using T_{sk}

While, generally, T_{sk} can be easily monitored, the T_{sk} in the current study did not provide data suitable for extrapolating to similar changes in the T_c. Consequently, the development of integrative models using T_{sk} to determine the heat balance during exercise has been investigated in human studies and in one equine study (87, 90, 91). However, no regression model could predict physiological stress load using single-point IRT T_{sk} in 17 human marathon runners in the field (92). A recent approach in human exercise research has investigated the application of models and algorithms using data and variables, such as HR and HR variability, to successfully estimate T_c (47, 90, 93–95). Physiologically, HR reflects the blood flow rate to the muscles (MH production) and blood flow to the skin (heat loss). For example, recent studies have concluded that combining continuous insulated T_{sk} and HR monitoring in 13 and 8 human athletes in a hot (35°C) and warm (25°C) environment, respectively, could provide a predictive model of T_{re} or T_c (using GI pills) (90, 94). In contrast in the current study, HR recovery in the endurance horses was not directly related to T_{sk}. Further investigation is required into the potential association of T_{sk} and HR for accurate predictive modeling of T_c in equine athletes.

Association Between Single-Point T_{sk} at the End-of-Exercise Period Compared With the T_c at the End-of-Recovery Period

An interesting finding of the current study performed with the endurance horses was the association of a greater T_{sk} at the end of exercise with a significantly lesser T_c at the end of recovery (60 min). Several theories could be considered to explain this association between T_{sk} and T_c: firstly, the raised T_{sk} indicates the launch of an active thermoregulatory response to anticipate the increased $T_{c,}$ and, once the MH is successfully dissipated, the T_c decreases. This argument can be coupled with the effect of cooling post-exercise, which may be more prominent when T_{sk} is greater and, ultimately, results in higher dissipation of MH and a reduced T_c. Several other field exercise studies in horses have investigated correlations between single-point T_{sk} and other variables (21, 32). For example, a recent equine study involving 8 endurance horses has investigated the association between endurance training intensity (1 h at 19 km/h vs. 2 h at 16 km/h vs. 3 h at 20 km/h) and T_{sk} using an IRT camera measured at different locations and at different time points. The study identified that the T_{sk} at the coronary band increased with training intensity unlike the maximum T_{sk} (32).

Aside from the variance in hot vs. cool T_a in these studies the differences in exercise intensity could explain the dissimilarity between the racehorse study results of Brownlow and Mizzi (5) and the current study involving endurance horses. For racehorses undertaking high-intensity, short-duration exercise, the dissipation of MH occurs post-exercise as opposed to endurance horses, which manage their MH throughout their submaximal long-duration exercise (2, 5, 21, 56). For example, the T_{sk} and its evolvement pattern can be related to acute blood flow variances associated with a different type of exercise intensity (25, 95). Overall, in our study monitoring endurance horses conducting exercise during cooler months, the mean T_{sk} at the end of exercise was 28°C, while none of these horses had a T_{sk} higher than 39°C.

The end-of-recovery period T_{sk} showed a considerable individual variation (range, 14.5–28.7°C) despite the application of a uniform cooling protocol. The T_{sk} during the 60-min recovery period revealed that the T_{sk} returned to the baseline only in over 50% of the 40-km recovery periods. This is in contrast to other studies, which found that after 20 min of treadmill exercise in a hot (32–34°C) and dry T_a condition, all T_{sk} values returned to baseline T_{sk} after 60 min, and, after 45 min in a T_a of 20°C (without cooling), respectively (28, 42). The main difference between the current study and other laboratory-based studies was the continuous T_{sk} monitoring during a field exercise in an uncontrolled T_a.

Limitations

As in any study, there are several limitations that should be considered. Obviously, throughout this "in-the-field" study, not all research conditions could be controlled for 100% of the time, such as weather conditions involving T_a and the degree of solar radiation, the training, and the dietary management of participating horses. These factors may have affected the individual T_{sk} and T_c time profiles. However, this applies to all "in-the-field" competition studies and, under ideal conditions, should not interfere with the reliability of a solid thermoregulatory monitoring proxy suitable for assuring the thermoregulatory wellbeing of competition horses in the field (74). Endeavors to assess the thermal environmental variables were limited to BOM and HOBO recordings of the T_a and the RH, with the HOBO device placed at one location. Other essential external variables, such as wind speed, were not included in the T_a measurements (17). The current study involved only one type and location of the wearable T_{sk} sensor based on IR technology. In the future, other thermo-physical measuring approaches will prove to be more robust. However, on all occasions, the involvement of a validated "gold standard" against which the performance of new individual monitoring devices is set should be an essential part of future studies (75).

CONCLUSION

While the method of monitoring T_{sk} may be non-invasive and straightforward, our results have clearly shown that T_{sk} monitoring on its own does not reliably estimate the T_c evolvement during a field exercise in endurance horses since a correlation between T_c and T_{sk} could not be identified. Notably, a high T_{sk} at a single point during a field exercise in a cool T_a did not identify the endurance horses with an increased T_c. Further research into T_c monitoring in different equine sports and under differing weather conditions must be undertaken to create a baseline for further fine-tuning hot weather policies. Accordingly, veterinarians, trainers, and owners can be advised to continuously monitor T_c to ensure the health and welfare of all horses.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by University of Adelaide Animal Ethics Committee. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

E-LV prepared and carried out the study design, data collection, preparation and creation of the database, descriptive and part of statistical analysis, interpretation of the data, and writing of the manuscript. CD was involved in study design, preparation and creation of the database, analysis and interpretation of the data, and writing of the manuscript. GH contributed to drafting and revising the manuscript. TM contributed to reviewing the manuscript and statistical analysis. The final manuscript was read and approved by all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.894146/full#supplementary-material

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Neonatal infrared thermography images in the hypothermic ruminant model: Anatomical-morphological-physiological aspects and mechanisms for thermoregulation

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Hypothermia is one factor associated with mortality in newborn ruminants due to the drastic temperature change upon exposure to the extrauterine environment in the first hours after birth. Ruminants are precocial whose mechanisms for generating heat or preventing heat loss involve genetic characteristics, the degree of neurodevelopment at birth and environmental aspects. These elements combine to form a more efficient mechanism than those found in altricial species. Although the degree of neurodevelopment is an important advantage for these species, their greater mobility helps them to search for the udder and consume colostrum after birth. However, anatomical differences such as the distribution of adipose tissue or the presence of type II muscle fibers could lead to the understanding that these species use their energy resources more efficiently for heat production. The introduction of unconventional ruminant species, such as the water buffalo, has led to rethinking other characteristics like the skin thickness or the coat type that could intervene in the thermoregulation capacity of the newborn. Implementing tools to analyze species-specific characteristics that help prevent a critical decline in temperature is deemed a fundamental strategy for avoiding the adverse effects of a compromised thermoregulatory function. Although thermography is a non-invasive method to assess superficial temperature in several non-human animal species, in newborn ruminants there is limited information about its application, making it necessary to discuss the usefulness of this tool. This review aims to analyze the effects of hypothermia in newborn ruminants, their thermoregulation mechanisms that compensate for this condition, and the application of infrared thermography (IRT) to identify cases with hypothermia.

KEYWORDS

cattle, goat, newborn/neonate, sheep, water buffalo, wild ruminants, thermoregulation, infrared thermography

Introduction

Hypothermia in newborn animals is caused by exposure to an ambient temperature significantly lower than that of the intrauterine environment (10–15°C lower than the core temperature) (1–3). The animal's body temperature is below the thermoneutral zone (4), which means heat loss exceeds heat production (5). This condition can affect the newborn's vitality by impeding the colostrum ingestion necessary to acquire the energy resources required to produce heat (6–9) and its importance lies in its impact on neonate mortality. Observed mortality rates in lambs are around 15%, while bovine calves range from 10 to 20% (10). Factors like the season of the year, environmental temperature (exposure to cold, rain, and wind) (11, 12), hypoxia and acidosis (in cases of dystocia), low birth weight, and inadequate nutrition of the mother during gestation are also elements that can induce hypothermia (3, 13, 14).

Unlike altricial species, precocial species, including ruminants, are more advanced neurodevelopmentally at birth. This provides them with highly efficient thermoregulatory mechanisms and an enhanced capacity to maintain homeothermy in diverse environments. The two main mechanisms are the vasomotor response and shivering or non-shivering thermogenesis (10). These mechanisms characterize the neonates of many animal species, but their nature and efficiency depend on the environment, species (precocial *vs.* altricial) (3), anatomical and physiological traits like coat type (hair, wool, fur), and the proportion of type II muscle fibers (15–17).

The physiological consequences and productive impact of hypothermia in newborns are potentially severe, so it is essential to recognize this condition promptly. Infrared thermography (IRT) has been proposed as a complementary, reliable, sensitive technique for assessing body temperature in animals (17, 18), but IRT's temperature recording in different anatomical regions has not yet been validated as an instrument for evaluating degrees of hypothermia. Moreover, available literature about the application of IRT in neonatal ruminants is required to determine its accurate application. This article aims to analyze the effects of hypothermia on newborn ruminants, their coping thermoregulatory mechanisms, and the usefulness of IRT for recognizing hypothermic conditions.

Effect of hypothermia on newborn ruminants, a precocial model

Newborn ruminants are precocial animals with advanced locomotor development that allows them to stand up within the first minutes, or hours, of life and begin to ingest colostrum. They also have thermoregulatory mechanisms that allow them to maintain or reestablish normothermia (19), but several factors can affect them (3). Differences in heat production efficiency have been observed among species. For example, lambs are reported to have a higher maximum caloric rate per unit weight than bovine calves (20–22), a difference that helps explain why small ruminants like lambs or kid goats are more susceptible to hypothermia than larger ones [e.g., domestic bovine calves (*Bos taurus* or *Bos indicus*) or water buffaloes]. Also, a large surface area relative to mass shows a disadvantage in retain heat (23).

Activating the responses to prevent a drop in core temperature has physiological consequences (1, 7, 24, 25), especially when hypothermia persists for extended periods because it triggers vasoconstriction as a circulatory reaction that prevents heat loss. However, vasoconstriction has adverse effects, such as decreasing blood flow to high-demand tissues in the neonate, and inducing cellular hypoxia (26). Moreover, in animal models at the histological level, even cerebral neuronal necrosis (histopathological hypothermia score = 2 vs. control = 0) has been reported by Petko et al. (27). Hypoxia also has metabolic consequences. Tyler and Ramsey (28) evaluated the effect of reduced blood oxygen in 12 Holstein calves at 72 h postpartum. They found that due to hypoxia, CO_2 levels did not exceed 45 mmHg, and glucose concentrations tended to decline in the hypoxic calves (110 vs. 105 mg/dl), while lactate levels increased between 6 and 42 h postpartum to values of 90 mg/dl in hypoxia group and 23 mg/dl in the control group. In a 28 blue and white Belgian calves' study, Cambier et al. (29) analyzed blood oxygen transport and tissue oxygenation. In hypoxic calves, acidosis and hypercapnia were the main effects caused by decreased oxygen affinity for hemoglobin in arterial blood, which was related to the amount of O_2 in the bloodstream (control group $= 77.2 \pm 9.9$ mmHg vs. hypoxemic group $= 47.9 \pm 12.9$ mmHg). According to those results, hypoxia caused the organisms to utilize anaerobic metabolic pathways that triggered lactate production, which induced a state of acidosis and hypoglycemia that reduced the animal's capacity for thermogenesis (21, 22, 30).

In response to the decreased oxygen supply to tissues, an increase in air volume in ventilation occurs with each inspiration that can produce tachycardia in animals to improve oxygen distribution (31). The increase in cardiac output and the production of erythrocytes are other compensatory responses to improve oxygen transport and nutrient delivery to tissues (26). These physiological consequences become more pronounced when the mechanisms cannot operate efficiently because the neonate has limited energy reserves (such as glucose and oxygen). This condition can arise, for example, when a newborn consumes and may severely deplete those reserves for purposes of thermoregulation during the first 12 h post-birth.

The ability of young ruminants to stand up and ingest colostrum in the first hours of extrauterine life allows them to acquire energy and maintain a thermoneutral state (3, 32). Nutrition in newborns has been discussed in depth by authors like Hammon et al. (33), who sustain that colostrum provides nutrients that include glucose, a biomolecule used to combat hypothermia. The supply of immunoglobulins through colostrum, and their absorption, are other elements that help maintain homeostasis in neonates. As mentioned above, acidosis can be an adverse effect of hypoxia derived from hypothermia. Some reports on bovine calves indicate that a blood pH of 7–7.20 and high pCO_2 concentrations (respiratory acidosis) are negatively related to immunoglobulin G1 absorption (IgG) (34). However, a study of 48 Holstein calves found no association between the degree of IgG absorption and increased $PaCO_2$ concentrations for 1 h (35). Therefore, it has been suggested that hypothermia generates metabolic consequences and affects newborns' underdeveloped characteristic immunological system.

Likewise, environmental factors such as the year's season may expose newborn ruminants to low temperatures. For example, for species like the water buffalo, there are reports of high mortality rates in autumn, when cold and rain predominate (36). However, this relation has not been specifically evaluated because incidences of stillbirths can be affected by both environmental and genetic factors. It has been suggested that backcrossing may help diminish mortality rates in newborns (37). Moreover, anatomical and morphological characteristics can impact the physiological effects of hypothermia in newborns or the animals' adaptive measures to prevent such consequences.

Anatomical-physiological characteristics of thermoregulation in ruminants

In ruminants, the degree of development at birth is considered similar within each species, though differences in rates of brain maturation may occur, as has been seen between lambs, which reach the maximum weight of cerebral parenchyma 50 days after birth, and kid goats where this occurs at just 20 days post-birth (38). Studies of sheep have shown that they lack awareness during the fetal stage and that the onset of this condition comes within 20 min post-birth. At that moment, these animals show voluntary responses or the involvement of the cerebral cortex in perceiving stimuli (39). The ensuing behavioral responses are recognized by mother-young interaction at 20–60 min post-birth. This time is called the sensitive period when newborns show a strong preference for their biological dams up to 24 h (40). Their interaction includes grooming, a behavior that promotes bonding and plays a role in thermoregulation as the dam cleans and dries the neonate's coat, thus reducing the amount of heat loss due to evaporation (41).

Another mechanism that helps prevent heat loss in neonatal precocial species is developing the locomotory system that allows newborns to stand up in the first minutes postpartum. Regueiro et al. (42) reported that newborn lambs stand up after an average of 22 min, and 53.9–87.9% began to suckle within 1 h of birth. Results for Australian Bush goat kids are similar, as they begin to stand at an average of 20.2 min and suckle at 50.8 min (43). Although neurological development is essential for maintaining the temperature of newborns, anatomical-physiological aspects are crucially involved in the thermogenic mechanisms that prevent heat loss, such as vasomotor control and heat production by contraction of the skeletal striated muscle or through the consumption of high-energy adipose tissue (1, 44).

In the first case, involuntary rhythmic contractions of skeletal striated muscle reflect the ratio of muscle fibers. The importance of a specific muscle fiber ratio lies in the degree of resistance and maintenance of the muscle contractions that can be produced. This has been proposed as a difference that may influence the efficiency of shivering thermogenesis, complemented by the individual morphology, percentage of body fat, diet and the degree of cold acclimatization (45). Recruitment of type I (dependent on aerobic metabolism) or slow-oxidative muscle fibers is associated with low-threshold (4–8 Hz), continuous shivering, and lipid consumption. In contrast, type II (fast-glycolytic) fibers respond to high-intensity bursts (0.1–0.2 Hz), produce burst shivering, and are specialized for carbohydrate utilization (46). Additionally, oxidative type I fibers show greater contractile efficiency than type II fibers, and their deeper location facilitates access to the bloodstream and a more efficient oxidative metabolism, unlike type II fibers, which are predominantly superficial (47). Type II fibers, called anaerobic muscles, play a critical role in exposure to cold environments because their glycolic fibers can be activated at a lower metabolic rate (45). Therefore, a high proportion of type II muscle fibers in ruminant neonates' first days of life can be considered an advantage due to their glycolytic metabolism and ATPase activity (48).

Because of their thermogenic characteristics, type II muscle fibers are more important for neonates. In Peinado et al.'s study of sheep (49), the percentage of type I and II fibers were similar on days 1–15 post-birth, but at 30 days, the percentage of type II fibers had decreased by 80%. Yates et al. (50) found that intrauterine growth restriction in seven fetuses of Columbia-Rambouillet ewes near the end of gestation reduced the proportion of type I muscle fibers. Their results showed that, in general, growth-restricted fetuses were 54% smaller and had lower blood glucose (0.69 ± 0.09 mM) and oxygen (2.15 ± 0.21 mM) levels than control animals (glucose= 1.05 ± 0.10 mM and $O_2 = 3.40 \pm 0.23$ mM). Regarding the semitendinosus and biceps femoris muscles, the authors reported a lower ratio of type I to type II/IIa fibers than controls, which correlated negatively with epinephrine concentrations ($r = -0.64$) but positively with insulin levels ($r = 0.62$). These results confirm that in the neonatal stage, metabolically active muscle tissue contraction participates primarily in thermogenesis since it contains high energy reserves (e.g., glucose) that are available to generate heat.

Glucose is a valuable energy reserve in newborn ruminants because skeletal muscle glucose is less abundant in these species due to the presence of deposits of brown adipose tissue (BAT), in contrast to animals like piglets (51). Dühlmeier et al. (52) compared glucose use in four skeletal muscle samples from five kids and nine adult goats at different stages of life. They found that GLUT 4 glucose transporter expression was significantly lower in the muscle of feeding kids than in adults and differed in each stage of life in the latter.

The placenta maintains fetal glucose levels during gestation, but at birth and during lactation newborns rely on glycogen stores in the liver to maintain adequate plasma concentrations (51). This led to the interpretation that newborn ruminants consume hepatic glycogen as the primary energy source for heat production (53). For example, in ovine fetuses, glycogen can be observed in small amounts in BAT, but the amount of glycogen at birth is markedly reduced (54). This limit on glycogen storage in the liver and muscles at birth means that goat kids, lambs, and calves have low glucose concentrations that make them susceptible to hypoglycemia since those limited reserves can be depleted rapidly within the first 12 h postpartum. This effect exacerbates if the onset of feeding is delayed (55).

Hypoglycemia at birth has been reported in 22.5% of goat kids, but figures are significantly higher for the Sahel breed, twins, and newborns with low birth weight (55). When neonates' glucose concentrations are inadequate, they begin to consume muscular reserves through the Cori cycle. Specifically, type II fibers can conduct glycogen, so a high proportion is critical for thermoregulation. Therefore, glycogen availability is essential for newborn ruminants since calves or lambs require an elevated metabolic rate to control their temperature within the first hours of life (56).

Adipose tissue regulates temperature and energy balance through its metabolic and cellular functions. In mammals, this tissue is divided into white (WAT, anabolic function) and brown forms (BAT, catabolic function) (57, 58). BAT develops in the fetal stage (59). At birth, it constitutes a key element for non-shivering thermogenesis (7, 60) because it contributes to the survival of neonate ruminants exposed to cold temperatures (61). It has been suggested that BAT has similarities to WAT. However, significant differences at the histological level include uncoupling proteins 1 (UCP1), a central nucleus, multilocular triglyceride vacuoles, abundant mitochondria with dense parallel cristae, high vascularization, and a rich cytochrome content, elements that explain its brown color (62, 63). The characteristics of these elements can change with the age of newborns. Observations of goat kids at 4 days of age showed that lipid vacuoles increased in size but decreased in quantity, while at seven days, only a single central lipid vacuole and a nucleus and cytoplasm at the periphery were found, with the appearance of univacuolar WAT (64). A similar effect has been reported in 2-day-old Merino lambs, where the proportion of large local fat increased, but there were no small locules at 32 days of age (54).

Although BAT is present in all ruminants, its distribution differs among species. For example, in goat kids, BAT pads are more common in the perirenal and inguinal regions (21, 64, 65). Studies of newborn piglets have shown a lack of WAT deposits, as those animals rely on BAT to produce heat in the main location sites, including the supraclavicular, neck, pericardial, and perirenal regions (65). Similarly, in wild ruminants like reindeer (*Rangifer tarandus* L), BAT is located mainly in the perirenal-abdominal region (66). In cattle, BAT is observed in the perirenal, subscapular, and retroperitoneal regions (59), and electron microscopy has shown that most of the adipose tissue in newborn calves is BAT. It represents 2% of body weight but is converted progressively into WAT during the first days of life (67). These differences among species in the location of BAT deposits suggest that degrees of thermogenetic efficiency depend on the amount and distribution of BAT and that a low amount of BAT increases susceptibility to hypothermia (13). Figure 1 summarizes the main neuroanatomical characteristics of newborn ruminants. In this figure, the lamb is used as an animal model to show the thermoregulatory characteristics of newborn ruminants, such as neurodevelopment and the limited hepatic glycogen reserves that make them susceptible to hypoglycemia. Specifically, the presence of brown adipose tissue (BAT) in the regions marked with numbers and the activity of type IIa muscle fibers function as coping mechanisms to produce heat by shivering and non-shivering thermogenesis.

Evidently, anatomical physiological differences among newborn ruminants constitute the physiological basis for the various thermoregulation mechanisms found in different ruminant species and contrast to those that characterize altricial species.

Thermoregulation mechanisms to combat hypothermia in ruminants

The thermoregulatory responses of precocial species encompass physiological, metabolic, and behavioral mechanisms that depend on the activation of the central and autonomic nervous systems. The goal is to achieve thermogenesis by activating BAT and shivering. The vasomotor response that constricts the blood vessels prevents heat loss by signaling the thalamus to the lateral parabrachial nucleus (68). Related thermoregulatory processes are explained in greater detail below.

Central and peripheral vasomotor control

Newborns exposed to cold suffer a drastic drop in core temperature that may exceed the body's ability to produce heat (5). The organism induces various responses to a marked deviation from the zone of thermoneutrality, activating immediate mechanisms to prevent heat loss through radiation.

One of these is the vasomotor mechanism, which changes the caliber of the superficial blood capillaries in the skin to prevent heat exchange with the surrounding medium (69–71). In this case, upon exposure to cold, sympathetic nerve fibers act on α2-adrenergic receptors to generate a vasomotor response that causes smooth muscle vasoconstriction of the superficial blood vessels (72). A study by Bligh et al. (73) used sheep and goats as models to study the effect of intraventricular injections of norepinephrine and acetylcholine on thermoregulatory performance and environmental temperature. They reported that administering norepinephrine lowered the respiratory rate and decreased body temperature when the animals were exposed to low ambient temperatures. In contrast, acetylcholine administration increased body temperature despite the low ambient temperatures. These results enhance our understanding that catecholamine neurosecretion tends to decrease heat loss in response to cold thermal signals as an immediate mechanism.

However, other results suggest that several factors can inhibit this mechanism, including the offspring's birth weight (74) and available energy resources (32). Studies have shown that newborns with low birth weight may have a lower average temperature, while those with higher birth weights do not exhibit the phenomenon of vasoconstriction during the first 12 h after birth (74). This may occur because vasomotor control is immature in animals with low birth weight and cannot function to reduce heat loss (75).

Similarly, the availability of energy resources at birth, such as colostrum, may provide the nutrients required to maintain heat production and compensate for low environmental temperatures (32, 76, 77). Caldara et al. (78) studied the effect of birth weight on body temperature in piglets. After recording the surface temperature of the animals using IRT and the humidity and temperature of the ambient air at 15, 30, 45, 60, and 120 min after birth, they found that surface temperature decreased at 15 min (32.55°C) post-birth but presented an upwards tendency at 30 min (35.05°C). Although a high negative correlation was recorded between humidity and the animals' surface temperature ($r = -0.82$ and -0.81) 15 min after birth, the weight of the piglets at birth presented a positive correlation with surface temperature ($r = 0.47$).

These results could be associated with those observed by our research team in a preliminary study of newborn water buffaloes (*Bubalus bubalis*) ($n = 50$), where the relation between birth weight (high birth weight: 48–59 kg *vs.* low birth weight: 38–47.9 kg) and surface temperature during the first 48 h of life was monitored in six thermal windows (ear canal, lacrimal gland, lacrimal caruncle, periocular region, nostrils, pelvic limbs). Figure 2 presents the thermal response of buffalo calves where (A) shows the comparison of the mean temperature at parturition in groups with high and low birth weights. At birth, results indicated that the group of high birth weight buffaloes at parturition had an average range of temperature of 0.4 to 2.9°C higher than that of the low-birth-weight group in all thermal windows, with statistically significant differences ($p < 0.0001$), except for the lacrimal caruncle. (B) Comparison of the mean temperature at 24 h after parturition in groups with high and low birth weights. At 24 h post-birth, both groups showed statistically significant differences ($p < 0.0001$) in all thermal windows. The high-weight group had a mean temperature of 0.4–2.5°C above the low-weight group. (C) Comparison of the mean temperature at 48 h after parturition in groups with high and low birth weights. At 48 h post-birth, significant differences ($p < 0.0001$) were only found in the nostrils and pelvic limb thermal windows, with an average difference of 0.7°C and 1.8°C, respectively. The effect that birth weight exerts on the thermoregulatory capacity of newborns is due to the limited capacity that low-birth-weight calves have (79). This is associated with newborns' fat reserves and body mass index thermoregulatory capacity (80). In lambs, low weight at birth is also accompanied by hypoglycemia (3), restricting

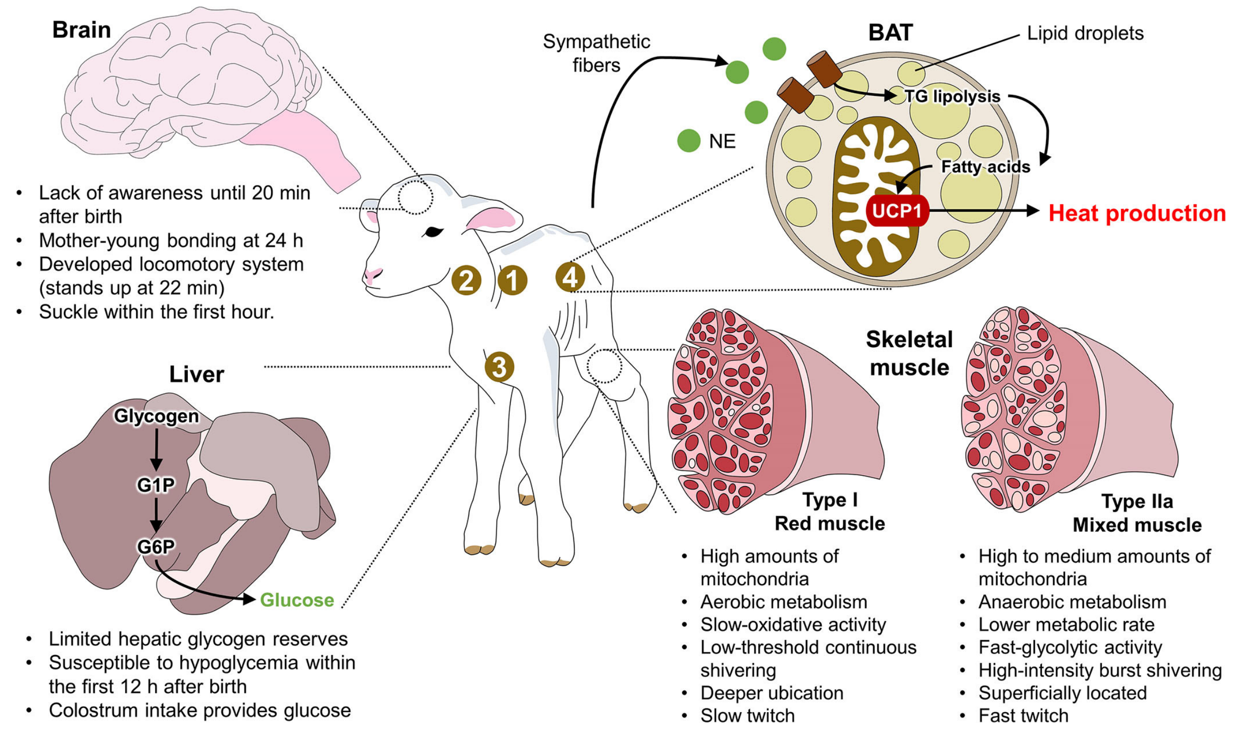

FIGURE 1
Anatomical traits of newborn ruminants. 1. supraclavicular region; 2. neck region; 3. pericardial region; 4. perirenal region; BAT, brown adipose tissue; G1P, glucose 1 phosphate; G6P, glucose 6 phosphate; NE, norepinephrine; TG, triglycerides; UCP1, uncoupling protein 1.

their energy resources to produce and maintain corporal heat. In addition to this, low birth weight can impact the health and vitality of newborns (81). Another relevant issue is the immaturity of the skeletal muscle in newborns, so shivering thermoregulation is not an effective mechanism in these cases to produce the required heat (82). Therefore, reducing the superficial temperature of the present buffalo calves reflects a vasomotor mechanism to prevent heat loss by vasoconstriction.

These findings indicate that the vasomotor mechanism constitutes an immediate compensation response triggered to prevent greater heat loss through exchange with the environment. It is important to note that the compensatory response of the vasomotor tone has a limit. It may prevent all sensible heat losses in the newborn while the organism initiates additional responses or thermoregulatory mechanisms that require greater energy expenditure.

Shivering thermogenesis

Shivering thermogenesis is the facultative thermogenic mechanism that produces heat at a resting level through active muscle contraction (1, 83–85). Some authors suggest that this ability is innate in precocial animals due to their degree of development at birth, but others, like Vanden Hole et al. (86), analyzed the level of movement and muscle contraction in newborn piglets in an attempt to show whether this is innate or matures rapidly in those animals. They evaluated gait patterns by analyzing spatial-temporal characteristics (stride frequency, step length, work factor) and left-right asymmetry step-by-step during gait in a corridor at different moments (0–96 h post-birth). Observations showed that the animals at 2 h of age had normalized values for the space-time variables, while asymmetry of the steps was around 10% within 8 h of birth. The authors affirmed that this means that the coordination of movements is not entirely innate in precocial animals but that a rapid neuromotor maturation occurs.

The neurophysiological shivering response has been described by researchers like Morrison and Nakamura (87) (Cited by Mota-Rojas et al.) (1), who described that the neurons of the dorsomedial hypothalamic nucleus (DMH) are activated in response to cold. These neurons allow the stimulation of neurons at the level of the raphe pallidus area (RPa) (88), which sends a signal to the sympathetic preganglionic neurons in the intermediolateral cell column (IML) that increases contraction at the muscular level to promote heat production by increasing the tremor amplitude of vibrations (89). This event is schematized in Figure 3. External factors such as a cold extrauterine environment, wet coat, and the consequent heat loss due to evaporation activate peripheral cold-sensitive

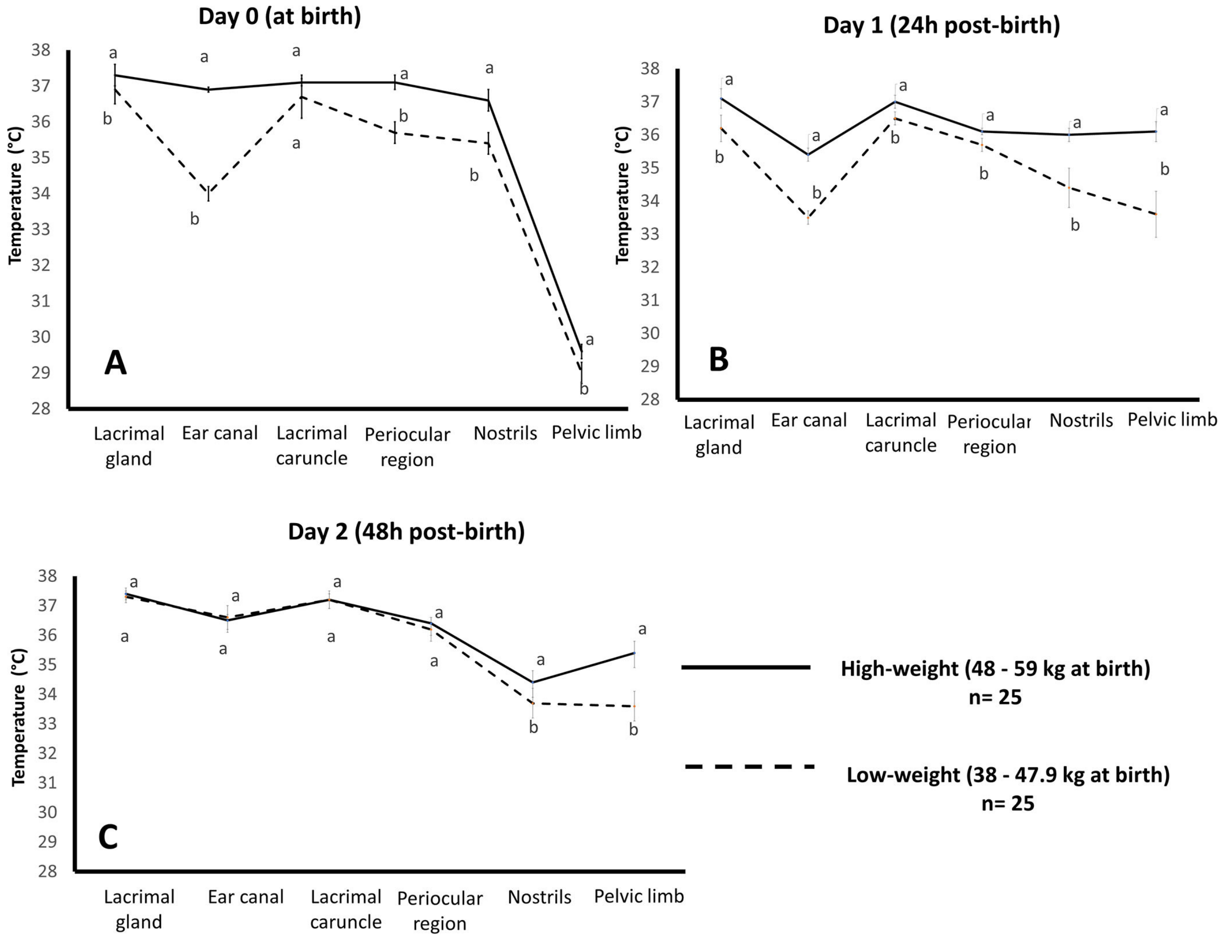

FIGURE 2
Effect of birth weight on the average temperature of water buffalo neonates ($n = 50$) in the first 48 h of life by evaluating six thermal windows. Data was obtained using variance analysis (ANOVA) by the mixed general linear model, with multiple comparisons of means using Holm-Sidak's test, Shapiro-Wilk normality test, and a significance level of $p < 0.05$. ^{a,b} Different literals indicate significant differences between groups of high- and low-weight buffalo calves ($p < 0.05$). Images were taken with a FLIR camera (FLIR Systems, Boston, MA, USA), 18 mm lens FOL, the emissivity of 0.95, IR resolution of 320 × 240, and a reflecting temperature of 20°C. The thermal images were processed by the software FLIR tools. **(A)** comparison of the mean temperature at parturition in groups with high and low birth weights, **(B)** comparison of the mean temperature at 24 h after parturition in groups with high and low birth weights, and **(C)** comparison of the mean temperature at 48 h after parturition in groups with high and low birth weights.

thermoreceptors. These receptors transmit signals to the spinal cord and such supraspinal structures as the MnPO, the main thermoregulatory center. Its projections to the DMH, RPa, and IML in the spinal cord integrate mechanisms that generate or preserve heat through shivering or non-shivering thermogenesis and vasomotor changes. Although the importance of shivering thermogenesis is evident, reports on newborn lambs suggest that around 50% of heat production comes from shivering thermogenesis and 50% from non-shivering thermogenesis (90). This may be mainly attributable to the anatomical magnitude of the newborn, that is, the degree of muscle development (84, 91).

The degree of muscle development depends on the mother's diet during pregnancy, her percentage of fat, and its distribution according to breed. For example, Hereford calves have been reported to have higher fat content than Simmental calves at 2 days of age (92). In this context, Lammoglia et al. (93) studied the effect of supplemental fat feeding in pregnant female bovines during the last 55 days of gestation on the cold tolerance of F2 calves (Piedmontese and Hereford), which 5 h after delivery were exposed to a temperature of 0°C for 140 min. The prepartum mothers had received diets containing 2.2% (low-fat) or 5.1% fat (high fat). Findings showed that glucose levels were higher in the offspring of the mothers that had received the high-fat diet and that rectal temperatures were 0.5°C higher in those animals than in the neonates of the low-fat diet mothers. The authors commented that although the mother's diet is important for improving offspring survival, the

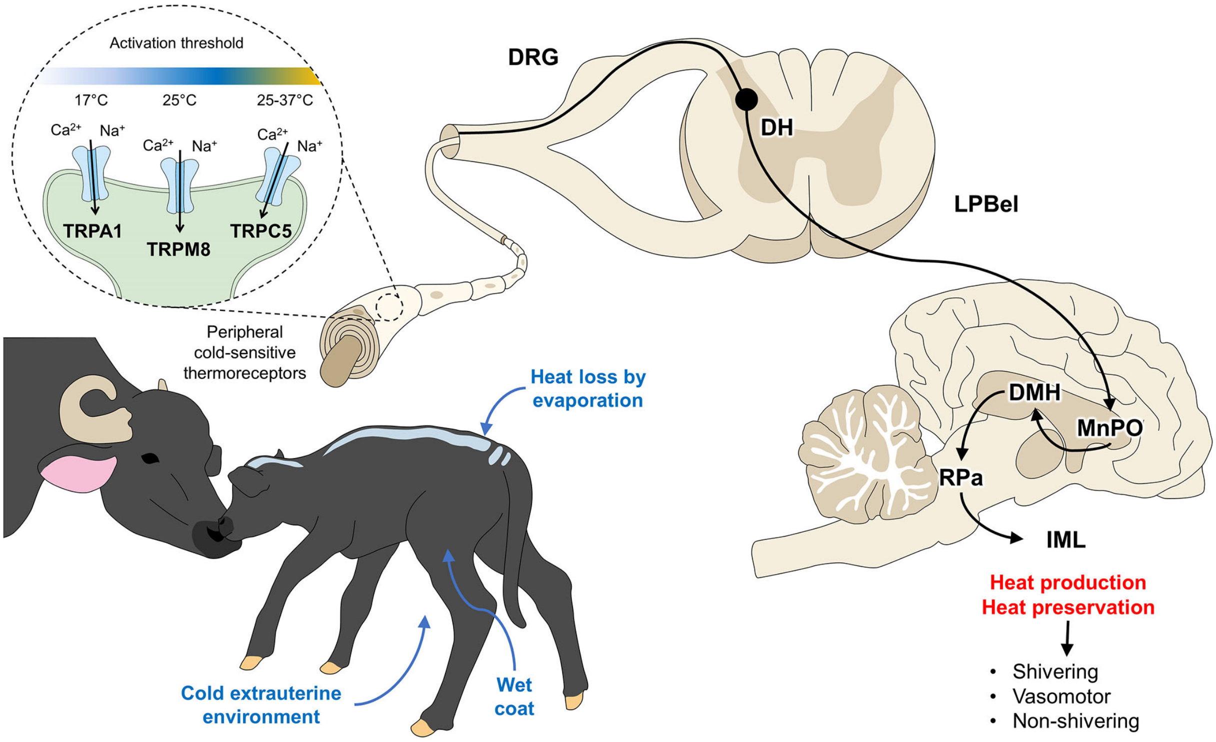

FIGURE 3
Hypothalamic response to hypothermia. Ca^{2+}, calcium; DH, dorsal horn; DRG, dorsal root ganglion; IML, intermediolateral column; LPBel, external lateral part of the lateral parabrachial nucleus; MnPO, median preoptic nucleus; Na^{+}, sodium; RPa, raphe pallidus. TRPA, transient receptor potential ankyrin 1; TRPC5, transient receptor potential canonical 5; TRPM8, transient receptor potential melastatin 8.

degree of muscular development also plays a key role because it enhances the proportion of thermogenesis through shivering.

A similar study design by Dietz et al. (94) evaluated pregnant fall-calving heifers fed diets with different fat levels (1.5, 4, and 5%). The calves were exposed to a temperature of 5°C for 90 min. Observations showed that the responses to cold and the frequency of shivering in the newborns were similar despite the variable levels of energy administered. The provision of energy resources is a fundamental factor for the newborns' success in temperature compensation but may be a limited resource given the high energy cost it represents for the animals. The authors also mentioned that the muscular contraction response could occur only for a short time when animals are exposed to a temperature of 10°C (14).

Shivering thermogenesis is a compensatory response that is efficient in the short term, but factors like the degree of muscle development and maternal nutrition during gestation may influence neonates' resistance to cold.

Non-shivering thermogenesis

Under hypothermic conditions, animals use NST as a short-term response, but if the condition is not overcome quickly, they may attempt to generate heat by consuming BAT (95, 96). This mechanism is called NST (97) and is critical for determining the offspring's metabolic adaptation to cold temperatures in the extrauterine environment (98–100). BAT is rich in energy cells that allow efficient heat production through sympathetic innervation (62, 68), which regulates the production of metabolic heat and vasomotor and piloerection responses to hypothermia (Figure 4).

Physiologically, BAT is activated by the release of norepinephrine from the nerve terminals of the sympathetic nervous system. Its thermogenic capacity is mediated by uncoupling protein-1 (UCP-1), which is found only in the mitochondrial adipocytes (1, 61, 65, 101). For this reason, BAT's heat producing-capacity is determined by the concentration of UCP-1 and the activity of the proton conductance pathway through which the electron transport chain uncouples the synthesis of adenosine triphosphate. The sympathetic fibers in BAT promote neurosecretion of catecholamines that stimulates the oxidation of fatty acids and the gene oxidation of UCP-1 for heat production under conditions of exposure to cold (101). These facts confirm the importance of food consumption during the first hours of life, the fact that newborns who are food-deprived for any reason have a limited ability to maintain their body temperature, and the importance of colostrum consumption.

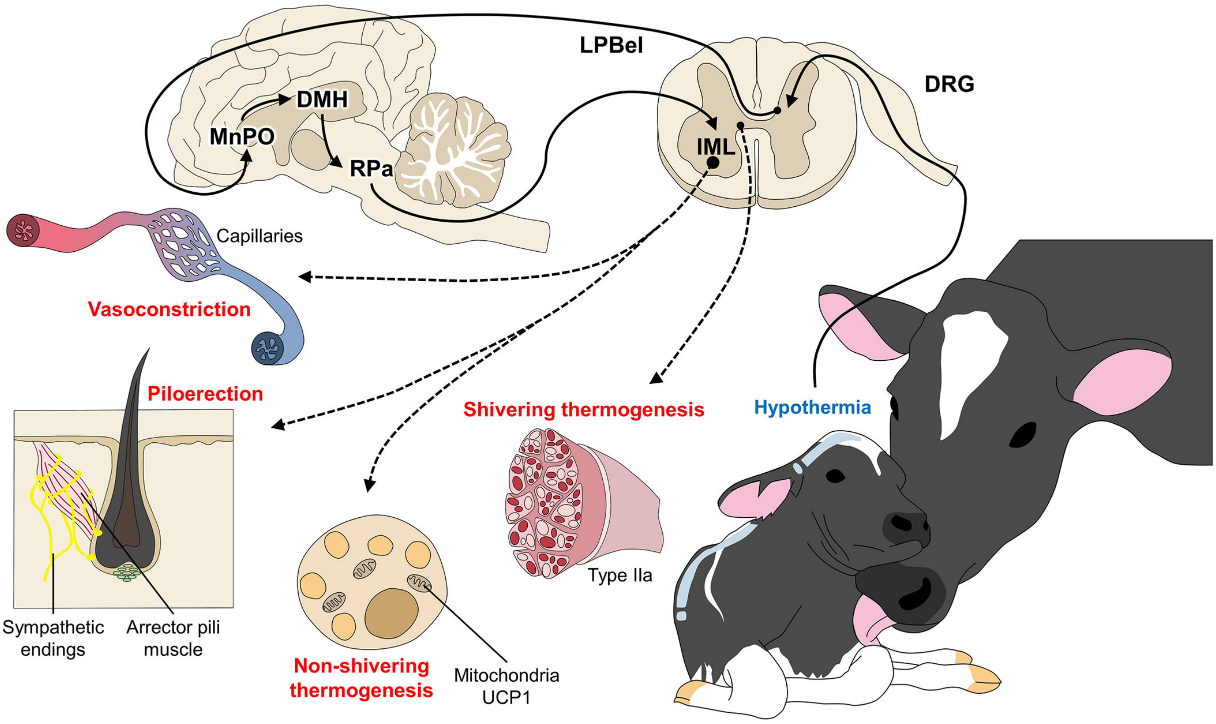

FIGURE 4
Peripheral responses as coping mechanisms for hypothermia. When the thermoregulatory center of the central nervous system (MnPO) perceives a decrease in body temperature, the organism of newborn ruminants (and other mammals) activates peripheral responses with two main objectives: **(A)** to prevent heat loss through vasoconstriction and piloerection; and **(B)** to produce heat by shivering and non-shivering thermogenesis, where BAT and skeletal muscle fibers participate in the return to a state of homeothermy.

Another factor that intervenes in thermogenesis is birth weight since low-weight animals may not have the energy resources necessary for heat production. As Dwyer (102) concluded in a study of the influence of birth weight on postnatal mortality in lambs, low birth weight animals had lower vitality scores, though they spent more time standing up. The fact that the act of standing up consumes abundant energy resources suggests that, in precocial animals, food ingestion can help maintain stable thermoregulation.

Colostrum is considered one of the main energy sources for newborns, and its thermogenic effect is associated with the production of metabolic heat through such processes as the digestion, absorption, and processing of nutrients. Moreover, as a source of lactose, amino acids, and triglycerides, colostrum is an important energy source for heat production *via* diet-induced thermogenesis and non-shivering thermogenesis (14). This occurs thanks to the increased plasma triglyceride levels that BAT metabolizes. This effect was explored by Silva et al. (103), who observed that male Holstein calves that consumed an amount of colostrum equivalent to 15 to 20% of their body weight presented less shivering than those that received only 10%. This finding confirms that the metabolic thermogenic response is additional and long-term in nature, though authors like Clarke et al. (63) have stated that the response degree depends on exposure to cold. For example, in newborn lambs exposed to temperatures of 1°C, heat production by BAT involved around 80% of adipose tissue.

Therefore, producing heat of metabolic origin represents a long-term way to compensate for a drop in body temperature but comes at a high energy cost. This situation is related to the availability of energy resources at birth and the weight of newborns, conditions necessary for the proper functioning of their thermoregulatory mechanisms.

Use of infrared thermography in assessing hypothermia in newborn ruminants

IRT systems have proven useful in assessing the thermal status of animals. The technique measures the radiation emitted from a body subject to changes in superficial microcirculation that increase or decrease these emissions (18, 97, 104–106). According to Villanueva-García et al. (105), the vasomotor effect caused by hypothermic states can be evaluated indirectly by tissue heat radiation, quantified by IRT. When peripheral circulation decreases, the heat radiated in some regions is also reduced. For the water buffalo, there is information on the application of IRT to assess thermoregulatory capacity under thermal stress conditions (17, 107). However, data of this kind have not been reported on newborn animals to analyze the mechanisms associated with temperature changes in extrauterine environments.

Evaluating degrees of hypothermia with IRT has been recommended on many occasions. However, authors caution that obtaining valid, reliable temperature values requires testing anatomical regions. For example, the auricular region is widely used in several ruminant and non-ruminant species to assess their thermal state (17, 108, 109) although concave surfaces are less likely influenced by external factors and may show a higher body surface temperature, while convex areas have the opposite effect, as shown in horses when measuring IRT in body regions such as elbow joint or the croup (110, 111). Likewise, specific key characteristics such as a high density of arteriovenous anastomoses, blood capillaries, and scarce –or no– hair or coat so that heat exchange with the environment can proceed through changes in the caliber of blood vessels (70, 112, 113). Figure 5 shows various thermal windows in newborn water buffaloes proposed in a preliminary study: (A) Pelvic limb. The windows in the pelvic limb are mainly in the femoral region, indicated by a circle that encompasses the cranial area at the level of the insertion of the quadriceps femoris and the tensor fascia lata, muscles irrigated by the femoral artery. At the distal level in the metatarsal region, the thermal response offered by the dorsal metatarsal artery is indicated with a circle. (B) Thermal facial windows. The windows of the auditory canal (B1), ocular region (B2), lacrimal gland (B3), and lacrimal caruncle (B4) are shown. Each window is delimited by regions like the auditory canal that, in turn, is delimited by the auricular folds of the auditory meatus. This ocular window is bounded to the upper and lower eyelids. The lacrimal caruncle window is delimited in the region of the medial canthus of the eye and allows the temperature evaluation that comes from the circulation of the infraorbital artery. (C) Nostrils. This window is delimited by the nostrils and allows its temperature evaluation together with the circulation of the maxillary artery that irrigates the area. Mota-Rojas et al. (17) described these regions and affirmed that in this species, the thermal windows of the lacrimal caruncle, ocular caruncle and corporals make it possible to assess thermal states.

Labeur et al. (114) evaluated the hip and back areas of 15 pregnant ewes grouped into two categories: sheared and unsheared. Both groups were exposed to a cold test for 1 hour. Findings showed that the temperature in the shoulder region was lower than in the hip and back areas. This difference could be related to adipose tissue deposits in these regions that constitute a resource for producing metabolic heat with no need for muscle contraction. Studies of this kind show that IRT could provide a way not only to assess levels of thermal comfort through the vascular reactions produced by cold responses but also to verify that regions with BAT deposits can cause increases in regional temperatures that reflect an organism's attempts to compensate for a temperature decrease and return to a thermoneutral state (115, 116). This coincides with the interscapular thermal window observations and BAT in laboratory animals (97).

The responses measured in various thermal windows differ. Figure 6 shows thermal responses due to the effect of age determined during a preliminary study of neonatal water buffaloes (n = 50) conducted by the authors. Note that while the temperature of the lacrimal gland remained constant during parturition and at 24 h and 48 h of extrauterine life, that of the lower limbs showed greater temperature variation during the 2-day period. In the same thermal window the lowest temperatures were found in the thermal windows farthest from the body core. This can be explained by understanding the central and peripheral circulation of the organism. The vital structures in which important metabolic functions are carried out are found in the central parts of the body (17, 18), meaning the heart, lungs, brain, stomach, and kidneys. To protect these systems, when exposed to cold environments or situations that promote heat loss (such as the parturition process and their wet coat), the organism shifts blood supplies from peripheral structures to vital organs (72). Vasoconstriction at peripheral areas such as the thoracic and pelvic limbs, nose and tail prevents heat dissipation and also reduces said structures' superficial temperature (26). The increase in the temperature of the pelvic limb and nostril window according to the age of the newborn, could be explained because the newborn gets dry, reducing heat loss by evaporation, so the calf can reach a thermostability after drying the coat (1, 15) and after colostrum intake provides them of glucose and energy reserves (55).

The above brings us back to discussing the possible influence of environmental conditions and the season when birthing occurs. A study by Deak et al. (117) reported the effect of season and the physiological state of several body areas in 24 Holstein cows evaluated in the rainy (October- March) and dry seasons (April- September). They observed that temperatures in the flanks, perineal area, and lateral udder were 1.1°C higher in the rainy season than in the dry season and that temperatures of the udder, perineal, and rectal regions differed in the reproductive phase. These results suggest that heat loss or gain pathways are closely related to the seasonand may support the hypothesis that the survival of water buffalo neonates can vary with this factor (113, 118).

De Souza et al. (119) studied 23 Zebu × Holstein crossbred calves to evaluate whether cutaneous evaporative thermolysis occurs heterogeneously on the body surface of ruminants. Using IRT, they assessed the animals' limbs, neck, thorax, abdomen, and back under ambient temperatures $<25°C$, 25–29°C, and $>29°C$. Findings showed that cutaneous evaporative thermolysis did not differ among those regions but that temperatures in the neck had the highest thermal ranges. They also found that exposure to temperatures $>29°C$ did not significantly increase evaporative thermolysis. Although those results confirm that heat loss is homogeneous throughout the

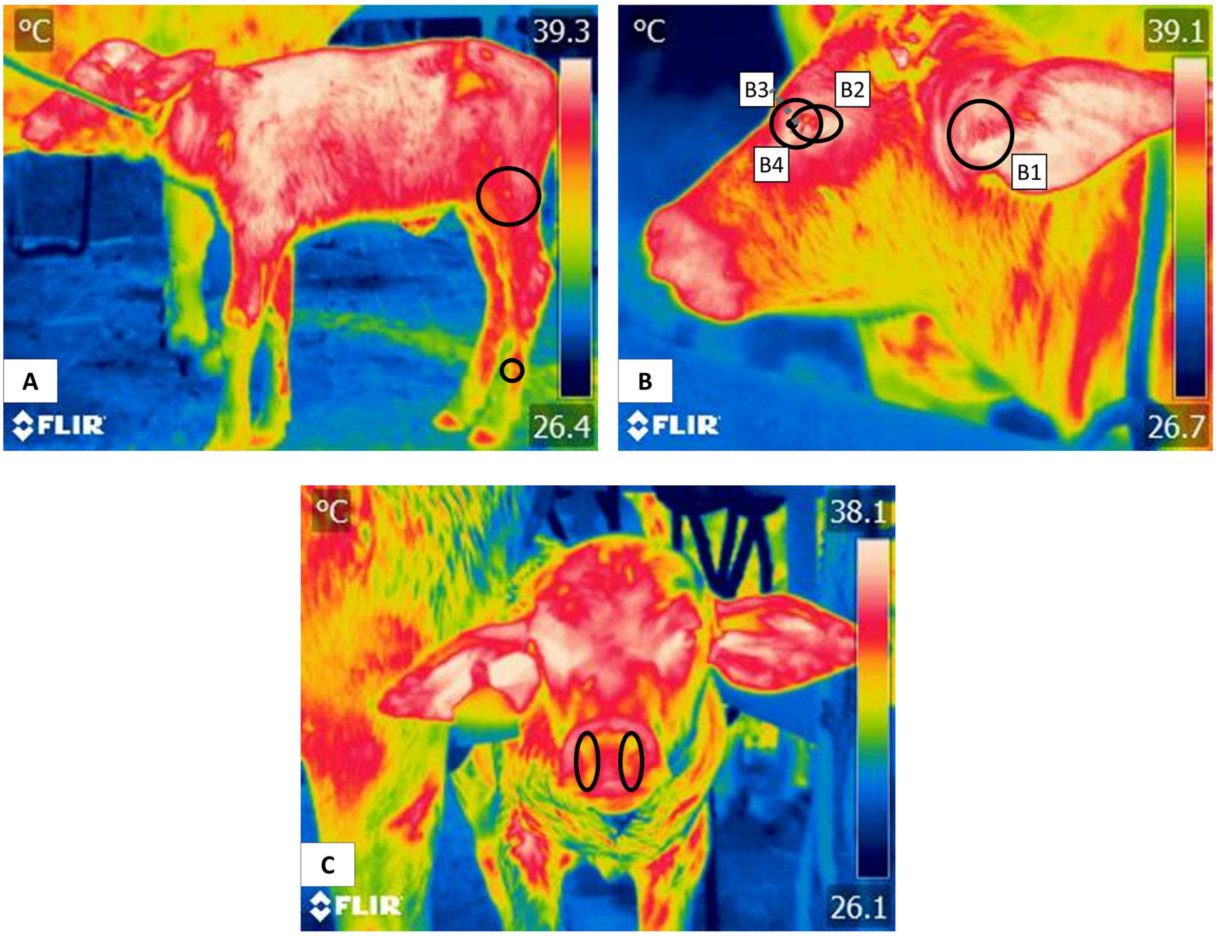

FIGURE 5
Thermal windows in newborn water buffalo. **(A)** pelvic limb, **(B)** facial window, **(B1)** auditory canal, **(B2)** ocular region, **(B3)** lacrimal gland, **(B4)** lacrimal caruncle, and **(C)** nostrils.

body, some regions may show slight differences due to the arrangement of the blood vessels. This finding may be related to such features as skin thickness or a high density of hair follicles that can impede heat loss, though differences also occurred in the regions that allow heat loss (15, 113). Hristov et al. (120) evaluated the efficiency of IRT for determining temperatures in calves. Their work compared reference regions in the chest, shoulders, and legs to rectal temperatures and respiratory rates. They found that temperatures in the shoulders and chest had correlations of $r = 0.9$ and $r = 0.65$ with rectal temperature and respiratory rate, respectively, demonstrating that peripheral regions of ruminants respond to core body temperatures. This finding may help us better understand the buffalo as a model of precocial species.

In conclusion, IRT is a valuable tool that can be applied to recognize hypothermia in newborn ruminants. There is ample opportunity for its use in sheep, goats, cattle, and water buffaloes due to the limited information on this species. However, there is also a clear need for additional information on the effectiveness and reliability of different thermal windows and their capacity to indicate the ability of newborns to reestablish thermoneutrality.

Perspectives

Although thermoregulation capacity in newborn ruminants such as lambs, kid goats, and calves can be similar to precocial species, some studies show anatomical-physiological differences between these species. For example, it is widely recognized that sheep have the greatest distribution of BAT in the hips and the interscapular region, which this species can use for heat production through NST (114). In contrast, kid goats have more type II muscle fibers in the neonatal stage than adults (49).

To date, it is not yet clear whether these differences could confer an advantage in some species as might be expected in large ruminants, where it is suggested that the

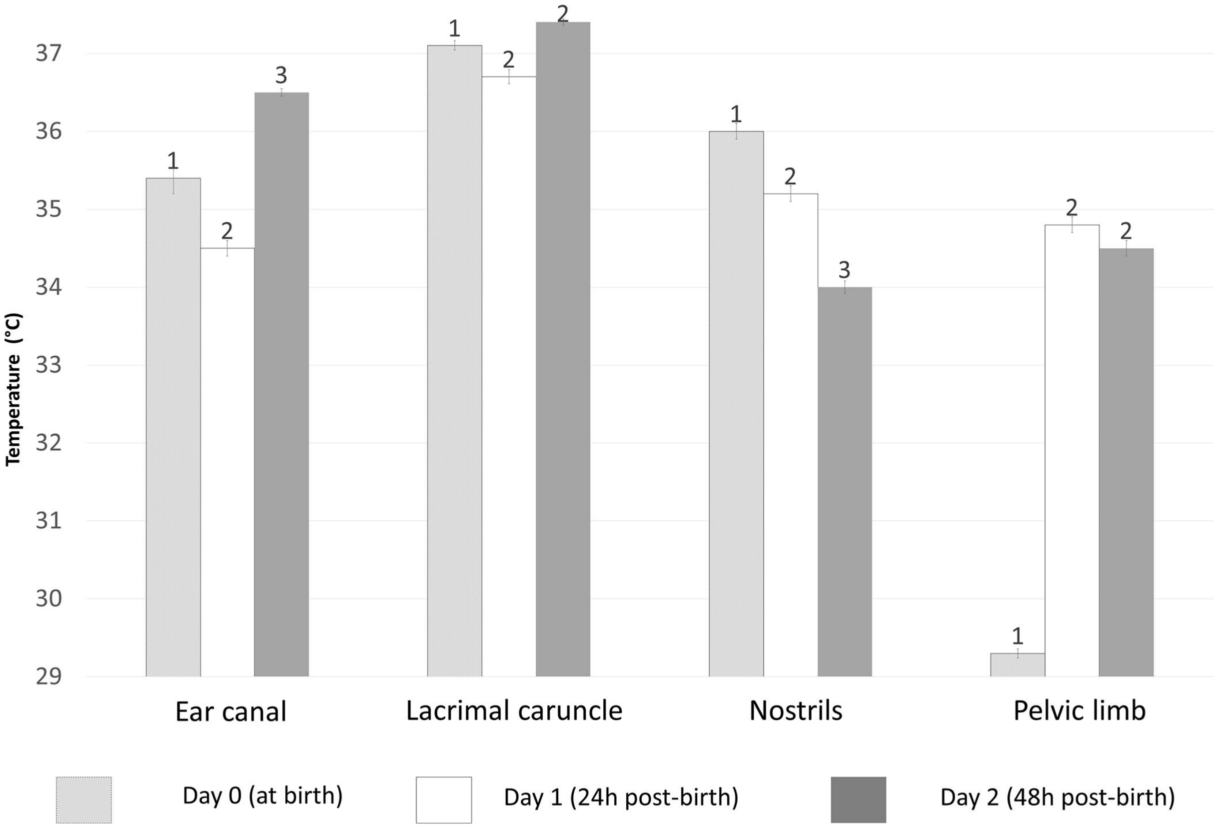

FIGURE 6
Effect of age measured in hours (0–48 h) on the temperature of newborn buffaloes in different thermal windows. In all events (hours), statistically significant differences ($p < 0.0001$) were observed in all measured times, except on the lacrimal gland and pelvic limb windows at 24 and 48 h post-birth. The average temperature at the ear canal differed from day 0 to day 2, with the highest value at 48 h post-birth (36.5°C) and the lowest at 24 h post-birth (34.5°C). The lacrimal gland window only showed differences of an average of 0.4°C from day 0 to day 1. The temperature of the nostril window showed a tendency to drop 0.8°C from birth to 24 h post-birth and 1.2°C from 24 h to 48 h post-birth. Meanwhile, a generally lower temperature was observed in the pelvic limb at birth (29.3°C) but it rose by 5.5°C at 24 h post-birth before decreasing by 0.3°C at 48 h post-birth. Data was obtained using variance analysis (ANOVA) by the mixed general linear model, with multiple comparisons of means using Tukey's test, Shapiro-Wilk normality test, and a significance level of $p < 0.05$. ^{1,2,3} Different numerals indicate significant differences between events according to the age of the buffalo calves ($p < 0.05$).

presence of large muscle masses improves heat production by shivering. Species like the water buffalo and its limited available literature make them a potential field for future research within these animals. The water buffalo is a species recently introduced into production in numerous areas, so there are large areas of opportunity for research on the newborns of this species since we lack knowledge on the efficiency of their thermoregulation mechanisms (37). Reports mention mortality rates for water buffalo calves in Pakistan that range from 0.09% (121) to 7.59 ± 13.49% (122) and contrast to figures reported for domestic cattle (0.6–9.2%) (36, 37). However, factors such as season have been associated with variations in mortality rates, indicating that more neonatal casualties are observed in winter and spring (123), possibly due to low temperatures and increased rainfall. Likewise, because this species is being introduced into regions with extreme climates, mortality rates could be closely related to climatological phenomena, as observed in the variability in its thermal responses (124). Therefore, the study of the thermoregulatory mechanisms in these animals and breeding strategies could prevent hypothermia or, where appropriate, counteract this condition in this species.

BAT is another relevant study area in these species due to the differences in its distribution. Some authors have found that the degree of BAT presentation may be related to the expression of genes responsible for BAT differentiation and its deposit in regions such as the back (125). Similarly, the excessive deposit of BAT is related to the nutritional status of the dam. Therefore, designing strategies aimed at the mother's nutrition and, consequently, the body condition and weight at birth of the fetus could increase the effectiveness of the thermoregulatory mechanisms of newborn ruminants and their survival (126).

Within the thermoregulatory system of newborns, the activation of peripheral thermoreceptors such as transient potential receptors (TPR) is the first step to initiating every thermal response. The key role of these receptors, particularly TRPA1, TRPM8, and TRPC5 are triggered by cold stimuli and promote the vasomotor changes or contraction of skeletal muscle (91). However, to date, the available data is focused on small rodents. Therefore, studies targeting these receptors are a wide area of opportunity to investigate the functionality of TRP in ruminants.

The concentration of endogenous energy sources in the form of liver is essential because it is the first metabolic pathway to produce heat (53). However, both muscle and liver glycogens are a limited resource in the postpartum period, and variable according to the pregnancy characteristics, such as malnutrition present in the mother, which generates hypoglycemia in the ewe fetus (55). In this sense, using the mentioned resource, when colostrum is not ingested in the first 24 h of life, could ensure constant plasma glucose levels in calves (53), increasing the newborn's ability to thermoregulate at calving adequately.

On the other hand, the behaviors and postural changes that most mammals develop during exposure to cold to avoid heat loss by transfer, such as grouping or approaching warm areas, are another relevant issue in ruminants (17). These behavioral changes are not evident in ruminants, and it is suggested that they may be due to the specific anatomical characteristics such as the presence of hair, wool, and the color of the coat, elements that prevent active loss of heat in cold environments (14, 16). Similarly, it is important to study whether the phenotypic characteristics of adults, such as hair length, skin thickness, or hair follicles, influence the thermoregulatory capacity and mechanisms of newborn ruminants, as observed in wildlife (127). As we have seen, precocial species like ruminants have certain traits that make them more or less well-adapted to cope with hypothermia. Anatomy-physiological differences among newborn ruminants constitute the physiological basis for the various thermoregulation mechanisms found in different ruminant species.

The application of IRT as a tool to assess thermal status in mammals has been described by different authors (1, 17, 18, 128). But it is relevant to note that in current agricultural settings, using a hand-held thermographic camera may not be plausible in most large production units. This failure could be resolved by the automatization and optimization of IRT technologies that could precisely inform about thermal changes (129). For example, Schaeffer et al. (130) automatically detected the onset of bovine respiratory disease (BRD) in 65 Herford X Angus calves by placing an IRT scanning station around a feedlot pen. The corresponding software controlled the camera, collected thermal data, analyzed the image, and stored the information in a database. With the daily automatic monitoring of the orbital area, every time the animal accessed the water station, the authors obtained an average temperature of 33.85 ± 0.66°C for all calves, while true positive (TP) and true negative BRD animals had average temperatures of 35.44 ± 0.58°C and 34.71 ± 0.57°C, respectively. Therefore, it was possible to automatically record a 1°C elevation in the temperature of TP calves during the experimental period. Likewise, Zaninelli et al. (131) analyzed cow udder health status automatically taken through thermographic images, obtaining a significant relation between udder surface temperature and somatic cell counts with a sensitivity of 78.6% and a specificity of 77.9%. Technologies based on algorithms to detect a specific region such as the eye or the cheek in calves help to determine the maximum temperature of the area and were validated by comparing its measurements with a manual camera. The images taken by the algorithm were highly correlated to the hand-held device. They showed correlations (R2) of 0.99 for the eye and between 0.85 and 0.90 for the cheek (132). These studies present the possibility to integrate these automated systems for a field application of IRT on commercial farms.

Finally, the potential for utilizing remote tools like IRT means that we must determine the effectiveness of the various thermal windows that have been described for large ruminants (17, 133). Although the ocular and auricular regions have been mentioned as areas that indicate body temperature, another promising option is the appendicular region since it has been shown to be more sensitive to hypothermia due to the vascular supply it receives (2, 18).

Conclusions

To confront the adverse effects of hypothermia, precocial species, including ruminants, are born with anatomical, physiological, and morphological characteristics that allow them to maintain their body temperatures. For example, one of the primary mechanisms is vasoconstriction of peripheral blood vessels to reduce heat loss through radiation. However, prolonged vasoconstriction can induce cellular hypoxia and impact the outcome of the newborn.

On the other hand, neonates have BAT deposits that serve as a substrate to NST to produce heat and prevent a sudden drop in core temperature, especially evaporative loss due to their wet coats at birth. The amount of BAT depends on the species and anatomical site (e.g., goats in the perirenal and inguinal region and cattle in the perirenal and subscapular). Moreover, its activation requires energy resources to produce heat by NST. Therefore, they can have consequences, such as inducing anaerobic metabolism that can lead to a state of acidosis. Finally, shivering thermogenesis requires muscular development of type II fibers in metabolically active muscles such as semitendinosus and biceps femoris, and its thermogenesis needs high energy reserves. One of them is glucose, an element that is limited in newborn ruminants and predisposes them to fast hypoglycemia. For this reason, critical factors like birth weight, available energy resources, and prompt colostrum ingestion need to be considered to prevent cases of hypothermia.

All these changes in microcirculation or metabolic activation of adipose tissue and their reflection of the superficial temperature of ruminant newborns allow the use of IRT as a valuable tool for recognizing hypothermia or identifying critical temperature drops during the first hours of life. The implementation of IRT on determining thermal windows such as ocular, auricular, flank, limbs, and neck, among others, have been shown to provide crucial information on the thermal status of newborn and the amount of heat radiation. However, additional validation is required to determine which ones are most appropriate for evaluation as a complementary step to installing preventive practices in ruminant neonates. Although, it should be noted that the lack of information on the application of IRT in newborn ruminants is a limitation and future studies may focus on this aspect.

Author contributions

All authors contributed to the conceptualization, writing, reading, and approval of the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Do intense weather events influence dogs' and cats' behavior? Analysis of owner reported data in Italy

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Climate change is a threat to global health and can affect both veterinary and human health. Intense weather events, including sudden and violent thunderstorms or periods of extreme heat, are predicted to rise in frequency and severity and this could lead owners to significantly change their habits and schedules based on the weather, could modify human management and could aggravate pre-existing behavioral problems in pets. The aims of the present study were to identify and quantify possible weather events impact on management, behavior, and behavioral problems of Italian dogs and cats, based on previous owners' experiences with their animals. Two questionnaires were prepared, one for dogs and one for cats, investigating owners' perceptions of the impact of weather events on their pets' behavior. A number of 392 dogs and 426 cats' owners answered the questionnaire. Our study showed that many behaviors in both species were equally modified by environmental temperature. Play and activity increased with cold weather and decreased with heat, and sleep increased with drops in temperature and with hot weather. In particular, the increase in activity in correspondence with the thermic drop was more significant in males, while the increase in playing behavior was statistically greater in the Sheepdogs and Cattle dogs – group1. Weather events did not affect aggressive and house soiling behaviors in both dogs and cats, but weather events, including wild thunderstorms, torrential rains influenced the pets' behavior. Understanding how pets modify their behaviors based on a different owners' schedule and to weather events can help to refine prevention strategies through societal changes and owner education.

KEYWORDS

dog, cat, behavioral problems, owners, extreme weather events

Introduction

Around the world, companion animals are part of human societies (1, 2), and provide people with companionship, improved mental and physical health (including reduced depression, increased levels of oxytocin and decreased blood pressure and cholesterol levels), and expanded social networks. Many of these roles depend on physical activity, but it has been shown that also children and teenage development may benefit from living with pets (3–6). Many aspects of human society benefit from pets: simple companionship, but also work services, such as visual and hearing assistance dogs, medical detection dogs, and military working dogs (7, 8). Worldwide, the statistics describing the numbers of companion animals are scarce. However, according to Vetnosis and European Pet Food Industry Federation, in 2014, there were 223 million registered companion dogs and 220 million registered companion cats worldwide. Many owners consider their dogs and cats as family members and show great concern for their wellbeing. Those owners are prone to invest considerable resources for food and water requirements, living spaces, health conditions, and even pet's emotions and feelings (9–11). Over the years, co-evolution shaped the relationship between humans and pets, influencing human management and pets' behavior. Intense weather events could affect both factors, modifying human management and aggravating pre-existing behavioral problems, since owners could significantly change their habits and schedules based on the weather.

Global climate change is a One Health crisis, threatening both animal and human health (12, 13). Climate change is listed among the World Health Organization's top ten threats to Global Health in 2019, with heat-related illness predicted to contribute to an additional 250,000 human deaths annually by 2030 (14). All organisms live within a limited range of body temperatures, and temperature extremes result in functional constraints. Thermal windows evolved to be as narrow as possible to minimize maintenance costs, resulting in functional differences, between species and subspecies in various climate zones and between populations of a species (15). Direct effects of climatic warming are related to decrements in the organism's performance in growth, reproduction, foraging, immune competence, competitiveness, and behaviors. Moreover, performance in animals falls below its optimum during cooling and warming (15). Since extreme weather events, including thunderstorms, torrential rains, and flooding, are predicted to rise in frequency and severity (16), increased attention is necessary to identify and implement adaptation strategies (17). During last years, changes in temperatures and precipitation patterns have been evidenced in Italy, with events of extreme heat, torrential rains lasting for days and sudden and violent thunderstorms increasingly frequent (18), likely leading owners to change their routine based on the weather conditions. Unfortunately, in these circumstances, pre-existing pathological conditions, such as pathological anxiety or fear, thunderstorm phobia, and cognitive dysfunction in older dogs could worsen (19). Changes in daily routines can create a condition of distress with consequent modification of the main behavioral patterns (20), worsening all forms of anxiety and exacerbating problems such as urinary marking (21, 22), separation anxiety (23) and cognitive dysfunction in elderly subjects (24). Furthermore, subjects who previously suffered from fears or phobias toward loud noises can worsen the symptoms during sudden and violent thunderstorms (25).

At present, there is no specific literature on the subject. To the authors' knowledge, only one study in English (26) has examined the potential environmental impacts related to a dog's entire life cycle. Few studies investigated the most common triggers of heat-related illness and injuries in dogs in the UK (7, 17, 27) and the environmental impacts of companion dog's and cat's food consumption (26). However, limited attention has been given to the environmental factors contributing to canine and feline behavioral changes.

Therefore, the present study aimed to identify and quantify the possible climate change impact on management, behavior, and behavioral problems in an Italian pet dog and cat population.

Methods

Data collection

Two questionnaires were created to obtain owner-reports of the impact of weather events on caretaking as well as their pets' behavioral changes and administered to owners online through social media channels, personal contacts, and information disseminated to veterinarians.

Participants were given written information about the aim and the procedures of the study and the right to withdraw. Written informed consent was obtained from each participant. Participation was voluntary. No sensitive data were collected in both questionnaires, and complete anonymity was guaranteed. The study was approved by the Ethics Committee (reference number 5021, date 18/05/2021). All methods were performed in accordance with relevant guidelines and regulations. To meet key requirements for enrolment the survey targeted Italian participants older than 18 years, primary caregivers of a least one dog or one cat.

The questionnaires included three sections (see Supplementary materials 1, 2). The first section contained demographic information on participants (age, gender, number of adults and children in the household), the dog/cat's characteristics and history, and the physical and social environment of the dog/cat. Questions inquired about the home environment (apartment vs. house, urban vs. rural), pets "management (strictly indoor or the number of walks outdoor, place to rest, daily routine), age (current, age at acquisition), sex, reproductive status (entire or neutered/spayed), breed, source of dog/cat (breeder, pet store, shelter, rescue, family, friends, or stray), and the number of dogs or cats in the household. The second section targeted questions on specific behavioral patterns (sleeping, feeding, drinking, activity level). The survey also investigated whether the weather events change modifies the owners" animal management and the animal behavioral patterns based on previous experiences with their dogs or cats, including the most common behavioral problems (such as aggression, urine marking, fear of loud noises, thunderstorm phobia, etc.) that could be influenced by intense weather events.

Data analysis

Data were entered into Microsoft Excel (Microsoft Corporation, 2010, Washington, DC), before being analyzed with SPSS statistical package (SPSS Statistic 27, IBM, Armonk, NY). Descriptive statistics (frequencies and percentages) were calculated to provide a general description of the sample. The data were not normally distributed, so were analyzed using non-parametric test: Kruskal-Wallis and X square test was used to investigate possible associations between the characteristics (e.g., age, sex, and breed) and management of pets and changing in behavior during weather events. Statistical significance was accepted at $p < 0.05$. A multivariate statistical analysis, principal component analysis (PCA) was used to analyze behavioral traits based on i) increased temperature and ii) after thunderstorms to determine the role of variables and detect common features. PCA plot was used to evaluate the distribution of the subjects according to the considered variables and contribution of the behavioral traits in the two settings. PCA analysis was conducted with the "princomp" function included in the default "stats" package, in R statistical environment version 3.6.2 (www.r-project.org). All PCA graphical representations were produced using R (www.r-project.org).

Results

We collected reports from 392 people who answered the dog questionnaire and 426 the cat questionnaire. In both cases, the highest percentage was represented by women (87% dogs and 87.3% cats) aged between 21 and 40 years (55.1% dogs and 49.7% cats) living in couple (dogs 44.4% and cats 46.7%) without children (80%). Most of the owners who answered the questionnaire lived in northern Italy (69.4% for dogs and 68.1% for cats) in an urban environment (65% dogs, 71.1% cats). Owners believed that weather events had an effect on their pet's behavior (65.3% dogs, 66.9% cats). In particular, the number of purebred cat owners who believe that weather events influenced their cats' behavior was statistically greater than that of European shorthair owners ($p < 0.05$).

Dogs

FCI classification was used to identify dog breeds. The most represented dogs were crossbreeds (44.4%), followed by Sheepdogs and Cattle dogs (except Swiss Cattle dogs) - group 1-(13%) and Retrievers-Flushing Dogs-Water Dogs-group 8-(9.2%). All other breeds were represented in a smaller percentage. Most dogs were adopted between 2 and 4 months of age (69.6%) and, to a lesser extent, after 5 months (28.1%). Many came from a private individual (34.4%), although a fair number were adopted from kennels (27.3%) or breeders (27%). Dogs included in the study were almost equally distributed between males and females (spayed female 39.3%, intact male 32.9%, neutered male 14.3%, and intact female 13.5%), and were mainly between the ages of 3 and 7 (37.8%) and between 13 months and 3 years (25%). Senior dogs aged 8 to 10 years represented a smaller percentage (15.6%). Most dogs (54.3%) were the only pet in the house. A specific daily routine was followed by most of the dogs (87%) even if the number and duration of walks varied in relation to the outside temperature both in periods of extreme heat (number 79.1%, duration 87.5%), and extreme cold (number 66.1%, duration 76.4%). The walk for 71.7% of dogs lasted from 15 to 60 min and was repeated two to four times a day (79.8%).

Dogs' play behavior and activity were considered normal by 79.1% of owners. These behaviors increased with sudden temperature drops (activity 60.7%, play 56.6%). In particular, the increase in activity in correspondence with the thermic drop was more significant in males ($p < 0.05$), while the increase in playing behavior was statistically greater ($p < 0.05$) in the Sheepdogs and Cattle dogs –group1. Most dogs had their own place to sleep (98%), evidenced by having a personal bed (45.7%), although some of them (20.2%) preferred the owners' bed. Sleeping behavior, defined as normal by most owners (92.1%), increased both when the temperature dropped (58.2%) and when the air temperature was excessively hot (66.6%).

Eating behavior, defined normal by most owners (74.5%), increased when there was sudden thermic drops (63%) and decreased with hot temperature (58.7%). Owners reported a significant decrease in appetite due to heat in intact males ($p < 0.05$).

Most owners (68.9%) defined their dogs' grooming behavior as normal. According to owners, this behavior tended to increase (39.3%) in the warmer seasons slightly.

In our sample, 41.1% of dogs never showed aggressive behavior toward owners, while inter-dog aggression was reported in 33.2% of dogs. House soiling was never reported by 59.9% of the owners, although a fair number of dogs (40.1%) have had episodes of inappropriate elimination in the home. Climatic change didn't seem to have any effect on aggressive and house soiling behaviors.

Owners defined 58.4% of dogs as very reactive/nervous in response to environmental stimuli, but only 11% of owners believed that their dog barked excessively, while 25% believed that their dog's vocalizations were normal.

About half (49.7%) of the dogs considered in our sample were not afraid of loud noises, gunshots and thunderstorms, while a smaller percentage sought for owner attention (21.4%).

In correspondence with heavy thunderstorms or high-intensity incessant rains, dogs showed an increase in nervousness and reactivity (45.9%), fearful behaviors (47.7%), tendency to hide (38.8%), and vocalizations (26.5%). There was also a reduction in activity (48%) and play behavior (33.7%).

Fear behaviors (14%) and vocalizations (7%) are significantly less present in dogs adopted from breeders ($p < 0.05$). Owners' and dogs' ages did not affect the behaviors considered in the questionnaire.

Cats

European shorthair was the most represented in the questionnaire (85%), followed by smaller percentages of cats of different breeds. Most of the cats were adopted between 2 and 4 months of age (75.6%) and, to a lesser extent, after 5 months (18.8%). Many cats came from colonies -feral cats living outdoors in groups- (34.3%), and about the same number came from a private individuals (31.9%), although a fair number came from rescues (23.7%); cats adopted from breeders were a small percentage (8.5%).

Our sample of cats was almost equally distributed between males and females (spayed female 47.2%, spayed male 44.6%, intact female 4.5%, and intact male 3.8%), mainly between the ages of 3 and 7 (35.9%) and between 13 months and 3 years (26.3%). Senior cats (over 10 years) represented a smaller percentage (12.9%). Most cats (42.5%) were the only pet in the house, although as many (41.8%) lived with another cat.

Most of the cats followed a specific daily routine (78.4%). Cats mostly (63.4%) lived in apartments, and 53.3% were exclusively indoor, while 46.7% had access outdoor. Number and duration of outside activities varied in relation to the outside temperature, both in periods of extreme heat (number 65.1%, duration 75.3%) and extreme cold (number 69.5%, duration 75.8%).

Cats' play behavior and activity were considered normal by 70% of the owners. Owners reported that these behaviors changed during weather events. In particular, extreme heat led to a decrease in the level of activity (76.6%), while this behavior tends to increase with cold temperature (66.1%). Play behavior also increased in relation to temperature lowering (63.8%).

Most cats had their own place to rest (93.7%), and in 38.7% the preferred resting place was the owners' bed. Sleeping behavior, defined as normal by most owners (91.1%), increased both when the temperature dropped (76.4%) and when the climate was excessively hot (77.2%). Eating behavior, defined as normal by most owners (76.5%) increased with sudden thermic drops (78.7%) and decreased with hot temperature (71.8%). Food was always available for most of the cats (81%).

Most owners (87.1%) described grooming behavior as normal. This behavior tended to slightly increase (46.9%) in the colder seasons. In our sample, 37.1% of cats never showed aggressive behavior toward owners, while inter-cat aggression was reported in 25.3% of cats.

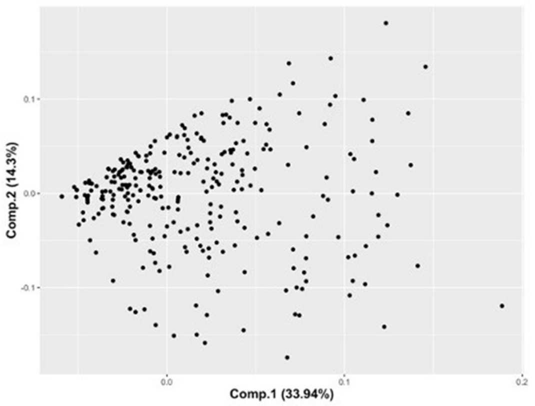

FIGURE 1
Score plot of dogs on the first (PC 1) and second (PC 2) principal components, with the increase in temperature.

Inappropriate elimination was never reported by 58.7% of the owners, although a fair number of cats (41.3%) have had episodes of house soiling. Marking behavior by rubbing their face or scratching on objects was shown by 70.7% of the cats considered in our sample. Climatic change did not appear to have any effect on aggressive and house soiling behaviors.

64.8% of the owners defined their cats as very reactive/nervous in response to environmental stimuli, but only 15.3% of owners believed that their cat vocalized excessively, while 61.5% believed that their cat's vocalizations were normal.

Most (60%) of the cats considered in our sample were afraid of loud noises, gunshots, and thunderstorms, while a smaller percentage (31.9%) did not show any behavioral reaction toward these stimuli. In correspondence with heavy thunderstorms or high-intensity incessant rains, cats showed an increase in nervousness and reactivity (46.8%), fearful behaviors (57.7%), tendency to hide (55.6%), and vocalizations (30.8%). Fear behaviors were significantly higher in cats older than 1 year of age and adopted from rescues ($p < 0.05$).

The age of the owners and the age of the cats did not affect the behaviors considered in the questionnaire.

PCA analysis

PCA results are shown using the first two components (PC1 and PC2) as axis (Figures 1–8). These two components together explained 48.2% of the variation for dogs and 49.2% for cats with the increase in temperature, and 65.5% of the variation for dogs and 62.5% for cats with the increase in thunderstorm.

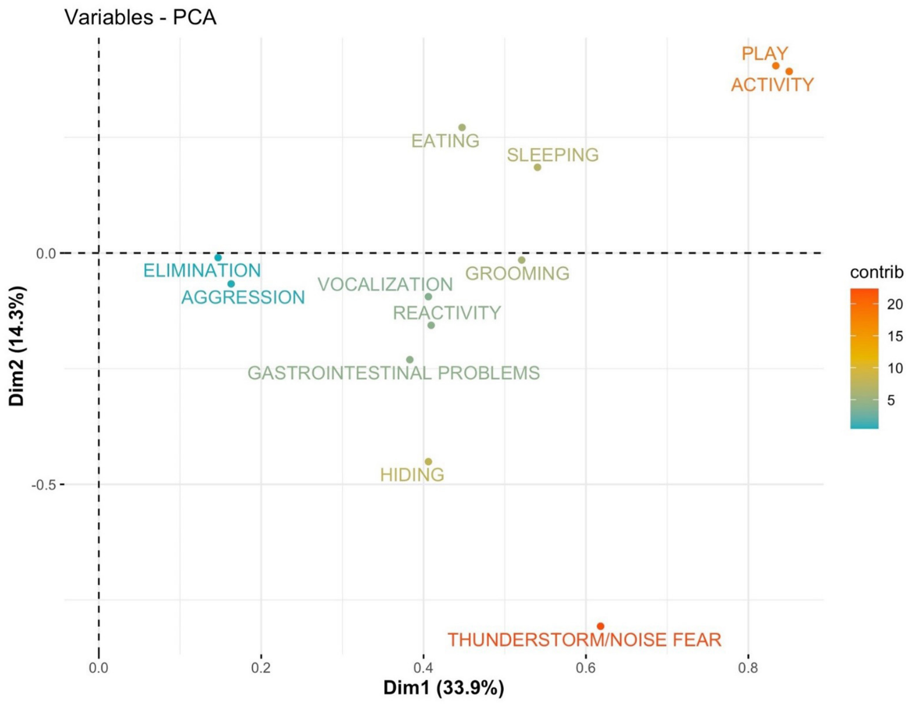

FIGURE 2
Loadings plot of the behavioral variables of dogs on the first (PC 1) and second (PC 2) principal components with the increase in temperature. The contribution of the variables is colored depending on their importance.

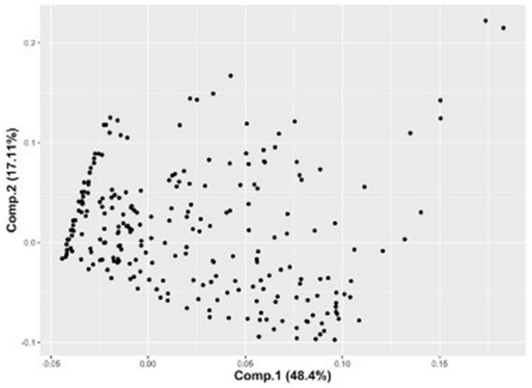

FIGURE 3
Score plot of dogs on the first (PC 1) and second (PC 2) principal components, with the increase of thunderstorms.

For both cats and dogs, the PCA analysis showed homogeneity of the sample: we did not find any clustering based on individuals' sex or age. Individuals with a similar profile are grouped together in the graph of individuals (cats or dogs) (Figures 1, 3, 5, 7). In the graph of variables (Figures 2, 4, 6, 8), positively correlated variables point to the same side of the plot, while negatively correlated variables point to opposite sides of the graph. The contribution of the variables is colored depending on their importance. With the increase in temperature, dogs were more differentiated by behaviors such as play and activity, thunderstorm and noise fear (Figure 2), while cats were more differentiated by behaviors such as play and activity (Figure 6). With the increase in thunderstorms, both dogs and cats were more differentiated by behaviors such as thunderstorm and noise fear, and hiding (Figures 4, 8).

Discussion

Veterinary and medical public health are threatened by global climate change, which is considered a One Health crisis (12, 13). Weather events can directly affect dogs' and cats' behavior, since their organism implements complex physiological mechanisms to restore body homeostasis. Moreover, owners could be induced to change their management routine based on the weather conditions, and this could be a factor that indirectly influences pets' behavior. It is known that changes in daily routines create a condition of distress that modifies the main behavioral patterns (18) and worsen pre-existing pathological conditions, such as anxiety, fear and loud noises or thunderstorm phobia. Behavioral

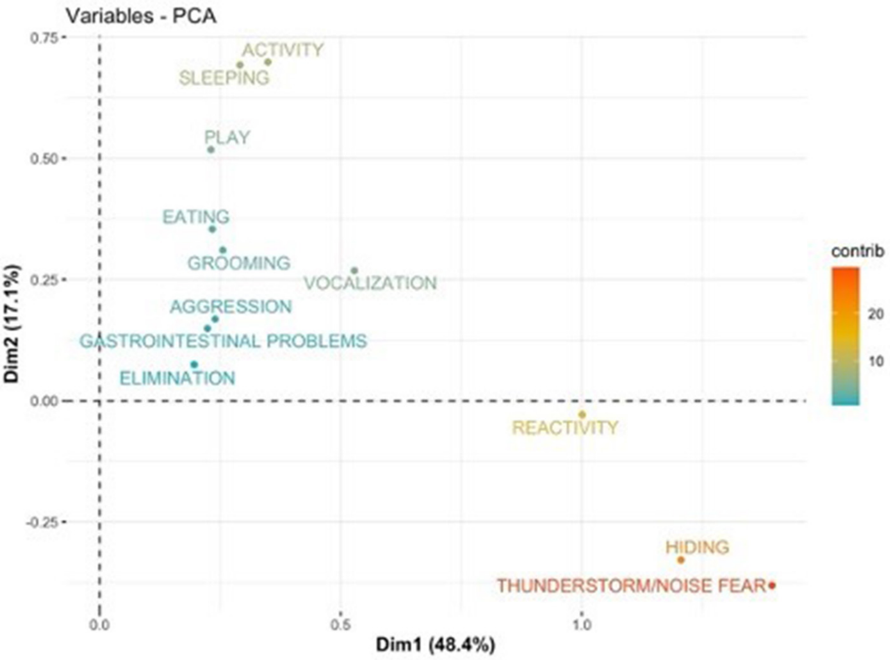

FIGURE 4
Loadings plot of the behavioral variables of dogs on the first (PC 1) and second (PC 2) principal components with the increase of thunderstorms. The contribution of the variables is colored depending on their importance.

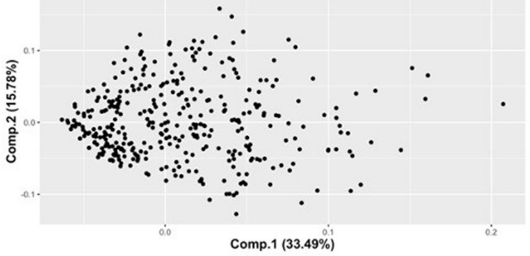

FIGURE 5
Score plot of cats on the first (PC 1) and second (PC 2) principal components, with the increase in temperature.

problems such as urinary marking (21, 22), separation anxiety (23), and cognitive dysfunction in elderly subjects (24) may also be exacerbated. In literature, limited attention has been given to the environmental factors that may contribute to canine and feline behavior change.

Our sample resulted to be very homogeneous in terms of both owners and pets. Our results showed that owners' and pets' age did not affect the behaviors considered in the questionnaire. As confirmed by the PCA analysis, little difference was found comparing dogs and cats, which were equally distributed between sex and age. Most owners followed a specific daily routine, even if the number and duration of outside activities were adapted to the outside temperature in extreme heat or cold periods. Heat-related illness is a potentially fatal condition inflicted on pets, bound to be more frequent as global temperatures rise. Older animals are at greater risk of developing a heat-related illness, even from sitting outside in hot weather. Extreme heat events are known to typically affect socially vulnerable patients, such as those with advanced age or chronic medical conditions, which may be confined indoors and be less resilient to natural hazards such as heatwaves (7, 28–30). Older dogs are more likely to suffer from underlying health conditions that impact thermoregulation, such as metabolic dysfunction or heart disease (31), which could increase the likelihood of environmental illnesses (32). Global warming will lead to the need to expand cooling strategies, which will inevitably include our canine and feline companions, as they can suffer fatal consequences when we fail to keep them safe (7, 27).

Our study showed that many behaviors in both species were equally modified by environmental temperature. Play and activity increased with cold weather and decreased with heat, and sleep increased with drops in temperature and with hot weather. Different factors appear to influence sleep in dogs, including diet and frequency of feeding (33), changes in housing conditions (34), changes in working routine (35), and activity levels during the day (36), but to our knowledge, no studies reported a relation of sleep and activity level to

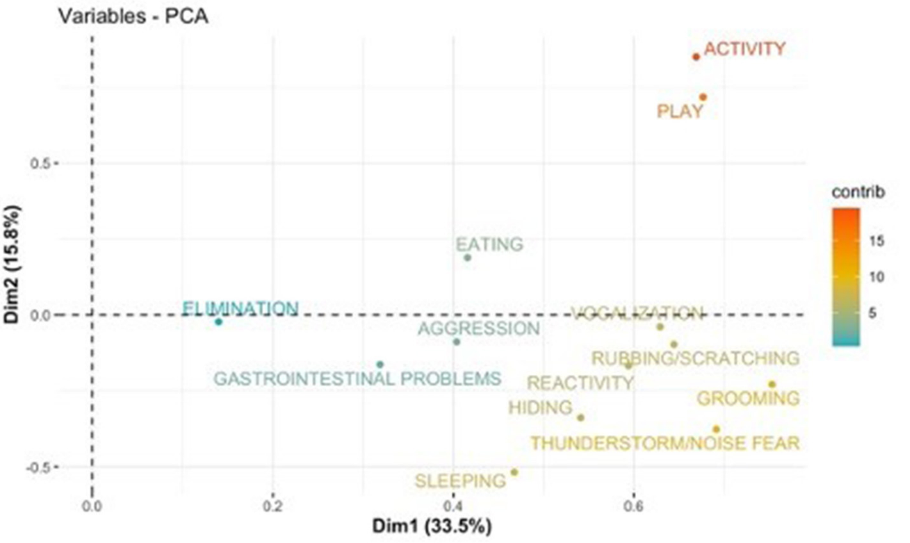

FIGURE 6
Loadings plot of the behavioral variables of cats on the first (PC 1) and second (PC 2) principal components with the increase in temperature. The contribution of the variables is colored depending on their importance.

environmental temperature. Our results are possibly related to the direct influence of temperature on behavior but also to the consequence of the temperature-induced changes in daily routine.

The increase in activity in correspondence with the thermic drop was more significant in whole males; this result may be related to testosterone, which exerts a positive action on energy metabolism (37) and it could also relate to the difference in activity level between males and females and between gonadectomized and no-gonadectomized subjects (38–41).

We found a statistically significant increase in Sheepdogs and Cattle dogs (group 1) playing behavior in the presence of thermic drop: this result could be related to breed activity needs.

A lower propensity to play in high ambient temperatures could be considered a factor of rapid environmental adaptation since Hall et al. (7) found that exercise was the most common trigger of heat-related illness in dogs. Heatstroke caused by exercise was just as likely to kill as heatstroke from a hot car. Respiratory diseases, such as brachycephalic obstructive airway disorder (BOAS), have been shown to accelerate the increase in body temperature during exercise (42), and brachycephalic dogs have intrinsically greater odds of developing heat-related illness compared to dogs with longer muzzle (27). Heat regulation problems are reported to affect around a third of brachycephalic dogs (42) and obesity has been reported as a significant risk factor for death in dogs presenting with heat-related illness (44).

Our survey evidenced that feeding behavior was also affected by weather events, with an increase of feed intake during the cold season and a decrease in the heat: this type of feed control is part of the body temperature and metabolism homeostasis system (21). In cats, an increase in grooming behavior was observed in correspondence with temperature increases, probably due to the evaporative cooling losses through the skin and the hair licking (43, 45).

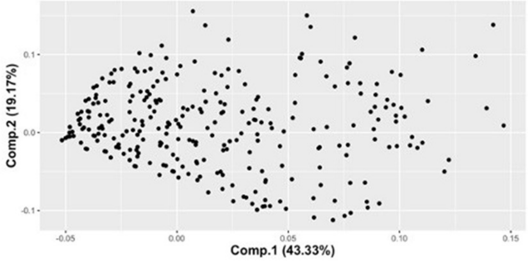

FIGURE 7
Score plot of cats on the first (PC 1) and second (PC 2) principal components, with the increase of thunderstorms.

Weather events did not affect aggressive and house soiling behaviors in both dogs and cats.

Weather events, including wild thunderstorms, torrential rains influenced our pets' behavior. In correspondence with heavy thunderstorms or high-intensity incessant rains, increased nervousness and reactivity, fearful behaviors, tendency to hide, and vocalizations were observed. When an animal lives in a stressful situation, physiological changes occur, to prepare the animals' response to the perceived danger. From a behavioral viewpoint, the most frequently observed signs of fear are avoidance, immobility, and flight (46–48). Behavioral signs of

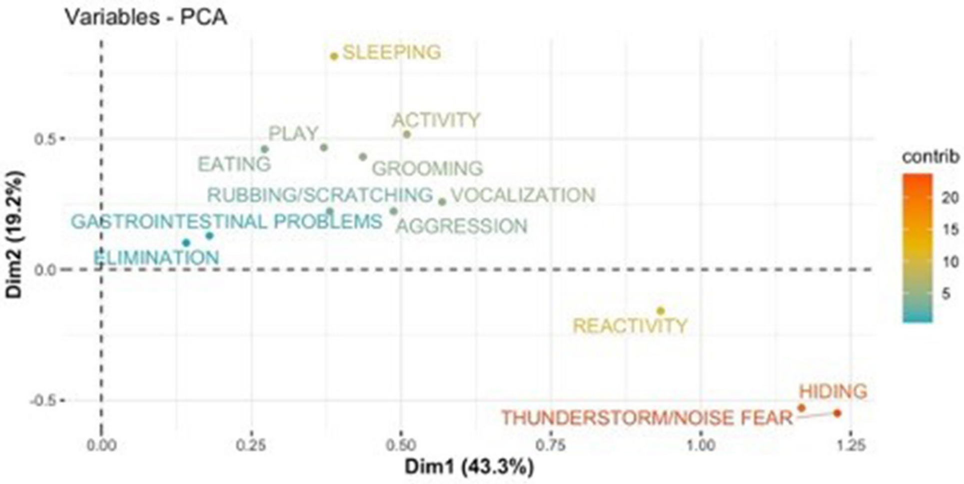

FIGURE 8
Loadings plot of the behavioral variables of cats on the first (PC 1) and second (PC 2) principal components with the increase of thunderstorms. The contribution of the variables is colored depending on their importance.

fear may include increased vigilance, reactivity, and motor activity (pacing). There may also be excessive demands for human attention and reassurance in sociable individuals (cats or dogs). Equally, a fearful animal may show behavioral inhibition, shyness, avoidance, reduction of locomotor activity (freezing), hiding, and running away (49, 50). Stress associated with fear and anxiety can have negative impacts on health, welfare, behavior, and lifespan (49, 50), depending on both the nature of the stressor (intensity, duration, persistence, etc.) and the coping skills of the individual (51–53).

Our results evidenced that fear behaviors and vocalizations during thunderstorms were significantly less present in dogs adopted from breeders and higher present in cats older than 1 year of age and adopted from rescues. Behavioral responses to noises have a relatively high prevalence in the owned dog and cat population and are related to early sensitive periods, suggesting that early experience is an essential factor in the development of fear responses (54–56). Rescues animals therefore could have higher risk to develop high sensitivity to noise fear.

The result of this study represents a step toward the improvement of owners' awareness on management tools that can limit their animal exposure to sudden weather events. Without appropriate mitigation strategies, as extreme weather events continues to increase, also the frequency of heatwave and heavy thunderstorm events and the number of dogs and cats experiencing environmental stressors will likely to increase.

This study highlights canine and feline behavioral modification related to weather events in Italy. The main limitation of this study relates to the use of a questionnaire and the use of self-reported data. Our data refer to owners' perceptions, who detect the behaviors of their pet in the daily routine and in the presence of intense atmospheric events. Since the behaviors are not directly observed, there is a level of uncertainty in the accuracy of the results. Moreover, survey participants were recruited *via* social media and veterinarians, meaning the study likely selected for a demographic of dog and cat owners more actively engaged with their pet's health.

Understanding how dogs and cats modify their behaviors based on weather events can help to refine prevention strategies through owner education and societal changes (57). Domestic dogs and cats often share their owners' home and leisure activities, including walking, running, and other sports (55). Dogs increasingly accompany their owners to the workplace (58) and are often included in travel and holiday plans. No other species more intimately intertwines with the human lifestyle, meaning dogs potentially face similar levels of both environmental and exertional heat exposure to humans. How dogs are transported, housed, and managed will also influence heat-related illness risk. As the frequency of extreme weather events such as heat waves or intense thunderstorms is increasing, society needs to prepare strategies to mitigate their effects (59) to protect both human and canine health (44).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee (reference number 5021, date 18/05/2021) University of Milan, Italy. The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by Ethics Committee (reference number 5021, date 18/05/2021) University of Milan, Italy. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

Conceptualization, methodology, supervision, project administration, funding acquisition, and investigation: CP and SC. Data curation: SC, AL, and GM. Formal analysis: GM. Writing—original draft preparation: CP. Writing—review and editing: CP, SC, AL, SM, and GM. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Heat tolerance, thermal equilibrium and environmental management strategies for dairy cows living in intertropical regions

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This review makes an attempt to characterize the physical attributes of heat tolerance, thermal equilibrium and thermal stress thresholds for dairy cows living in tropical environments, with a particular emphasis on pasture-based systems. Under such circumstances, the radiant heat load is the principal climatic factor that determines rates of heat and mass exchanges between cows and the environment. This fact may explain why simple mechanistic models based on air temperature and humidity are not adequately predicting thermal stress thresholds for cattle in tropical regions. To overcome this limitation, the Index of Thermal Stress for Cows (ITSC) and Index for the time spent in shade (ITS), which account for various sources of thermal radiation, were proposed to predict autonomous and behavioral thermoregulation of cows. Overall, the evolutionary adaptation of cattle in tropics favored animals that have cutaneous surface with a skin well protected against penetration of ultraviolet solar radiation (UV), covered by a coat surface with high thermal conductivity. For Holstein breed, although predominantly black animals absorb greater levels of short-wave solar radiation, they may present better protection of skin than white ones. However, dark-colored cows in tropical pastures have potential to absorb as much as 640 W m^{-2} of thermal radiation. This amount of heat load would require close to 1,300 g h^{-1} of cutaneous evaporative water loss through sweating to prevent increases to body temperature, where cows do not have access to shade. Cows are motivated to reduce time spent grazing and to seek shade when solar irradiance exceeds

550 W m^{-2}, levels that in equatorial latitudes are likely to occur between 08:00 and 16:00h. This information may help producers improve the welfare of cows, as they can determine more comfortable hours for them to graze, for example, by employing nocturnal grazing. Over the daytime, cows should have access to areas with shade and this could include shade provided *via* solar panels, which has the potential to improve thermal comfort and sustainability of dairy production in tropical areas.

KEYWORDS

dairy cows, thermoregulation, evolutionary adaptation, thermal equilibrium, solar radiation

Introduction

Over the last century the dairy cattle populations originated from temperate climates, and that were introduced in tropical regions have acquired phenotypic characteristics that have supported thermal tolerance, *via* either natural or artificial evolutionary adaptation (1, 2). Cows in tropical environments may be exposed to higher radiant heat load than cows in temperate climates, as high levels of solar irradiance in tropics are nearly constant throughout the year, and most cows are kept in open field conditions (3–5). The most appropriate cattle phenotype for better thermal tolerance includes a set of radiative and physical properties of body surface characteristics (1, 6) that protect skin against deep penetration of ultraviolet radiation (UV) and facilitates mass and heat transference to the environment (7, 8).

The combination of a light-colored coat surface over a well-pigmented skin results more incident short-wave solar radiation being reflected, and less being transmitted throughout the skin (9). Regarding the morphological aspects of coat surface, the combination of well-settled and thick hairs favors less resistance to the diffusion of heat and water vapor throughout the boundary layer (10). While these characteristics of skin and coat surface are prominent in *Bos indicus* cattle breeds as Nellore (9), Holstein cattle and other *Bos taurus* breeds, with exception of Jersey, have skin pigmentation accompanying the coat color. In other words, predominantly white (or red) Holstein cows have a low-pigmented skin, which make them more susceptible to skin damage when exposed to high levels of short-wave solar radiation. This may explain reasons for greater abundance of predominantly black Holstein cows in these regions, as they are better protected against negative effects of UV transmission (1).

Dark-colored cows may however absorb heat by thermal radiation twice as much as the light-colored ones (Figure 1), therefore would need to sustain greater rates of cutaneous evaporation to maintain the thermal equilibrium (11), if they are kept in tropical pastures without access to shade. The thermal radiation is the principal meteorological factor that influences thermal equilibrium of cows in tropical conditions, and consequently heat stress thresholds (12–14). This fact implies that more simplistic mechanistic models based on air temperature and humidity are very misleading to predict impacts of the thermal environment on thermoregulation and productive performance of cows in tropical areas (5, 15), as usually has been done (16). One topic of this review is to provide more appropriate approaches to improve the characterization of the thermal environment faced by a cow in a tropical environment.

FIGURE 1
Pictures of purebred white and black Holstein cows taken in a commercial farm, Latitude = 7° S. Source: Courtesy of Severino Guilherme C.G. dos Santos.

Previous reviews have highlighted physiological responses of thermoregulation and productive performance outcomes of dairy cows exposed to heat stress events in temperate climates, yet few attempt to characterize the physical attributes of heat tolerance, thermal equilibrium, heat stress thresholds, and ameliorative strategies for dairy cows living in intertropical zones, particularly in Brazil, where thermal radiation is abundant and constant throughout the year. This review aims to address these topics with an emphasis on Holstein cattle. This breed was introduced in tropical areas many years ago, and currently, at least in Brazil, is the most used breed in dairy production systems. Ultimately, concerning the possible adaptive strategies to ameliorate negative impacts of solar radiation on cows, we want to highlight the Animal Agrivoltaic

(17), a solution that uses photovoltaic panels to generate renewable electrical energy and to project shade for animals.

Evolutionary adaptation of dairy cattle in Brazilian tropical regions: An emphasis on heat tolerance traits

Cattle were introduced to Brazil during the early colonization and exploration attempts; the surviving animals then reproduced and adapted to the new environment, being known as "crioulo", "local" or naturalized (18). Zebu cattle (*Bos indicus*) were imported from India at the end of the nine-teeth century and largely disseminated over the Brazilian territory, crossing with naturalized breeds (19). With the aim at improving dairy productivity, during the 1920s and 1930s, there were attempts to introduce several European cattle breeds as Holstein, Jersey, and Brown Swiss (20). Although more productive, these animals were not adaptable to the new environment, especially to the thermal conditions (2).

Around the 1940s Brazilian farmers began to cross the Gir (an imported *Bos indicus* breed) with the Holstein (20–22), in order to attain the hybrid vigor, and to develop a high producing yet heat-tolerant breed. Indeed, milk yield of Holstein x Gir crosses averaged 2,574 kg per 305 days, significantly higher than the 1,600 kg produced by Gir in India (23, 24); this superiority increases with the Holstein contribution (e.g., from 1/2 to 7/8) (22). Most dairy cattle population in Brazil is currently represented by crossbreds (mainly Holstein x Gir), mostly kept under pasture based-systems (21). Furthermore, as a result of almost 100 years of evolution in tropical regions, the phenotypic aspects of purebred Holstein cattle are much different when compared with contemporary Holsteins living in temperate climates, particularly that associated with heat tolerance (8–10). For instance, the effective thermal conductivity ($W\ m^{-2}\ K^{-1}$) of body surface of Holstein cows living in Brazil was reported to be much higher than those in temperate conditions, which is explained due to the lower coat thickness of the Brazilian cows (2.4 vs. 25 mm) (1).

Heat tolerance is determined by the relationship between metabolic heat production and the ability to dissipate body heat (2). The coat color is the primary determinant of the amount of radiant heat absorbed over the body surface, sourced from short-wave solar radiation (i.e., spectral range from 300 to 3,600 nm). Although black-haired cows absorb more incident radiation (*circa* 91%) than the white-haired ones (close to 35%) ((9), Figure 2), Holstein cattle population in Brazil is mostly represented by black animals (6, 8, 9, 25, 26, 60). This information seems to evidence that adaptation of Holstein cattle in tropical regions favored selection of traits that are of greater importance to animal fitness than a body surface that absorbs less radiant heat.

Light coats of European cattle breeds, with exception of Jersey, coexists with a low-pigmented skin (9, 10) in response to a gene action that blocks melanogenesis (27). Breeds or animals with this characteristic are likely to be more susceptible to the harmful effects, e.g., skin burns and neoplasia (Figure 2), of the ultraviolet radiation (spectral range between 200 and 390 nm). Mixed colored Holstein cows in equatorial regions of Brazil were reported with serious cutaneous tissue damage only in white spots (25). Because black spots are over a well pigmented skin, less UV radiation is transmitted throughout dermis and epidermis (Figure 2).

Black cows however present higher absorption of thermal radiation and therefore have more body heat to be dissipated than white-haired cows, particularly if they are exposed to solar radiation. The thermal balance of the body surface is determined by a combination of radiative properties and arrangement of individual hairs, which in turn, is a function of density and inclination angle of the hairs at the skin surface (28). A body surface with a less-dense coat, thick, short, and well settled hairs characterize most cattle breeds well adapted to tropical conditions, including Holstein (7). These characteristics favor less still air to be trapped within the boundary layer, which in turn, gives less resistance to the diffusion of heat and water vapor, and enhances dissipation of heat and mass (mainly through sweating) to the environment (1).

The set of black and well-pigmented skin ($h = 0.75$), hair diameter ($h = 0.63$), sweating rate ($h = 0.43$), age at first calving ($h = 0.23$), and milk yield ($h = 0.34$) were found to have relatively high values of heritability, and favorable genetic correlations between them (29–31). Breeding programs therefore can select more heat tolerant, but still productive Holstein and Jersey cows to be raised in open field conditions of tropical regions. Moreover, with advances of genomic tools, it was possible to identify a specific gene that confers animals with a short and sleek hair coat, the SLICK haplotype (*slick hair*), which allows faster selection in order to enhance thermal conductivity of the body surface, and heat tolerance of cows. Holsteins cows with slick hair gene had superior thermoregulatory ability and lesser decay in milk yield during the summer, when compared to wild-type Holstein cows that did not have the slick hair gene (32). Further discussion about other possible genes that confer better thermal tolerance of dairy cattle can be found at Silpa et al. (33).

Thermal equilibrium of cows in tropical areas: An emphasis on thermal radiant environment

Living organisms are physical systems in which thermal energy is produced continuously by means of metabolic processes and exchanged (gains and losses) with the surrounding environment (34). The thermal equilibrium of a cow can be

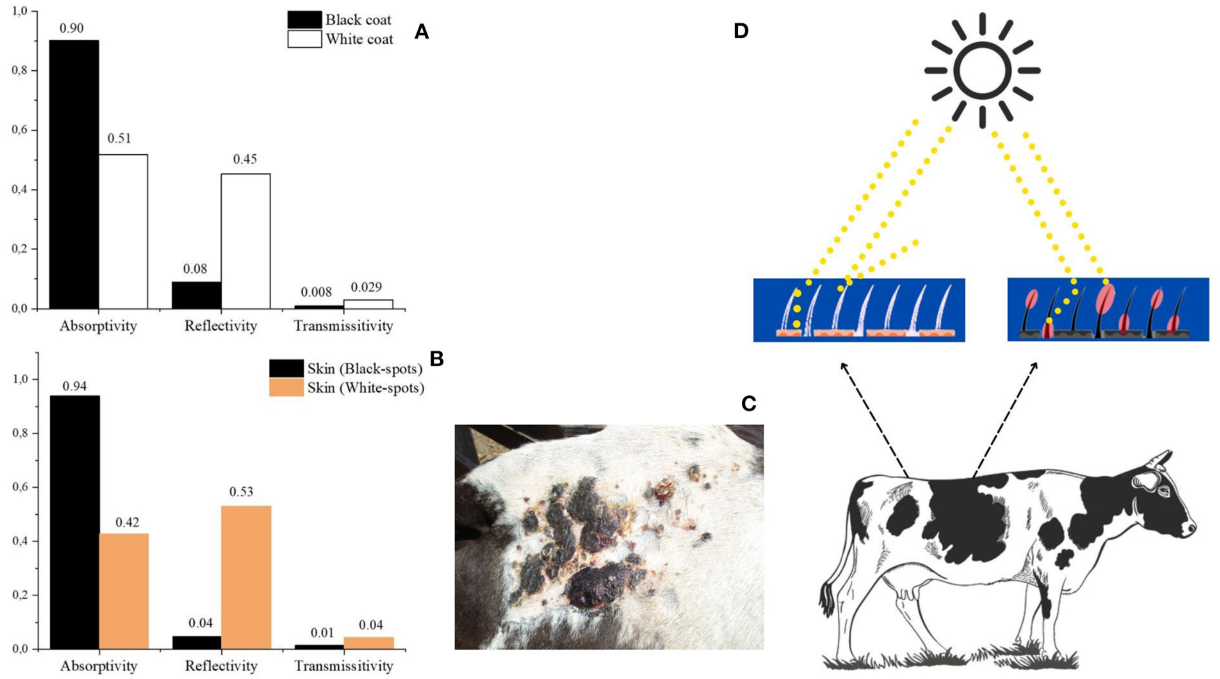

FIGURE 2
Radiative properties of the coat **(A)** and skin **(B)**; skin burn **(C)** in the white spot of Holstein cow managed in open pasture of a tropical region (Source: Courtesy by Roberto G. da Silva). Measurements of the radiative properties were performed with a radiometer [DaSilva et al. (6)]. **(D)** Dotted yellow lines represents the electromagnetic photons of energy that can be reflected, transmitted and absorbed (red circles) as heat over the coat and skin surface.

described as:

$$\mathrm{M} + \mathrm{R_C}\alpha + \mathrm{S} \pm \mathrm{R}_L \pm \mathrm{C_S} \pm \mathrm{K} - \mathrm{C}_R - \mathrm{E_S} - \mathrm{E}_R = 0 \quad (1)$$

Where ***M*** represents the metabolic heat production; for lactating Holstein and Jersey cows (Average milk yield of 25 kg) in tropical regions the metabolic heat production was reported to be between 180 and 230 W m^{-2} (34, 35). $\boldsymbol{R_C}$ is the heat absorbed by short-wave solar radiation, which depends of the absorptance of the coat surface ($\boldsymbol{\alpha}$); $\boldsymbol{R_L}$ is the thermal energy exchanged by long-wave radiation; ***S*** is the rate of thermal energy storage; $\boldsymbol{C_S}$ is the heat exchanged by surface convection; $\boldsymbol{C_R}$ is the heat transferred by respiratory convection; $\boldsymbol{E_S}$ is the heat transferred by cutaneous evaporation; and $\boldsymbol{E_R}$ is the heat transferred by respiratory evaporation.

The sensible heat transfer encompasses conductive (***K***), convective ($\boldsymbol{C_R}$ and $\boldsymbol{C_S}$) and radiative heat transfer ($\boldsymbol{R_C}$ and $\boldsymbol{R_L}$, Figure 3), while the evaporative heat transfer encompasses cutaneous ($\boldsymbol{E_S}$) and respiratory ($\boldsymbol{E_R}$) evaporation. The direction and rate of heat transfer by each of the three sensible routes depend on a temperature difference between the surface of the animal and that of the environment. However, the temperature of the surrounding air is not the most relevant meteorological variable for determining rates of sensible heat exchanges by a cow (at standing position) in a tropical pasture (16). In this case, sensible heat exchanges, particularly by long-wave radiation, are totally dependent of a mean radiant temperature, an environmental temperature that accounts all sources of thermal radiation emitted from the surrounding environment, i.e., ground-surface, objects, trees and sky (14).

Holstein and Jersey cows kept indoor partially offset the metabolic heat production by means of surface convection and long-wave radiation when the temperature of the surrounding air ranges between 15 and 25°C. Under such circumstances, evaporative heat transfer, mainly through cutaneous surface may account for only 20–30% of the metabolic heat dissipated to the environment. On the other hand, when the air temperature is above 28°C, the rates of cutaneous evaporative water loss become the principal route of heat elimination, by accounting for 80% of the total metabolic heat production (35, 36). Indeed, the upper critical temperature for dairy cows is predicted to be within 25 and 28°C, depending on its levels of metabolic heat production (37). This temperature threshold is however not relevant for cows outdoors, especially if they are exposed to high levels of thermal radiation. Recent research shows that Holstein cows kept outdoor in a tropical environment, and experiencing same level of air temperature of their counterparts kept indoor, increased by 50% the rates of cutaneous evaporative water loss, were more vasodilated, and stored more body heat (Fonsêca et al.; in preparation).

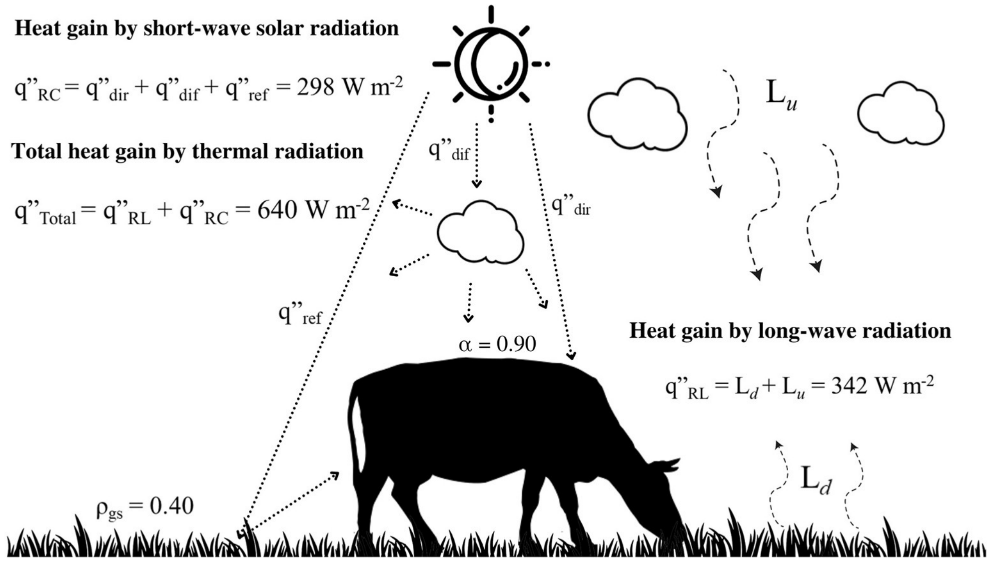

FIGURE 3
Representation of the thermal radiation absorbed by a dairy cow in a tropical pasture, at 12:00h, January, 20, Lat. 5° S (DaSilva et al. (14)). The ρ_{gs} is the reflectance coefficient of a green grass, and α is the absorptance coefficient of a black coat surface. The rates of total long-wave radiation (q''_{RL}, W m^{-2}) are the sum of downward radiation, emitted from the sky (L_u), and upward radiation emitted from the ground surface (L_d). The rates of heat absorbed by short-wave solar radiation (q''_{RC}) encompass direct (q''_{dir}), diffuse (q''_{dif}), and reflected (q''_{ref}) solar radiation.

This result emphasizes thermal radiation as the principal meteorological component at determining thermal balance of cows in tropical environment. A black-haired Holstein cow in a pasture have potential to absorb as much as 640 W m^{-2} of thermal radiation during the hottest hours of the day, e.g., from 09:00 to 16:00h (14; Figure 3). The short-wave solar radiation may account to 298 W m^{-2} or 47 % of the total thermal radiation absorbed, while long-wave radiation (342 W m^{-2}), most of which emitted from the ground surface, accounts to 53%. This amount of heat load can represent threefold the metabolic heat produced by a cow ($\sim$ 200 W m^{-2}), which in turn, would need to dissipate close to 850 W m^{-2} in order to maintain their thermal equilibrium. For doing so, cows would need to produce and to evaporate up to 260 g h m^{-2} or 1,300 g h^{-1} of sweat (Surface area of a cow, $m^2 = \sim 5$).

By adjusting behavior, cows however can manipulate rates of heat exchanges with the surrounding environment; for example, by seeking shade, they can avoid absorption of high amount of radiant heat load, thus decreasing requirements for evaporative water loss through cutaneous surface. Moreover, by lying down in a shaded surface, cows may be able to dissipated body heat by conduction depending on ground surface temperature. The critical level of short-wave solar radiation that motivates cows to stop grazing and to seek shade is between 500 and 700 W m^{-2} (38). In the absence of shading resources, cows can also employ conductive and convective cooling by lying down in sources of water (12, 13; Figure 4). It is possible to see that the two cows in the Figure 4 can lose body heat due to a positive thermal gradient between body and water surfaces. However, this behavior can be critical in terms of dissemination of diseases as mastitis, especially soon after the post dipping procedures.

Predicting heat stress responses of dairy cows living in tropical regions

Several indexes have been extensively used to predict the environmental thermal comfort threshold for cattle, such as the temperature-humidity index, THI (39); effective temperature, ET (40); black globe humidity index, BGHI (41); equivalent temperature index, ETI (42), heat load index, HLI (43). Overall, some of these indices were based on animal responses under temperate region, and in controlled climatic chambers, which in turn do not well represent complexity of the thermal radiant environment faced by dairy cows in tropical regions (34). The effectiveness of five indices against thermoregulatory responses of Holstein cows kept in open field conditions of an equatorial region of Brazil were tested (5). Unsurprisingly, results confirmed that THI and BGHI were not correlated with rectal and respiratory rate of cows. On the other hand, the Heat Load Index [HLI; (44)] presented significant correlations with thermal responses of cows, a single variable who accounts effects of air temperature, humidity, wind speed and solar irradiance, which was initially developed for feedlot cattle in Australia.

Although the importance of air temperature and humidity for the heat exchange processes between animals and

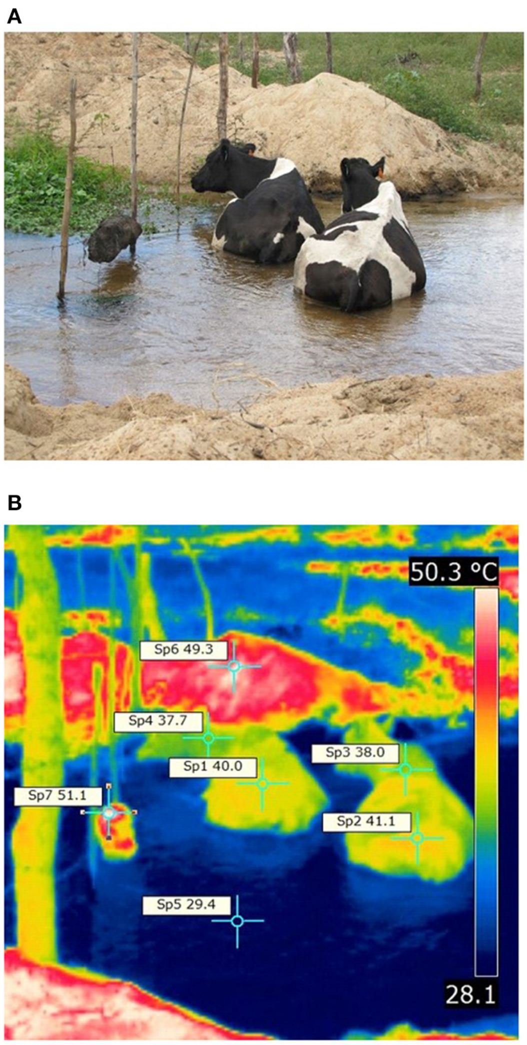

FIGURE 4
Holstein cows employing conductive and convective cooling with the water river **(A)**. Thermogram taken in an equatorial latitude **(B)**. Source: Courtesy by Alex S. C. Maia, innovation group of thermal comfort and animal welfare (INOBIO-MANERA). Dotted yellow lines (D) represents the electromagnetic photons of energy that can be reflected, transmitted and absorbed (red circles) as heat over the coat and skin surface.

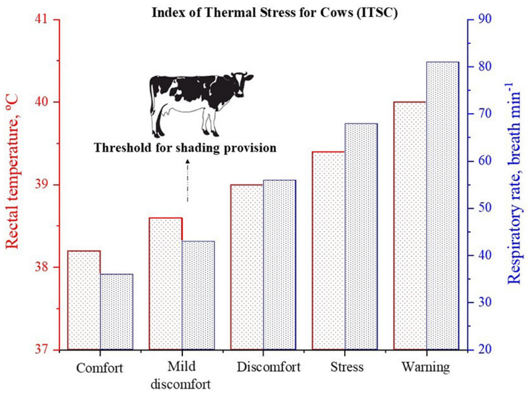

FIGURE 5
Mean of rectal temperature and respiratory rate of Holstein cows ($n = 840$), according to the heat stress thresholds of the Index of Thermal Stress for Cows (ITSC). Mean values were sourced from DaSilva et al. (5). Comfort (ITSC $\leq$ 150), Mild discomfort (151 $<$ ITSC $\leq$ 200), Discomfort (200 $<$ ITSC $\leq$ 250), Stress (250 $<$ ITSC $\leq$ 350), Warning (ITSC $>$ 351).

environment, thermal radiation assumes greater importance for cows kept in tropical pastures. A Thermal Stress Index for Cows (ITSC) was proposed by DaSilva et al. (5) based on meteorological measurements in an equatorial region, in addition to physiological responses of 814 Holstein cows. By using a principal component analyses the physiological variables respiratory rate (R_R, breath min^{-1}), rectal temperature (T_R, °C), skin temperature (T_{Skin}, °C), body surface temperature (T_S, °C), cutaneous (E_C, W m^{-2}) and respiratory (E_R, W m^{-2}) water loss of cows were summarized into a single variable, the ITSC. The ITSC was then fitted against air temperature (T_A, °C), wind speed (U, m s^{-1}), humidity (P_V,kPa), and effective radiant heat load (ERHL, W m^{-2}) (a meteorological variable who accounts effects of short-wave and long-wave radiation), by employing multiple regression analyses. The best fitted equation chosen based on higher R^2 in order to determine values of ITSC was:

$$\text{ITSC} = 77.1747 + 4.8327\,\text{T}_\text{A} - 34.8189\,\text{U} + 1.111\,\text{U}^2 + 118.6981\,\text{P}_\text{V} - 14.7956\,\text{P}_\text{V}{}^2 - 0.1059\,\text{ERHL}$$

The values obtained for ITSC were fitted against physiological responses of cows, and significant correlations were observed with rectal temperature ($r = 0.472$), respiratory rate ($r = 0.793$), skin surface temperature ($r = 0.755$) and sweating rate ($r = 0.570$). These correlations derived five heat stress thresholds for cows (Figure 5). For instance, when value of ITSC is greater than 200, cows must be provided with access to shade. Even for cows managed in free-stalls under semi-intensive production systems, with occasional or free access to external paddocks, values of ITSC may indicate when they should be housed again. Complementary to the ITSC, another thermal stress index was proposed to predict the time spent in the shade by dairy cows in tropical areas, the shade time index (ITS) (45). The ITS can be calculated based on local values obtained for air temperature (T_A, °C), relative humidity (R_H, %), solar irradiance (S_R, W m^{-2}), and black-globe temperature in the shade (T_G, °C), as follow: IST = – 14.32 (0.79T_A) – (0.56R_H) + (0.041S_R) + (1.58T_G). In addition to the ITSC, the IST can be employed in order to improve daily management of cows kept under pasture based-systems, for example, by determining more comfortable hours for them to graze.

Heat abatement strategies for dairy cows in tropical environment: An emphasis on shade use

Shade is any region of space not traversed by a radiation beam (34). Benefits coming from shade use by animals are due to the abatement of radiant heat gain through two main sources. First, from direct and diffuse short-wave solar radiation, where on hottest times, by seeking shade, animals could avoid levels of impinging solar irradiance as much as 1,200 W m^{-2}. Second, animals in shade may also have decrease radiant heat gain due to less long-wave radiation emitted from a shaded surface, which in turn, is directly proportional to its absolute temperature. Ultimately, animals could also avoid heat gain by conduction when lying down on cooler shaded surfaces. The provision of shade (natural or artificial) is therefore of particular importance at alleviating heat stress of cows kept under pasture based-systems of tropical areas. In particular, by taking into account shade availability and level of solar blockage provided by the shade structure (46–48).

A cow should be provided with minimum of 3 m^2 of shaded area, otherwise benefits of its use is likely to be minor, thus reducing cow's motivation to keep in the shade (34, 49). High density of animals in the shade may impair air displacement, thus decreasing efficiency of the convective evaporation over the body surface of animals. Studies have shown that cows with access to 9.6 m^2 of shade/cow spent twice as much time in shade compared to the availability of only 2.4 m^2 shade/cow, had lower panting scores and expressed less agonistic behaviors (50, 51). A cow also should be provided with a shade structure that blocks as much as possible the direct and diffuse solar radiation (52). Indeed, they spent greater time within the shade projected from structures that blocked 100% of solar radiation against shade-cloth structures that blocked 50 and 70% (45).

One good option is a shade provided by photovoltaic panels, by using the concept of "Animal Agrivoltaic", recently developed by the Innovation Group of Thermal Comfort and Animal Welfare [Inobio-Manera; (17)]. The Animal Agrivoltaic is a type of co-generation system that provides high quality shade for animals, production of sustainable animal protein, and generation of renewable electrical energy in the same area. Cows in the shade projected by photovoltaic panels can experience nearly by 100 W m^{-2} less radiant heat load than if they were in a shade projected by shade-cloth structure that blocks 90% of solar radiation (Figure 6). The possibility of such integrated systems cast the interest of policy makers and governmental agencies in Brazil and around the world, as a potential solution to offset carbon emissions derived from livestock production systems (17, 53, 54). Another strong motivation for the implementation of sustainable co-generation systems using photovoltaic panels is the continuous decrease in the price of photovoltaic panels (from US$ 3.90 *Wp* in 2006 to US$ 0.39 *Wp* in 2016; 5% expected annual price drop; (55)) as well as the development of new technologies expected to increase the efficiency of energy conversion (from 18 to 45% using Single-Junction GaAs, Thin-Film Crystal).

FIGURE 6
Holstein cows using shade projected from photovoltaic panels Latitude = 21° S (Source: Courtesy of Alex S. C. Maia, Innovation Group of Thermal Comfort and Animal Welfare (INOBIO-MANERA).

Even in the shade cows may experience levels of air temperature higher than its body surface, a condition that makes heat to be absorbed by the animal from the surrounding environment. Under such circumstances, spraying water over the skin can enhance mass and heat transfer through the cutaneous surface, thus avoiding body heat storage and thermal stress (56, 57). Although several studies have been showed benefits of sprinklers on thermoregulation and performance of dairy cows kept in housed conditions (58, 59), applicable solutions are needed to be proposed for cows managed in open pastures. For instance, shading and water spraying cows with a simple hand-held pump every 1 *h*, from 10:00 to 15:00, decreased body heat storage, requirements for evaporative water loss through panting, and increased milk yield by 3.0 kg day^{-1} cow^{-1}. This type of solution can be practical in small dairy farms (57). However, further studies should determine optimum amount of water that must be sprayed in order to offset requirements of cows for evaporative cooling mechanisms through sweating and panting.

Final remarks

This review highlighted the significance of the thermal radiant environment as the principal meteorological stressor for dairy cows living in intertropical regions, and discussed about more appropriate mechanistic models in order to predict heat stress thresholds for them. A coat surface with high thermal conductivity over a well-pigmented skin is the best phenotype for cows kept in open pasture, and for Holstein, a combination that only occurs in black-haired cows. As black cows receive large amount of thermal radiation sourced from short-wave and long-wave radiation, the provision of shade is then mandatory, particularly when solar irradiance exceeds 550 W m^{-2}, levels that motivate cows to stop grazing. Ultimately, shade could be provided by solar panels, as they can efficiently buffer the radiant heat load on cows, generate clean and renewable electrical energy, thus improving sustainability of dairy cow production in tropical environment.

Author contributions

Conceptualization, methodology, and writing original draft—manuscript: SS and VF. Writing original draft—figures: VF, SS, MM, and AM. Writing—review and editing: VF, ES, AL, VS, AM, GM, and SG. Visualization: VF and AL. Supervision: ES and VF. Funding acquisition: ES and SG. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Genotype by heat conditions interaction effects on growth and litter traits in rabbits

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Heat stress has severe impacts on rabbit performance because they have difficulty getting rid of excess heat. The interaction between genetic and environmental factors plays a vital role in the adaptation process. The current study aimed to evaluate the effects of interaction between the genotype and heat conditions (G×H) on litter size and growth traits. Two rabbit lines were used in the current study, Egyptian maternal line (APRI) and New Zealand White (NZW). The rabbits were raised under normal (22°C) or heat stress (35°C) conditions. The does were raised in individual cages, and their kits were reared under the same conditions. Negative effects of heat conditions were noted for litter traits, as heat stress had significantly reduced the number of born alive, total born, and marketed number rabbits by about 16, 11, and 25%, respectively. Moreover, growth traits were reduced under heat stress conditions compared to normal temperature conditions. Significant differences between genotypes were observed, APRI rabbits were higher than NWZ rabbits in the total born, number of born alive, number weaned, and the number of marketed rabbits by 10, 8, 11, and 10%, respectively. Genotype by heat conditions interaction effects were observed, APRI litters under normal conditions showed significantly higher litter traits than NWZ litters, whereas relevant reductions in litter traits of APRI line than NZW litters when reared in heat conditions. For growth traits, the differences between the two lines under normal conditions were reduced when animals of the two lines were raised under heat stress, for BW_{28}, BW_{42}, BW_{63}, ADG_{28-42}, and DF_{28-42} while these differences were increased for ADG_{42-63}, DF_{28-42} and feed conversion during the whole fattening period. Based on the observed G × H interactions, it is important to select the animals under the same production conditions to raise rabbits that can cope with the expected global warming conditions.

KEYWORDS

G x E interaction, heat stress, litter size, growth traits, rabbits

Introduction

Recently, global warming is leading to an increase in the atmospheric temperature by about 0.07°C per year, with predicted increases ranging from 2.6 to 4.7°C by the year 2100 (1). Rabbit is a good source of meat and can contribute to filling the gap between the production and consumption of animal protein. Nevertheless, rabbit is susceptible to heat stress (HS) conditions (2, 3). This change in atmospheric temperature will generate heat stress conditions in tropical and subtropical regions like the south of the Mediterranean, especially in the open production system, which is the most common farm type in Egypt (4). Rabbit is sensitive to high environmental temperatures and has a low capacity to cope with high ambient temperatures (5), which negatively affect health, behavior, physiology, reproduction, welfare, meat quality, and production performance of rabbits (6, 7). Therefore, heat stress is associated with economic losses to the rabbit industry. Moreover, this impact could be measured either directly *via* the performance of the animal, or indirectly through biological stress markers (8, 9).

On the other hand, the responses to heat stress differ between animals of different genetic backgrounds, in which different breeds and lines respond differently to the different environments (10, 11). In general, local or indigenous animals have more capability for adaptation to the changes in local environmental conditions compared with exotic animals, this can be attributed to the genetic compositions that enable them to adapt the local environmental conditions (12, 13). To cope with the rapid changes in the environmental conditions, a better understanding of the role of G × E interaction in environmental adaptation is needed which is one of the main components of robustness. The G × E interaction is observed when the effects of the environmental factors differ from one breed to another, and the presence of G × E interaction may result in the re-ranking of genotypes (14), which makes one breed more sensitive to thermal stress than other. Moreover, in cattle, Misztal et al. (15) indicated that the selection for milk production, without considering the HS, will produce animals more susceptible to HS. With the desire for lines for robustness in the rabbit industry, an Egyptian Hispanic project developed a long and extensive genetic research program to develop new lines of interest for both extensive and intensive rabbit production. During this project, the Spanish maternal line (Line V), which is adapted to hot conditions, was crossed with local Egyptian rabbit breeds to produce lines that maintain a reasonable productive capacity and adaptation to heat. By this project, three rabbit lines were founded called Alexandria, Moshtohor, and APRI (16–18).

In rabbits, reproductive performance of does is important, and litter traits are the most important traits, which affect the economic benefits of rabbit production that provide the needed animal protein with low capital outlay and time (7). In this way, Cartuche et al. (19) reported that feed conversion rate during the fattening period, litter size, weaning survival, fattening survival and daily gain during the fattening period are the most economic traits in rabbits. These traits are severely affected when rabbits are subjected to heat stress conditions. Therefore, the purpose of this study was to compare the genetic response of Egyptian maternal line and exotic rabbit breed under normal and heat stress conditions.

Materials and methods

The study was carried out at the experimental rabbit farm of the Faculty of Agriculture, Kafrelsheikh University (Kafr Elsheikh, Egypt). This study was conducted after the approval of the Animal Care and Ethics Committee of Kafrelsheikh University (approval number, KFS1345/10) and also approved by IACUC at Cairo University (CU/II/F/19/19).

Animals and management

The animals used in the current study belonged to line APRI and New Zealand White breed (exotic breeds raised at the commercial level in Egypt many years ago). APRI line is an Egyptian maternal line developed in 2008, by crossing bucks of Baladi Red rabbits (a local breed) with does of V line rabbits (18). After its foundation, the generated rabbits were then selected for weaning weight (20).

The first mating of the does was done around 18 weeks of age. The does were then served 10–12 days post kindling by its assigned buck. On day 12 post-mating, the does were palpated to detect pregnancy, and non-pregnant does were returned to a new mating. The mating system was designed to avoid inbreeding. The maximum number of weekly services allowed to a buck was two. Five days before the expected day for kindling, the nest boxes were prepared. Litters born were examined and recorded for total born and number born alive. The litter born stayed with their mothers, without fostering, for about 35 days the young rabbits were weaned and then individually identified by tattooed number in the ear.

All rabbits were fed *ad libitum* commercial pelleted diet (17.5% crude protein, 15%—16% crude fiber, 2.5% either extract, 0.6% minerals mixture, 67.4% soluble carbohydrates, and 2300:2500 kcal/kg diet). Water was also provided *ad libitum* from nipple drinkers. The rabbits were housed in cages, where 100 cages were distributed equally for each rabbit line. Sixty cages were used to raise does under the normal environmental temperatures (NC), with an average of 18–26°C. Whereas, the heat-stress room included 40 cages, to keep the does under heat conditions (HC, 33–37°C). The heat conditions were applied for 7 h daily from 9:00 to 16:00, where it peaked daily temperatures during the summer season in Egypt, followed by the daily temperature cycle.

At first mating age, 100 does were used in this experiment, females were distributed almost the same between the two genetic groups, and animals were housed in individual cages. When one of the does died or was culled, it was replaced by another doe of the same genetic line. After weaning, during the fattening period (i.e., from 28 to 63 days of age), the kits produced under each environmental condition were grown under the same condition as their mothers. All weaned rabbits (2037 rabbits) were housed in collective cages (50 × 60 × 30 cm) of six animals in the same environmental conditions (normal or heat stress) until they reached the marketing age (around 63 days).

Traits and statistical analyses

The considered traits were litter traits including total born (TB), number born alive (NBA), number weaned (NW), number marketed (NM), in addition to growth traits including individual body weights [at weaning (BW_{28}), at 42 days of age (BW_{42}), at marketing age (BW_{63})], average daily weight gain [from weaning to marketing (ADG_{28-63}), from weaning to 42 days of age (ADG_{28-42}), from 42days of age to marketing (ADG_{42-63})], daily feed intake (calculated by dividing total feed consumption by the number of animals in each cage, and to avoid the biased calculation for feed intake, if an animal died in a cage during any week, the feed intake was calculated immediately including the dead and live animals) was calculated from weaning to marketing (DF_{28-63}), from weaning to 42 days of age (DF_{28-42}), from 42 days of age to marketing (DF_{42-63}), and feed conversion ratio during the fattening period (FC_{28-63}).

All the obtained data were subjected to analysis, the univariate mixed model was fitted to a total of 504 parities for APRI and NZW were analyzed.

Litter traits were analyzed using the following univariate mixed model:

$$Y_{ijkl} = LRYS_i + PS_j + p_k + e_{ijkl},$$

where Y_{ijkl} is the record l^{th} of litter traits being analyzed (TB, NBA, NW and NM), corresponding to the k^{th} doe which was in the physiological status j^{th} and belongs to the line-room-year-season combination i^{th}; $LRYS_i$ is the fixed effect of the line-room-year-season combination [combination of each line by room by year season (every 3 months): 10 levels]; PS_j is the fixed effect of the doe physiological state (three levels depending on the parity order and lactation state at mating, one is for nulliparous does, two for primiparous, and three for multiparous lactating); p_k is the random permanent environmental plus non-additive genetic effects of the doe; and e_{ijkl} is the random residual of the model.

The model for growth traits was:

$$Y_{ijkl} = LRYS_i + OP_j + \beta(NBA)_k + lo_l + e_{ijkl}$$

where: Y_{ijkl} is the record of the growth trait of animal l; $LRYS_i$ is the effect of line-room-year-season combination, line of animal l and the year-season of parity (one year-season every 3 months: nine levels for all lines); OP_j is the effect of the order of parity (five levels: 1^{st}, 2^{nd}, and $>2^{nd}$), NBA_k is the number of born alive in the litter in which the young rabbit was born and β is the regression coefficient on this covariate; lo_k is the random effect of the litter in which the animal was born and e_{ijkl} is the residual effect. Sex effect was not included because sexual dimorphism in rabbits is thought to either not exist or to arise only late in life (21). The prior distribution for the permanent environmental effect (**p**) and litter effect (**lo**) were $\mathbf{p} \sim N(0, \mathbf{I}\sigma_p^2)$ and $\mathbf{lo} \sim N(0, \mathbf{I}\sigma_{lo}^2)$, respectively, where **I** is an identity matrix.

Feed intake and feed conversion data were analyzed using the following model:

$$Y_{ijkl} = LRYS_i + \beta(N)_j + e_{ijk}$$

Where, Y_{ijkl} is the record trait; $LRYS_i$ is the effect of line-room-year-season combination; N_k is the number of young rabbits per cage β is the regression coefficient on this covariate and e_{ijk} is the residual effect.

A linear model was used to analyze the studied traits to test the significant differences between the levels of fixed effects and the probability of the estimated contrast being different than 0 by using the program Rabbit developed by the Institute for Animal Science and Technology (València, Spain) applying the previous model without the random effects. After some exploratory analyses, results were based on Markov chain Monte Carlo chains consisting of 60,000 iterations, with a burn-in period of 10,000, and only 1 of every 10 samples was saved for inferences.

Results and discussion

Descriptive statistics including overall means and standard deviations of the studied traits are presented in Table 1. All the entire data was considered.

Effects of genotype and heat conditions

The observed least square means of litter and growth traits for genotypes and environmental conditions are presented in Table 2. The present results are similar to production levels previously reported for studies carried out in similar production conditions by APRI line (16–18). As expected, litter traits were negatively impacted by heat conditions, which are in agreement with most of the studies on this subject in environmental conditions. In both genotypes, TB, NBA, and NM were reduced with relevant values due to heat stress (ranged from 15 to 25%). Concerning the effects of heat conditions on the growth traits, as expected, heat stress remarkably affected all growth traits, with important reductions. The observed reduction in body weight was associated with heat-stress conditions at all ages. The reduction was 3% at weaning age, and increased with age, reaching 12 and 14% at 42 and 63 days of age, respectively. Bassuny et al. (22) reported a reduction of 7% in body weight at 28 days of age due to the increase in ambient temperatures. In addition, there was a negative effect of heat stress on the average daily gain and daily feed intake. The observed reduction in ADG ranged from 18 to 23%. Furthermore, HC environment limited the feed intake (−14% to −21%) of animals and negatively affected feed conversion (+5%) compared with NC. Similar results were obtained in previous studies (16, 23–25). The deleterious effect of heat stress on the reproductive traits in mammals is well documented (26, 27). Since rabbits are susceptible to heat stress conditions (3), lower litter traits are usually associated with an increase in ambient temperatures (28, 29).

TABLE 1 Descriptive statistics [mean, standard deviation (SD), and extreme values] of the studied traits.

Traits	Number	Mean	SD	Minimum	Maximum
TB	504	8.78	2.80	1	16
TBA	504	7.88	2.96	0	14
NW	504	6.69	2.90	0	13
NM	504	5.69	2.90	0	13
BW$_{28}$	2037	575.72	151.83	138.16	1020.16
BW$_{42}$	1847	992.19	198.39	409.28	1690.53
BW$_{63}$	1672	1620.46	282.86	712.70	2615.32
ADG$_{28-42}$	2037	29.93	8.61	−8.73	53.87
ADG$_{42-63}$	1672	29.92	5.89	8.19	58.90
ADG$_{28-63}$	1672	29.91	6.59	5.17	55.03
DF$_{28-42}$	340	67.75	18.25	21.14	128.39
DF$_{42-93}$	340	121.67	21.93	28.61	214.91
DF$_{28-63}$	340	113.44	20.58	30.51	182.96
FCR$_{28-63}$	340	3.83	0.39	1.81	11.21

TB, total born; NBA, number born alive; NW, number weaned; NM, number marketed; BW, body weight (g); ADG, average daily gain (g/day); DF, daily feed intake (g); FCR, feed conversion ratio (g feed/ g body weight); SD, standard deviation.

Independent of environmental conditions, APRI rabbits showed important increases in TB, NBA, NW, and NM of 10, 8, 11, and 10% compared to NZW, respectively (Table 1). The superior reproductive traits of APRI rabbits could be due to the foundation of this line which was founded by the crossing between Red Baladi with the Spanish maternal line (V line) selected for litter size at weaning which has high values for litter traits (30). Moreover, after the APRI line foundation, it has been selected for litter weight at weaning (20).

Moreover, the NZW rabbits showed significantly higher body weights at all ages compared with APRI rabbits. It is worth mentioning that although the APRI line had fewer

TABLE 2 Effects of heat conditions and genotypes on the litter and growth traits^{a}.

Trait	Environmental conditions		Genotypes	
	Normal	Heat stress	APRI	NZW
TB	9.21 ± 0.17	7.74 ± 0.21	8.89 ± 0.19	8.06 ± 0.21
NBA	8.34 ± 0.19	6.82 ± 0.26	7.88 ± 0.33	7.28 ± 0.23
NW	7.00 ± 0.20	5.95 ± 0.23	6.80 ± 0.20	6.13 ± 0.23
NM	6.18 ± 0.19	4.63 ± 0.26	5.66 ± 0.21	5.16 ± 0.24
BW$_{28}$	586.78 ± 4.64	568.05 ± 5.48	557.76 ± 4.24	598.50 ± 5.13
BW$_{42}$	1049.8 ± 9.95	922.1 ± 12.43	901.9 ± 9.9	1070.0 ± 9.66
BW$_{63}$	1727.8 ± 12.79	1476.5 ± 19.34	1490.9 ± 12.86	1713.2 ± 19.04
ADG$_{28-42}$	33.19 ± 0.48	25.30 ± 0.91	24.69 ± 0.48	33.85 ± 0.66
ADG$_{42-63}$	32.31 ± 0.30	26.46 ± 0.69	28.06 ± 0.39	30.62 ± 0.46
ADG$_{28-63}$	32.69 ± 0.34	25.93 ± 0.74	26.75 ± 0.62	31.91 ± 0.83
DF$_{28-42}$	73.8 ± 0.86	58.4 ± 1.80	57.3 ± 1.16	74.9 ± 1.49
DF$_{42-63}$	127.4 ± 1.16	109. 2 ± 3.12	117.0 ± 2.06	120.6 ± 2.30
DF$_{28-63}$	120.4 ± 1.03	100.0 ± 2.60	105.3 ± 1.58	116.9 ± 2.01
FCR$_{28-63}$	3.72 ± 0.02	3.92 ± 0.05	3.98 ± 0.02	3.66 ± 0.03

TB, total born; NBA, number born alive; NW, number weaned; NM, number marketed; BW$_{28}$, body weight at 28 days of age (g); BW$_{42}$, body weight at 42 days of age (g); BW$_{63}$, body weight at 63 days of age (g); ADG, average daily gain (g/day); DFI, daily feed intake (g); FCR, feed conversion ratio (g feed/ g body weight). ^{a}The presented vales are posterior least-squares means ± SE.

TABLE 3 Response of genotypes to environmental conditions for litter and growth traits.

Traits	APRI			NZW		
	Normal vs. heat	HPD$_{95\%}$	P	Normal vs. heat	HPD$_{95\%}$	P
TB	1.92	1.11, 2.75	1	0.90	0.03, 1.87	0.97
NBA	2.22	1.30, 3.05	1	0.54	−0.38, 1.58	0.86
NW	1.84	1.03, 2.75	1	0.09	−0.89, 1.01	0.58
NM	1.99	1.11, 2.85	1	1.00	−1.16, 0.91	0.97
BW$_{28}$	4.78	−10.09, 19.57	0.72	37.59	21.07, 55.5	1
BW$_{42}$	84.93	66.39, 102.1	1	183.18	164.2, 202.7	1
BW$_{63}$	230.62	203.7, 255.6	1	281.67	252.6, 311.5	1
ADG$_{28-42}$	5.74	5.00, 6.47	1	10.39	9.55, 11.26	1
ADG$_{42-63}$	6.95	6.36, 7.52	1	4.69	4.02, 5.36	1
ADG$_{28-63}$	6.47	5.88, 7.12	1	6.98	6.27, 7.64	1
DF$_{28-42}$	11.11	9.51, 12.79	1	20.87	19.01, 22.74	1
DF$_{42-63}$	19.22	17.18, 21.11	1	15.83	13.57, 18.12	1
DF$_{28-63}$	22.6	17.65, 24.69	1	20.78	18.51, 23.1	1
FCR$_{28-63}$	−0.23	−0.27, −0.18	1	−0.13	−0.18, −0.08	1

TB, total born; NBA, number born alive; NW, number weaned; NM, number marketed; BW, body weight (g); ADG, average daily gain (g/day); DF, daily feed intake (g); FCR, feed conversion ratio (g feed/ g body weight). HPD$_{95\%,}$ Marginal Posterior highest density region covering 95% of the density; P, probability of the difference being greater than zero.

values in body weight measurements, it was superior in the reproductive traits measurements, this is mainly due to the negative phenotypic and genetic correlations between growth and reproductive traits. Ezzeroug et al. (31) reported highly negative genetic correlations between growth and litter traits in rabbits, where weaning weight was negatively correlated with litter size at birth (−0.85), number born alive (−0.92), and litter size at weaning (−0.85). Furthermore, NZW rabbits were significantly superior to the APRI line in average daily gain and daily feed intake during all periods. The average daily gain in rabbits is a very important trait in rabbit breeding, and consider one of the selection criteria in the paternal line (32, 33). Drouilhet et al. (34) reported that ADG has a high genetic correlation with BW at 63 days of age (0.81), and a low correlation with weaning weight (-0.2). Marco-Jiménez et al. (35) commented that the ambient temperature needed for rabbits is ranging from 14 to 22°C, and exceeding this range will prone rabbits to stress affecting their litter traits and the growth of young rabbits negatively. They observed significant differences between reproductive performance and body weight traits of young rabbits reared under normal conditions and those of animals under HS conditions. Within this context, Boddicker et al. (36) observed that HC during the first half of gestation may have a negative consequence on postnatal offspring growth and development. Furthermore, Habeeb (37) and Bakr (38) observed that heat conditions had a negative and significant effect on daily feed intake and body weight at weaning. Decreased feed intake results in a lower supply of nutrients, thus reducing the weight and growth rate of rabbits. This effect is in agreement with that observed by Savietto (39) and Savietto et al. (24) where heat stress reduced dry matter and digestible energy intake encountered in rabbits. Jimoh et al. (40) reported that heat conditions decreased feed intake which results in a lower supply of nutrients, thus reducing body weight, growth rate, and reproduction and increasing mortality in rabbits. Similar results, for growth traits, were obtained by Ragab et al. (41).

To evaluate the response of genotypes to environmental changes, the contrasts of each genotype under the two environmental conditions for litter traits and growth performance were calculated (Table 3). Despite their higher litter traits, APRI does showed higher reductions due to HC compared with NZW for all litter traits with relevant values, including TB (1.92 in APRI compared with 0.90 in NZW), NBA (2.22 vs. 0.54), NW (1.84 vs. 0.09), and NM (1.99 vs. 1.00), these differences indicate that the two breeds responded differently to HC, and on the whole, APRI rabbits were more sensitive to HC (the probability of contrasts being higher than 0 was 1 in the most traits) high than NWZ rabbits which seemed to be more adapted to climatic stressors. Moreover, the observed reductions in body weight were associated with HC and increased with age. The reductions were higher in NZW compared with APRI rabbits for BW at 28, 42, and 63 days of age, ADG from 28 to 42 and from 28 to 63 days of age. Similar responses for daily feed intake were observed by the two genotypes. The different responses and environmental sensitivity of genotypes were described previously by many authors (42–44), which could be due to the capacity of does to manage their body reserves usage in providing rabbits with adaptive and maintain their litter size during a stress period.

TABLE 4 Effects of genotype by heat interaction on litter and growth traits.

Traits	Normal conditions			Heat conditions			Pc
	APRI vs. NZW	$HPD_{95\%}$	P	APRI vs. NZW	$HPD_{95\%}$	P	
TB	1.18	0.47, 1.89	1	0.15	−0.92, 1.13	0.61	0.97
NBA	1.19	0.54, 1.93	1	−0.50	−1.57, 0.53	0.82	0.89
NW	1.26	0.59, 1.99	1	−0.48	−1.52, 0.53	0.82	0.79
NM	0.87	0.11, 1.65	0.99	−0.13	−1.16, 0.91	0.6	0.65
BW_{28}	−50.47	−64.03, −35.16	1	−17.66	−36.51, 0.24	0.97	0.73
BW_{42}	−203.9	−219.0, −186.4	1	−104.80	−126.6, −84.03	1	0.66
BW_{63}	−237.6	−262.9, −215	1	−186.54	−217.9, −155.4	1	0.93
ADG_{28-42}	−10.93	−11.53, −10.12	1	−6.23	−7.11, −5.31	1	0.93
ADG_{42-63}	−1.63	−2.18, −1.08	1	−3.90	−4.59, −3.17	1	0.68
ADG_{28-63}	−5.34	−5.92, −4.79	1	−4.83	−5.55, −4.11	1	0.70
DF_{28-42}	−21.17	−22.75, −19.58	1	−11.44	−13.32, −9.36	1	0.76
DF_{42-63}	−1.64	−3.51, 0.14	1	−5.03	−7.58, −2.69	1	0.71
DF_{28-63}	−11.88	−13.82, −10.08	1	−10.19	−12.60, −7.75	1	0.69
FCR_{28-63}	0.25	0.20, 0.29	1	0.39	0.33, 0.45	1	0.78

TB, total born; NBA, number born alive; NW, number weaned; NM, number marketed; BW, body weight (g); ADG, average daily gain (g/day); DF, daily feed intake (g); FCR, feed conversion ratio (g feed/ g body weight). $HPD_{95\%}$, Marginal Posterior highest density region covering 95% of the density; P, probability of the difference being differ than zero; Pc, probability of the difference between the two contrasts being differ than zero.

Genotype by heat conditions interaction

Genotype by environmental conditions interaction can be observed when the difference in the performance of two genotypes depends on the environment in which the performance is measured. Therefore, G × E interaction plays an important role in the adaptation and resilience of genotypes. Table 4 shows the effects of genotype × heat conditions interaction on litter and growth traits. The presented differences between APRI and NZW genotypes under normal conditions, confirm the goodness of crossing local breeds with selected lines, such as V-line that has been selected in Spain in a climate similar to that of Egypt and used in the development of APRI line. Moreover, the obtained results indicate interaction effects on all litter traits. It can be noticed that the differences between the two lines under NC were reduced under HC, which reflect the different response of genotypes to environmental changes. Under NC, APRI litters showed relevant higher values for TB (+1.18), NBA (+1.19), NW (+1.26), and NM (+0.87) than NZW litters when reared under the same conditions, while there were relevant reductions in litter traits of APRI line in HC with significant differences (different HPD_{95} and probability of the difference between the two contrasts being greater than zero is close to 1) in NBA and NW, in which the NZW compensated for the previous differences under NC and showed higher values (0.5 and 0.48, respectively) compared with APRI litters. These results are signs of the G×H interaction and confirmed that the environmental conditions produce different physiological consequences on female rabbits, whereas the NZW breed was more flexible and less sensitive to heat stress due to the fact that NZW has been colonized and raised for many years under Egyptian conditions. The superiority of the APRI line is due to the foundation and selection process after its foundation. But, as confirmed in previous studies, does at higher production levels are more sensitive to heat stress. Marai et al. (28) reported that heat stress has negative effects on the growth of embryos, pregnancy rate, litter size, litter weight, and milk yield of does. In a similar study in rabbits, Savietto et al. (24) observed important genotype by environment interaction, where the does of the V line (which was used to develop the APRI line) showed higher litter traits than does of LP lines and the opposite occurred under heat stress conditions.

Genotype by heat condition interaction effects on growth, feed intake and feed conversion traits are presented in Table 4. Under normal conditions, NZW does had higher body weight and growth rates at different ages than APRI rabbits, these differences were significantly different than 0 to with values of 50.47 g at weaning and 237.6 g at marketing with ADG of 10.89 g/day at the first two weeks after weaning. However, these differences were changed when animals of the two lines were subjected to heat stress for BW_{28}, BW_{42}, BW_{63}, ADG_{28-42}, DF_{28-42} and DF_{28-63}, while these differences were increased for ADG_{42-63}, DF_{42-63} and feed conversion during the whole fattening period. As commented before, these changes of differences between the two lines, in the different environment conditions, are a good indicator for G × H, and the probability of the difference between the two contrasts being greater than zero is higher in the most traits. Therefore, it is important to take into account the environmental conditions when we will rear a line or genotype. In the same context, Sánchez et al. (45) concluded that the selection of animals should be realized under the conditions in which they will produce. Therefore, under tropical conditions, the genetic improvement of rabbits by crossbreeding between local and exotic breeds should be followed by selection in conditions similar to commercial production conditions in order to select well-adapted animals to tolerate heat stress and increase their ability to survive, grow and reproduce.

Conclusion

Crossbreeding between local and exotic breeds helped to improve rabbits' performance. The observed results confirmed the existence of G × H interactions that affected growth and litter size traits, as different genotypes responded differently under different environmental conditions. Our results also indicated that heat stress, as a part of the environmental factors, has negatively impacted the gestation and fattening period in both genotypes, and therefore, G × H interaction should receive more attention whenever designing breeding programs.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Animal Care and Ethics Committee of Kafrelsheikh University, Egypt (license number, KFS1345/10).

Author contributions

MR: formal analysis, methodology, validation, supervision, and writing—original draft. IE: conceptualization, resources, methodology, and writing. HY: writing—review and editing. MA: visualization, formal analysis, and writing—original draft. MH: methodology, validation, investigation, and writing—review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Thermoregulation mechanisms and perspectives for validating thermal windows in pigs with hypothermia and hyperthermia: An overview

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Specific anatomical characteristics make the porcine species especially sensitive to extreme temperature changes, predisposing them to pathologies and even death due to thermal stress. Interest in improving animal welfare and porcine productivity has led to the development of various lines of research that seek to understand the effect of certain environmental conditions on productivity and the impact of implementing strategies designed to mitigate adverse effects. The non-invasive infrared thermography technique is one of the tools most widely used to carry out these studies, based on detecting changes in microcirculation. However, evaluations using this tool require reliable thermal windows; this can be challenging because several factors can affect the sensitivity and specificity of the regions selected. This review discusses the thermal windows used with domestic pigs and the association of thermal changes in these regions with the thermoregulatory capacity of piglets and hogs.

KEYWORDS

hypothermia, heat stress, infrared thermography, piglet, hog

Introduction

The regulation of body temperature in homeotherms is ensured by mechanisms of thermolysis and thermogenesis (1). Thermoregulatory adjustments can be induced by changes in environmental temperature and various physiological situations, including age, fasting, food intake, and stress conditions (2–4). Therefore, the evaluation of body temperature represents a valuable tool to monitor animals' physiologic status, welfare, and stress responses. Under stressful conditions, the activation of the sympathetic system and the hypothalamic-pituitary-adrenal axis (HPA) releases effector hormones such as catecholamine and glucocorticoids, respectively. Stress-induced hyperthermia, a condition triggered by the sympathetic system, consists of increasing core body temperature and the consequences on the thermoregulatory mechanism of animals (5).

The thermoregulation mechanisms of domesticated swine face challenges during all stages of growth due to certain anatomical-physiological characteristics of this species (6) that make these animals sensitive and susceptible to neonatal hypothermia, or hyperthermia in adulthood (7, 8), events that can trigger not only physiological alterations but also a predisposition to pathologies and mortality due to thermal stress (9). This impact productive and reproductive parameters and the quality of meat, milk, and other products of animal origin (10). The anatomical features that influence thermoregulation in swine include fine hair that helps reduce heat loss to a small degree under exposure to cold climates (11) and protects the skin from direct solar radiation in hot environments (12). The lack of functional sweat glands (13, 14) and low amounts of brown adipose tissue (BAT) at birth also impact the thermal response of domesticated pigs to diverse stimuli (15). However, some of these characteristics –like the lack of hair and heat-insulating fat behind the ears and near the sternum– make certain body regions candidates as thermal windows that can reflect heat exchange between the animal and its environment (16).

Infrared thermography (IRT) is used to measure the amount of heat that a body radiates (17, 18) as a result of vasomotor control that dilates or constricts peripheral capillaries (8). The discovery of this relationship suggested that IRT could be useful in veterinary medicine to evaluate circulatory changes caused by inflammatory or infectious processes, wounds, thermal stress, and stressful events (19). IRT could help perform detailed analyses of thermoregulation and the compensation mechanisms involved in returning to a state of homeothermy. However, achieving this goal requires identifying adequate anatomical regions and the information they can provide depending on their vasculature and location.

To date, the ocular, auricular, and nasal regions are most often employed to quantify the heat that animals dissipate or conserve (17, 20, 21). In species like swine and canines, appendicular regions are recognized as being more sensitive to temperature decreases due to prominent blood vessels that contract to prevent active heat loss (7, 22). IRT can detect changes of this kind in the surface vasculature of the skin, depending on the anatomical components of the region (15, 23). The use of IRT is, however, still controversial because studies have shown variability in indices of sensitivity and specificity, two parameters that can be affected by diverse factors, both internal (e.g., presence of hair, bare skin, and hair length, among others) (24), and external, that limit its use in veterinary medicine (11, 24).

This review aims to analyze (i) the thermoregulation mechanisms of piglets and adult pigs (hogs); (ii) the thermoregulatory adjustments that pigs of different ages confront; (iii) evidence for the use of various thermal windows with domesticated swine; (iv) the factors that affect their validation; and (v) possible limitations on applying IRT with this species.

Review methodology

The search was conducted in Web of Science, Scopus, PubMed, and CAB Reviews databases. The keywords used to find the literature were: "pig thermoregulation", "pig surface temperature", "infrared thermography", "pig thermal windows", and "infectious or inflammatory disease". The inclusion criteria for the articles and books cited in the present review ($n = 174$) were those regarding the changes in surface temperature in domestic pigs in response to environmental, physiological, or biological stressors. In addition, articles between the years 2000 and 2022 were considered. Studies that did not meet the inclusion criteria or reported the use of infrared thermography in other species were excluded. Figure 1 describes the overall methodology for this review.

Thermoregulatory contrasts between piglets and hogs, anatomical-physiological aspects, and differences in thermo-stabilization

The anatomical-physiological particularities that affect thermoregulation in pigs include scarce fine hair (25), apocrine glands distributed throughout the body, and the absence of eccrine sweat glands (26). However, these characteristics differ in distinct stages of development and growth. For example, due to the absence of BAT and microfibril mass and low fat and glycogen reserves, the piglet's thermoregulatory immaturity (27) helps explain why newborns are vulnerable to environmental challenges when the temperature is around 18–20°C. Piglets require the activation of compensatory mechanisms such as

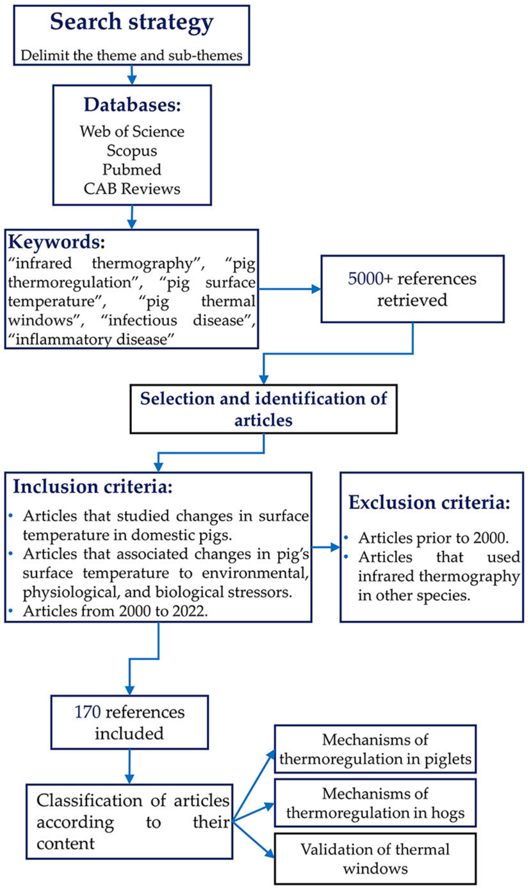

FIGURE 1
Flow chart showing the criteria used during the search of scientific articles for the overview.

shivering thermogenesis, piloerection, and vasoconstriction in the first hours and days of life to impede heat loss from vital organs.

In hogs, in contrast, the disposition of adipose tissue is considered a factor that affects their heat-dissipating capacity, especially during exposure to high environmental temperatures. Hogs rely on peripheral vasodilation and panting to dissipate excess heat. However, due to their few functional sweat glands and the subcutaneous layer of backfat that impedes heat loss, they are susceptible to thermal shock that can also lead to death (Figure 2) (28–30).

Regarding behavioral thermoregulatory responses such as panting, pigs use a wide repertoire of changes to maintain their core temperature. For example, newborns huddle as the primary method to prevent heat loss, representing around 61.2–73.8% of their time (31). In sows, responses to cold environments include postural changes (sternal recumbency) (32), decreased locomotion (33), and an increase in food and water intake (34). In contrast, wallowing is the first reaction, when exposed to heat stress, and reduction in feed intake and motivation to search for warm places are frequently observed (27, 35).

A significant difference between piglets and hogs is the thickness of the skin and its supporting structures. The dermis and subcutaneous tissue of hogs measures around 3 mm, 21–26 μm (36). The thickness of the epidermis varies from one anatomical site to another, but ranges around 30–140 μm, which can be considered a barrier against cold temperatures (37). The skin is thicker and has numerous crests in the shoulders and snout but is thin around the dorsum and hips (38). In contrast, studies of one-day-old piglets report a skin thickness of 0.3–1.4 mm, a stratum corneum of 11–48 μm, and an epidermis of 33–68 μm (39). This influences their thermoregulatory capacity, tolerance to extreme climates, and interaction with the vascular anatomy that, in neonates, is similar to that of human skin (36).

Two other factors related to the skin are glandular structures and the presence of hair follicles. Piglets have a capillary: follicle ratio of 730/cm^2, but reports on hogs estimate just 10.16 follicles/cm^2 (36). The importance of these structures during the first days of life lies in their heat-conserving function generated by piloerection. In this regard, while swine are considered a species with scarce hair distribution, a study of Hampshire hogs weighing 100–120 kg reported the union of an arrector pili muscle with the outer root sheath (40) that supports the effects of piloerection and heat retention.

Another important characteristic that distinguishes swine from other species is the structure of their sweat glands. Unlike human sweat glands of the eccrine type and function mainly to cool the skin, pigs have apocrine glands that end in the epidermis, near hair follicles (41). They have been loosely described as simple, though the ones in the snout are considered more complex, as they are coiled and distributed in a ratio of 1:1 with respect to the follicles (40, 41).

Adipose tissue is another essential element for thermoregulation that differs significantly among swine of distinct ages. Neonate piglets have small fat deposits (around 15 g Kg^{-1}) available in the abdomen and on the back (42), limiting their capacity to produce heat through food consumption (43, 44). Research has shown that piglets –which lack BAT at birth– use the process called "browning of white adipose tissue" (WAT) to perform non-shivering thermogenesis (45). Mersmann et al. (46) reported that the piglet's organism has 4% fat immediately postpartum. Other authors mention just 1%, but this can increase to 16.4% during the first 24 days of life (47, 48). In pigs, the fat-producing process begins in the fetal stage. From 0 to 68 days of gestation, the fat content has been reported to be around 0.06 g/d, but increases to 1.09 g/d at day 69 (47). As the age of the animal advances, the fat percentage increases to 9.3–24.3% (49), with an average of 30% of extra-muscular fat (44). This means that mortality by hypothermia is not a major challenge in hogs as it is in piglets. Instead, the increase in the thickness of the layer of adipose tissue reduces emissions of cutaneous heat into the environment through

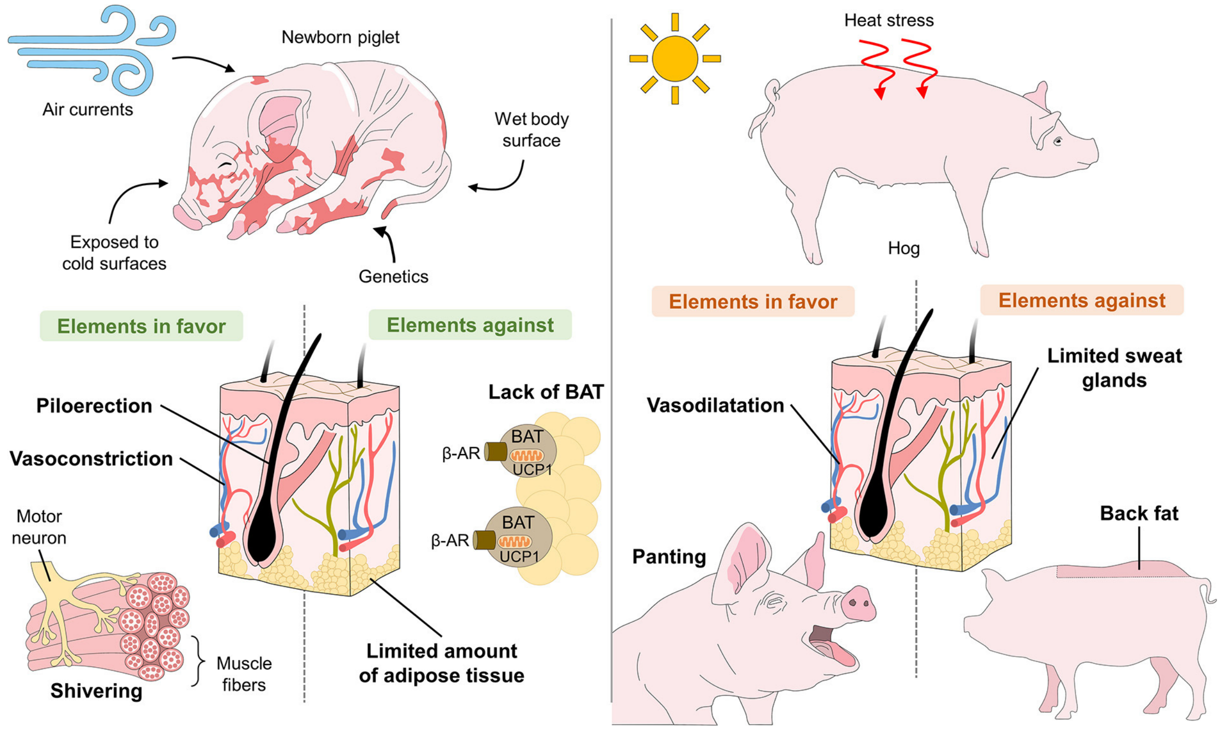

FIGURE 2
Anatomical-physiological challenges of piglets related to hypothermia and of hogs related to hyperthermia.

vasodilatation. This situation becomes complicated, however, when we consider the reduced functionality of sweat glands in swine, and the limited effectiveness of thermoregulatory behaviors like panting. These elements can cause imbalances when hogs are exposed to high environmental temperatures of 24–30°C and leave them susceptible to hyperthermia (known as an elevation of body temperature caused by an imbalance of thermoregulatory mechanisms that leaves them unable to eliminate heat at the same rate as it is produced) or heat stroke (50, 51).

Regarding the characteristics of the muscle required to perform shivering thermogenesis, skeletal muscle fibers are classified into "slow-twitch" (type I) and "fast-twitch" (type II with subsequent subtypes IIa, IIb, IIx), according to the isoform of myosin heavy chain and the contraction speed, where type IIb is the fastest (52). Vanden Hole et al. (53) determined that the percentage of type II muscle fibers in the pelvic limbs of piglets represents 95.58% of total muscle fiber. It has been reported that piglets have predominantly secondary muscle fibers, that these correlate positively with muscle weight ($r = 0.39$) (54), and that low temperatures of 15 ± 1°C in 21-day-old piglets increase the expression of type IIa fibers (55). In hogs, an immunohistochemical study of muscle tissue samples showed a higher proportion of type-II muscle fibers (56), which can increase quadratically with parity (3^{rd} birth, $r2 = 0.44$; 4^{th} birth, $r2 = 0.54$). Other studies of animals weighing 100 kg showed that the longissimus muscle expresses three isoforms of type II muscle fibers but not IIb, which were not observed in Lefaucheur et al.'s work (56). These fibers' distribution consists in islets of type I fibers surrounded by peripherally-located IIa- and IIb-type fibers (56). In hogs, the percentages of type I, IIa, and IIb fibers are 4, 8, and 88%, respectively (57), lower than the figures for piglets. The importance of the predominance of certain fibers is that while type II are fast, they require ATP production to initiate non-shivering thermogenesis, a substrate that may be limited in piglets. In contrast, type I rely on oxidative metabolism to thermoregulate (58), and a shift toward oxidative muscle can occur in piglets (55). Finally, reduced muscle irrigation, added to the absence of microfibril mass that controls the potency of muscular contraction, also compromises the mechanism of shivering thermogenesis (43), leaving piglets susceptible to neonatal hypothermia.

Concerning the metabolic mechanisms that pigs use for thermoregulation, glycogen reserves in skeletal muscle (around 30–35 g Kg^{-1}) and liver are the main sources of energy in the first hours of life (43, 59). Glycogen concentrations in the skeletal muscle, front and hind legs, and liver of 32 neonates of a Topigs x German Pietrain breed were studied in the first 96 h of life. In that study, low birth weight in the piglets (body mass = 0.79 ± 0.26 kg) was associated with reduced

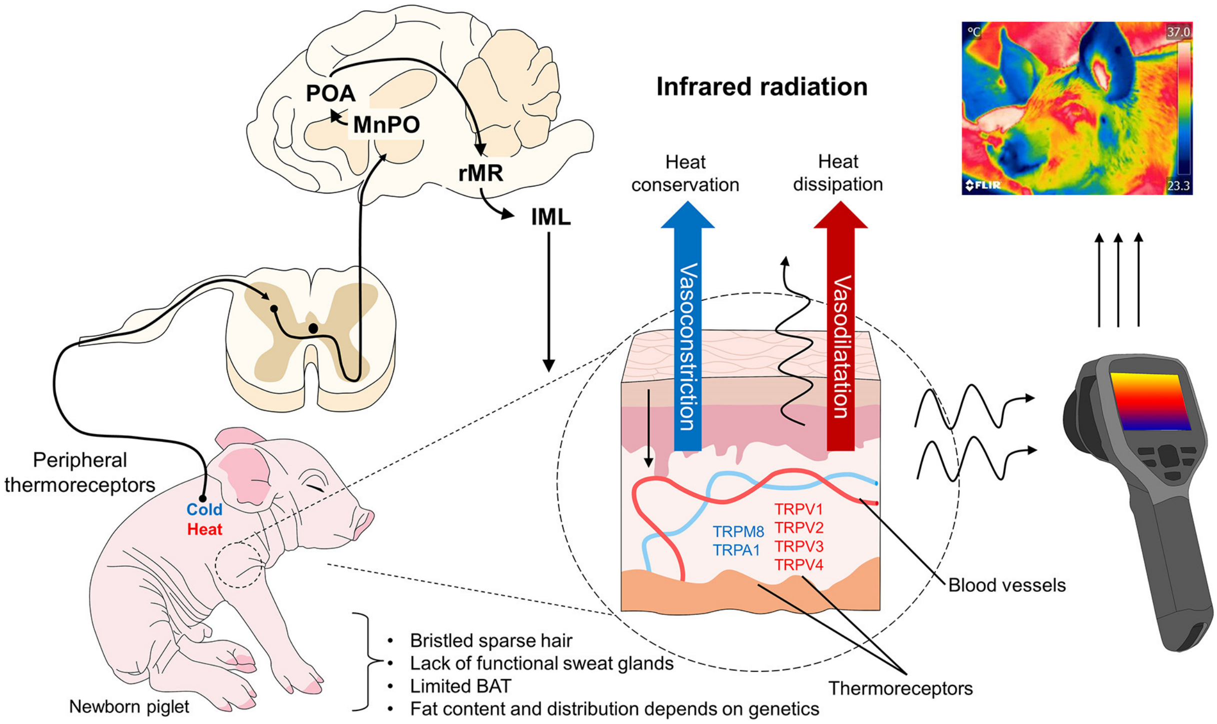

FIGURE 3
Thermoregulatory mechanism in domesticated swine. When peripheral thermoreceptors are activated to recognize cold or hot stimuli, the information is transmitted to the hypothalamus (MnPO) and other brain structures involved in the sympathetic control of peripheral blood vessels. When exposed to cold environments, these vessels vasoconstrict to conserve heat, but under exposure to hot temperatures, they vasodilate to dissipate heat. Both responses influence the amount of infrared radiation observable using techniques like IRT, though this also depends on the thermal window evaluated. IML, intermediolateral column; MnPO, median preoptic nucleus; POA, preoptic area; rMR, rostral medullary raphe; TRP, transient receptor potential vanilloid (V), melastatin (M), ankyrin (A).

glycogen utilization in the hind legs, as those reserves were not utilized until 8 h postpartum. Moreover, those newborns had as much as 50% less glycogen than normal-weight piglets (mass body = 1.37 ± 0.29 kg). An evaluation of the amount of glycogen in the liver showed that low-weight piglets did not utilize their reserves for 96 h, in contrast to the normal-weight ones that consumed half of that glycogen in the first 8 h of life ($P = 0.0238$) (60). Another energy reserve available at birth is glucose. Staarvik et al. (61) evaluated this in one-day-old piglets, finding average blood glucose concentrations of 5.48 mmol/l. Interestingly, they also found that male piglets had higher glucose levels than females, while those born in large litters had glucose concentrations as much as 0.07 mmol/l lower. Because glycogen and glucose are energy reserves that can be depleted quickly, consuming colostrum is essential for providing the energy newborns require for thermoregulation and the passive immunity needed to reduce the risks of low vitality (43, 59).

Recognizing the anatomical differences between newborn and adult pigs allows us to determine the resources each age group utilizes when exposed to environmental challenges like thermal stress, and also helps identify the responses that occur when those mechanisms are activated. Moreover, this information serves as a guide for planning strategies to prevent, diagnose, and manage affectations of the thermoregulation of swine in relation to their physiological stage of development.

Thermoregulation in the newborn piglet

At birth and during the first 48 h of life, piglets rely on shivering thermogenesis as their principal thermoregulation mechanism (62) (Figure 3) to compensate for low environmental temperatures of 20–22°C (43, 63), which can cause a drastic drop in body temperature, perhaps as great as 2°C (15, 64). The placental fluids (including amniotic liquid) that cover piglets together with the high body conductance and the high specific surface area at birth worsen this temperature drop due to the evaporation of 50% of those liquids that usually occurs in the first 5–30 min post-birth (65, 66). Hence, the main challenge for piglets is hypothermia. Their immature thermoregulatory center also plays an important role during birth; in fact, neuroimaging studies suggest that their thalamus and hypothalamus do not reach maturity until week 5 of life (67). This immaturity added to their relative physiological and metabolic immaturity, helps explain the incapacity of neonate pigs to thermoregulate when facing environmental challenges (15).

Because they do not have a mature thermoregulatory center (67, 68), newborn piglets first mobilize energy reserves (glycogen, fat, skeletal muscle, in descending order) to produce heat and survive in the extrauterine environment (7). The lack of microfibril mass that controls the potency of muscle fiber contraction (56) and the predominance of secondary muscle fibers help piglets produce heat through a muscular contraction so they can remain metabolically active. This may confer a particular advantage for achieving thermoregulatory success, but only in a limited way. Causes of thermoregulatory incapacity in piglets include reduced muscular irrigation, low mitochondrial mass responsible for the oxidative potential of muscle and, hence, the energy supply, and the level of bioavailability of carbohydrates as an energy source that leads to a biochemical modification of the oxidation of non-structural fatty acids, compromising the mechanism of shivering thermogenesis and leaving piglets susceptible to neonatal hypothermia (43).

The total amount of fat in piglets at birth and usually 42% of the limited muscle glycogen reserves are used in the first 12 h of life to satisfy its energy requirements. Nonetheless, the expenditure depends on factors such as body mass and vitality since piglets with low vitality have less muscle volume and liver glycogen stored at birth (69). Therefore, for this species, colostrum intake is critical for their survival. Reports show that colostrum provides around 5.4% of fat and 2.0% of lactose as bioavailable carbohydrates that support the newborn's survival. However, colostrum also gives piglets passive immunity by supplying immunoglobulin G, substantially reducing susceptibility to infections by agents like *E. coli* (70, 71). Under these conditions, piglets that are born weak with body weights <800 g (72) are rarely able to ingest colostrum and milk, their two main sources of energy, because of (i) low energy reserves in their muscles that impede initial attempts to suckle, and (ii) insufficient insulation given their higher surface/body mass ratio that can lead to hypothermia (73).

- Inanition and hypothermia hinder locomotion, increasing the risk of crushing and death in the first 24 h post-birth. The piglet's organism may consume glycogen reserves in the liver or muscles during the first 6 h of life, striving to achieve thermoregulation despite hypoglycemia and limited glycogen reserves (74), but this can trigger metabolic acidosis, which that can end in coma or death by cardiac arrhythmia (7, 75). Studies show that piglets' 10–13% of perinatal mortality is due to underlying events like crushing and cold stress (43). Due to the reported correlation between piglet survival and the degree and duration of postnatal hypothermia (76), swine producers are interested in studies of hypothermia that may help prevent this condition that causes significant economic losses (77) as the leading cause of perinatal death (7, 78). The main threat to neonate piglets is a cold extrauterine environment that provokes active heat loss through evaporation and exposure to low temperatures and cold objects or fluids. Their limited thermoregulatory capacity exacerbates this due to scarce energy resources that are quickly depleted in such a challenging environment. Therefore, the timely recognition of hypothermia is crucial to avoid dire physiological consequences.

Thermoregulation of hogs at the slaughterhouse

In contrast to piglets, hogs may be exposed to elevated environmental temperatures that affect growth, food conversion, reproduction, health, and welfare. Though hogs have a mature thermoregulatory center, they are susceptible to heat stress because they have few functional sweat glands (30/cm^2) (79). Hogs radiate heat by convection and evaporation through vasodilatation by increasing their respiratory rate and changing postures (80). However, when temperatures exceed their body's thermoneutral zone (81), they cannot lose heat at the same rate as acquired. Stressful situations can also compromise the mechanism of peripheral vasodilatation and, hence, thermoregulation (82). Moreover, the effectiveness of the evaporative thermoregulatory response varies with the degree of humidity in the environment: the higher the humidity (50% or more), the less effective evaporative cooling will be. Hogs can suffer heat stress at lower temperatures than when the air is drier because less liquid evaporates from the respiratory tract and skin in humid environments (80).

It is important to understand that the magnitude of heat production and exchange in hogs depends, as well, on the stage of growth, gestation, lactation, diet, stocking density [1 *vs.* 2 m^2/pig (83)], the air movement index, and convection and conduction methods (80). Animals in stages marked by high metabolic activity (e.g., early growth, lactation) tend to be more susceptible to heat stress, so sows, boars, and finishing hogs weighing over 50 kg may begin to experience the adverse effects of heat stress at temperatures that barely exceed 20°C (79).

Another critical characteristic of hogs is the layer of subcutaneous fat that insulates the skin but impedes thermolysis, leaving them especially sensitive to heat stress (84). The thickness of the hog's back fat is another relevant trait. Autochthonous, non-selected breeds like Iberian pigs have a thick layer of subcutaneous fat that can increase their sensitivity to high temperatures compared to leaner breeds (85). According to Usala et al. (86), the heritability of back fat depth is greater in conditions free of heat stress ($h^2 = 0.34$ *vs.* 0.28). In line with these results, studies have proven that hogs weighing over 51.4 kg are more susceptible to heat stress than lighter animals, likely due to a limited heat-dissipating capacity because they have more adipose tissue (87). This corroborates the idea that certain anatomical features of hogs promote greater susceptibility to heat stress.

During transport to an abattoir, hogs may be exposed to stressors like dense loading densities and high ambient temperatures that can compromise homeothermy (88). When unable to maintain a balance between heat gain and loss, hogs suffer heat stress that can trigger myocardial and circulatory insufficiency, perhaps ending in death (89). The development of these insufficiencies is due to metabolic disorders like an increased flow of Ca^{2+} and consumption of adipose tissue, which foster a sustained temperature increase (90, 91) that can lead to oxygen deficiency, electrolyte imbalance, and oxidative stress caused by the activation of myeloperoxidase and eosinophil peroxidase enzymes which serve as indicators of ischemic changes (92).

Adverse effects of hyperthermia for the health of hogs

In hyperthermic conditions, blood irrigation of the splanchnic tissues is channeled toward the periphery to dissipate heat. When this increase in body temperature is sustained for a certain time, it is classified as acute hyperthermia (93). Greater susceptibility to acute hyperthermia has been registered in hogs raised in tropical zones and during summertime in regions with temperate climates. This predisposition is greater than in other species; one of the factors is the lack of functional sweat glands. Heat dissipation occurs through two evaporative mechanisms (respiratory and dermal), but hogs dissipate $<50\%$ of the heat produced *via* respiratory evaporation. Evaporation through the skin involves two types of processes: one passive, when water diffuses through the skin, and the other active, called sweating. However, hogs have a very low density of sweat glands ($30/cm^2$) compared to species like bovines ($800–2,000/cm^2$), and the few they have are not stimulated by heat stress, so little heat is lost by sweating. The second factor is subcutaneous fat. Heavier hogs (e.g., gestating or lactating sows and hogs of commercial weight) require greater energy consumption in relation to their productive stage but also present a lower heat-dissipating capacity because of a low surface: mass ratio (volume) and larger amounts of fatty subcutaneous tissues that impede heat dissipation (93, 94).

At the organ level, during hyperthermia, the gastrointestinal system is affected by hypoxia of the intestinal mucosa because blood flow (nutrients and blood) is diverted to the periphery. This damages the intestinal tract by reducing the height of the villi and the depth of crypts, increasing permeability, and intensifying the inflammatory response (93). There are also reports of tachycardia, hypertension, and supraventricular or ventricular arrhythmias, possibly indicative of cardiovascular damage after an hyperthermia episode (94, 95). Chen et al. (96) identified polymorphonuclear leucocytes in heart tissue sections from hogs affected by heat stress, suggesting that the animals were in an acute stage and may have developed myocardial lesions. As environmental temperatures rise, efficiency is compromised because maintaining a stable body temperature becomes the priority, so nutrients are channeled to achieve euthermia, pushing the synthesis of products (meat, milk) to a second plane. Hyperthermia also affects numerous intracellular signaling pathways responsible for survival and productivity (97). The effects of high temperatures on production can vary widely. Sows may present late-onset puberty and long intervals between weaning and estrus. In addition, the proportion of impregnated sows that give birth may be low. In boars, seminal quality may decrease (94), causing economic losses in the industry due to reduced efficiency, higher outlays for veterinary care, low meat quality because of increased lipids and reduced proteins, and higher mortality, especially especially under challenging stages for dissipating heat (e.g., gestation, fattening) (97).

Unfortunately, common hyperthermia is not the only condition threatening existing swine populations, for recent increases in demand for pork have intensified the breeding of genetic lines that reach the meat-production stage more quickly. While this process produces pigs with a higher muscle-to-fat ratio, it also brings undesirable traits, including a recessive, monogenic hereditary autosomal syndrome called malignant hyperthermia or porcine stress syndrome (PSS) (98). This pathology is described as a hypermetabolic reaction of skeletal muscle caused primarily by alterations of calcium channels in skeletal muscle cells and the central nervous system (Purkinje cells). If untreated, it can be fatal. Studies have described a mutant ryanodine 1 receptor gene (RYR1) that can cause episodes of malignant hyperthermia under adverse or stressful conditions, such as high environmental temperatures, intense exercise, reproductive activity, transport to the abattoir, or the application of inhaled halogenated anesthetic agents (95). Unlike the hyperthermia described earlier, which develops progressively, malignant hyperthermia is characterized by a sudden increase in body temperature and metabolic index. It usually ends in death. The dysfunction of calcium channels in skeletal muscle cells caused by mutations of the RYR1 gene produces a high concentration of intraplasmatic calcium because the Ca^{2+}- releasing channels anchored to the membrane of the endoplasmic reticulum stay open (99). This disorder has become more rare due to the extensive removal of carriers of this gene mutation (100–102); the environment and the facilities where the animals are kept continue to be a factor in the development of disrupted thermal states.

The evolution of PSS passes through 3 phases. The first is marked by increased skeletal muscle metabolism, greater oxygen and glucose consumption, hypercapnia, and higher CO^2 concentrations at the end of expirations that lead to hyperventilation. As energy supplies are consumed, the hog presents hypoxemia and hypoglycemia. Then, attempting to revert this, it intensifies glycolysis, which can produce lactacidemia. In the second phase, local hypoxia, an acid-base imbalance, and fever lead muscle cells to suffer necrosis. Combined with edema, this can trigger compartment syndrome. The rupturing of skeletal muscle fibers releases their content into the bloodstream (rhabdomyolysis). The electrolytes that enter the blood can cause fatal organ damage, while increases in plasma myoglobin and creatine phosphate kinase (CK) levels can trigger acute renal insufficiency. In stage three, the hog suffers systemic metabolic disorders, core hyperthermia, arrhythmia, heart damage, and continuous rupturing of myocytes that, finally, cause disseminated intravascular coagulation (DIC), multiple organ failure and death (95).

Nevertheless, even hogs that do not present the RYR1 gene mutation can die of heat stroke. A hog exposed to excessive heat may develop hyperthermia and activation of the sympathetic-adrenal-medullary axis (SAM), as in other stressful situations. Activation of this axis culminates in increased catecholamines synthesis and secretion, increasing gluconeogenesis, tachycardia, and tachypnea (103). It is important to understand that tachypnea –that is, panting– helps eliminate heat by evaporating liquid from the respiratory tract (27). Hyperventilation and greater oxygen consumption lead to hypercapnia and hypoxia, aggravated by hyperlactatemia due to increased anaerobic glycolysis. Finally, all these conditions together produce metabolic acidosis that affects heart function and, possibly, death (Figure 4) (103).

In recent years, besides changes in pig farm management and handling, alternative tools have been proposed to aid in assessing the thermal states of piglets and hogs. One of these complementary tools is IRT (104, 105).

Infrared thermography (IRT) as a tool to evaluate the thermal and physiological state of swine

IRT measures surface temperature non-invasively by quantifying the radiation emitted by a biological body that reflects modifications in peripheral blood circulation (18, 23). Using IRT with humans and animals requires identifying body regions with specific characteristics called thermal windows, characterized by a high density of blood capillaries, arteriovenous anastomosis, and glabrous skin. In veterinary medicine, the eye, auricular pavilion, and tail are usually the foci of IRT used in species like rats, dogs, and large ruminants (23, 75, 106). However, the usefulness of IRT has been questioned because physiological and circulatory responses can differ among individuals and species depending on the region chosen due, for example, to the properties of hair in dogs or the skin thickness of large ruminants (11).

In newborn piglets, the entire body is considered a thermal window because of its low-fat content. In contrast, in adults, as the fat levels increase and reduce heat emissivity (50, 51), the main thermal windows proposed are the eyes (50, 107), ears, vulva, udder (108), armpit, back, shoulder, and snout, all of which have successfully been correlated positively with average rectal temperature values. However, the sites with reports of greatest reliability and accessibility are the base of the ear, shoulder, and udder (50, 108). Scientific evidence for thermoregulation in swine demonstrates that environmental temperatures play a particular role, so any attempt to determine the efficiency of thermal windows must consider this external factor.

To date, IRT has been used to detect febrile viral states, diagnose pathologies of public interest in pigs (109, 110), or inflammatory processes such as lameness in reproductive females (111).

Thermal window: Orbital zone (periocular area and lacrimal caruncle)

Two thermal windows have been identified in the orbital region: the lacrimal caruncle and periocular area. The infraorbital and supraorbital arteries pass through this region as ramifications of the maxillary artery, supplying blood to the eyelids and ocular muscles (112). The infraorbital artery also has ramifications of the facial nerve, sympathetic fibers that permit vasomotor control by activating the autonomous nervous system (ANS) (113) (Figure 5).

The ocular window has been studied mainly to determine the thermal state of animals and evaluate body temperature non-invasively due to its contiguity to the CNS (114, 115). This was proven in a study by Barbieri et al. (107), who evaluated if this thermal window could help to determine body temperature in 108 pigs. They obtained temperature readings with IRT and a calibrated digital rectal thermometer. The mean rectal temperature was 38.9 ± 0.4°C (min = 37.9°C; max = 40.1°C), while the mean ocular temperature was 36.7 ± 0.1°C (min = 34.8°C; max = 38.8°C). In general, the authors observed a moderate correlation between ocular and rectal temperatures ($r = 0.58$, $P < 0.01$), meaning that surface ocular readings and rectal values increased; however, the correlation varied from strong in recently-weaned pigs ($r = 0.73$, $P < 0.01$) to weak in fattening animals ($r = 0.23$, $P < 0.05$). This correlation may be because food intake and the age of the individual are factors that affect the animal's thermoregulation level. This situation has become evident in piglets, where the colostrum intake

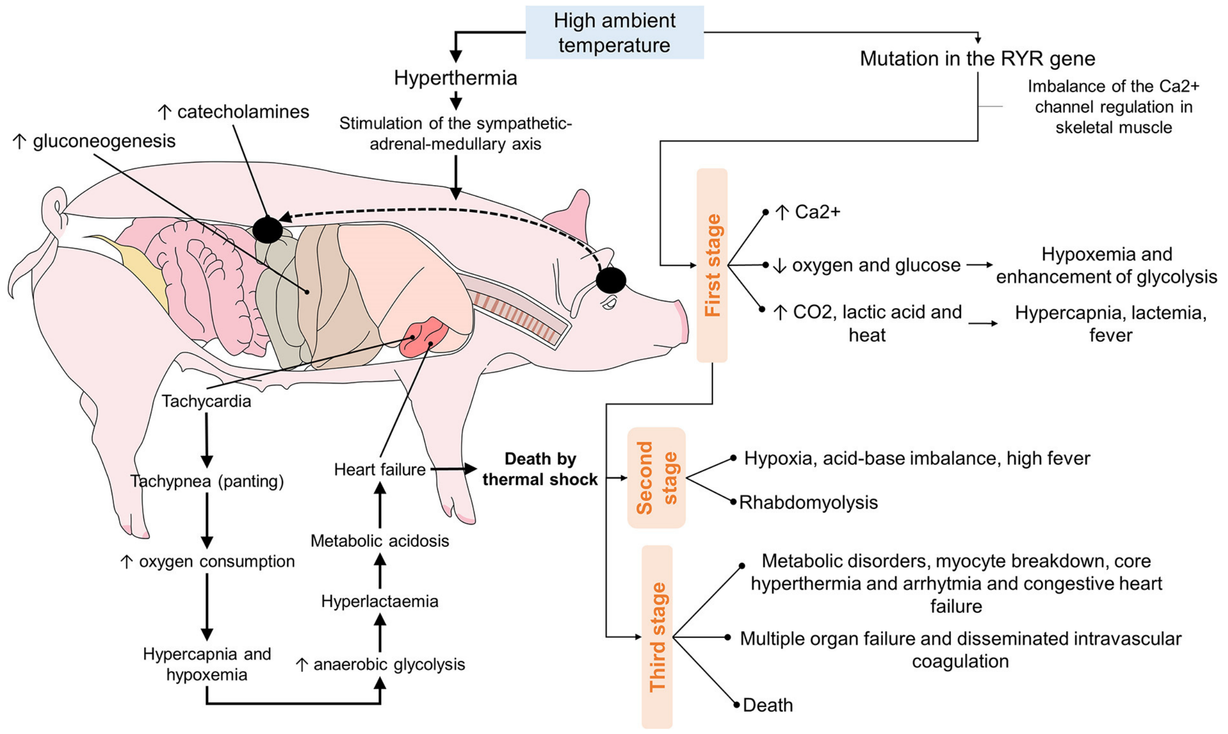

FIGURE 4
Sequence of the events that culminate in the death of hogs due to the effects of high ambient temperatures.

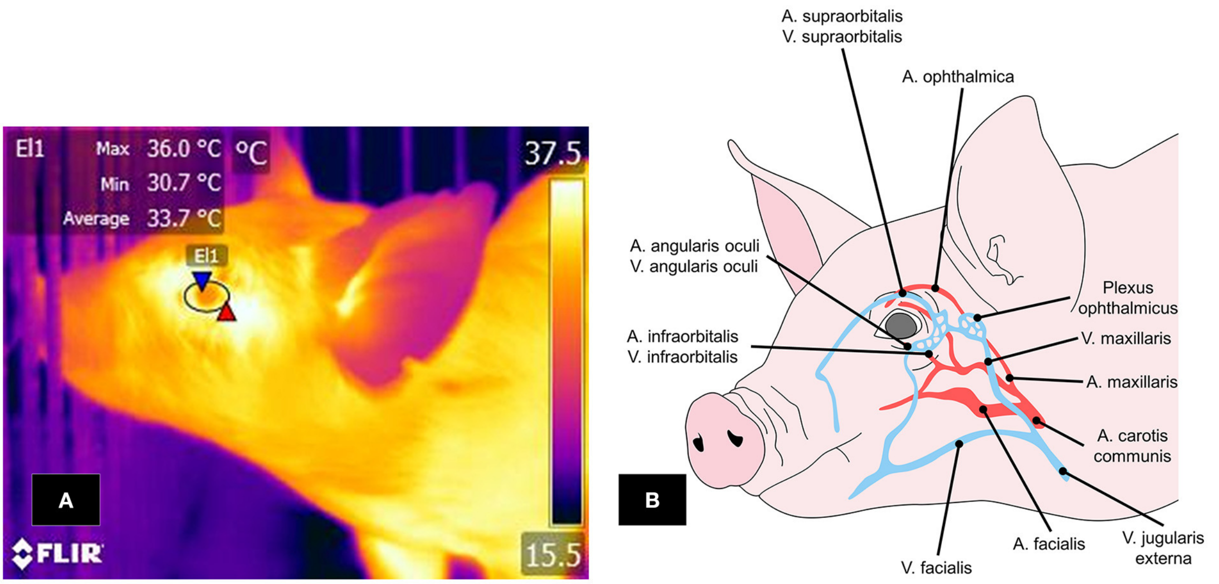

FIGURE 5
Ocular window in pigs. **(A)** The periocular window is outlined by an ellipse (El1) that surrounds the entire ocular surface, including a few millimeters of the upper and lower eyelids. **(B)** Irrigation to the ocular region, mainly by the supraorbital and infraorbital arteries and veins, which are branches of the maxillary artery and vein. IRT can detect changes in the muscle tone of these vascular structures and the heat radiated through them.

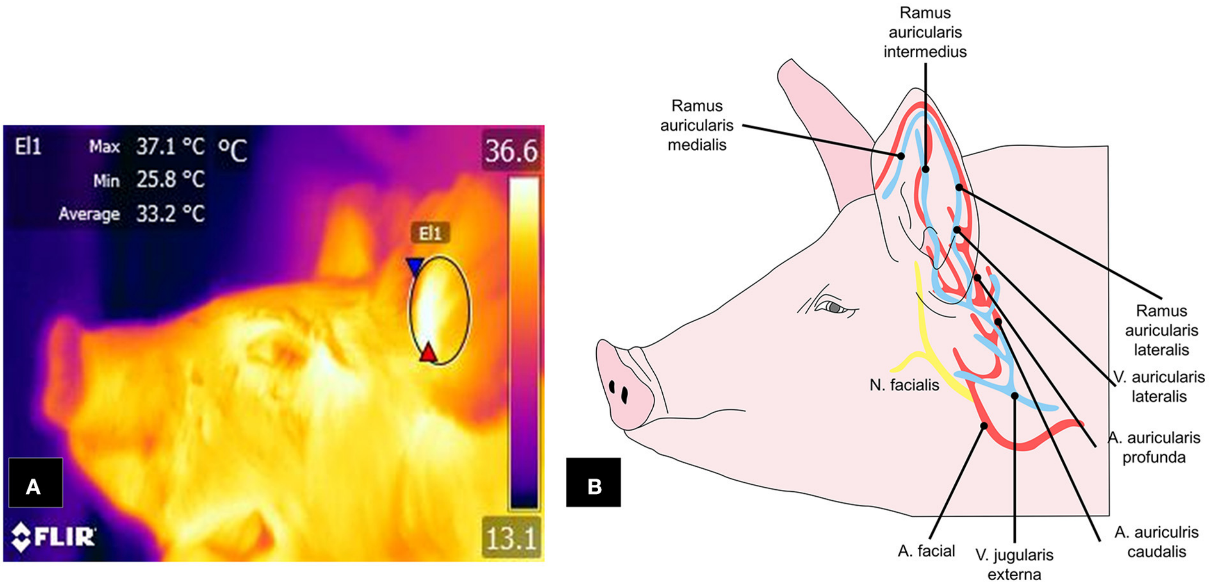

FIGURE 6
Auricular window in pigs. **(A)** The window is delimited by an ellipse (E1) that includes the central cartilage. **(B)** Circulation from the facial artery and external jugular vein, with their respective branches, spreads into the *ramus auricularis medialis*, *intermedius*, and *lateralis*, which are responsible for transporting blood to the ear pavilion and that respond to activation of the ANS.

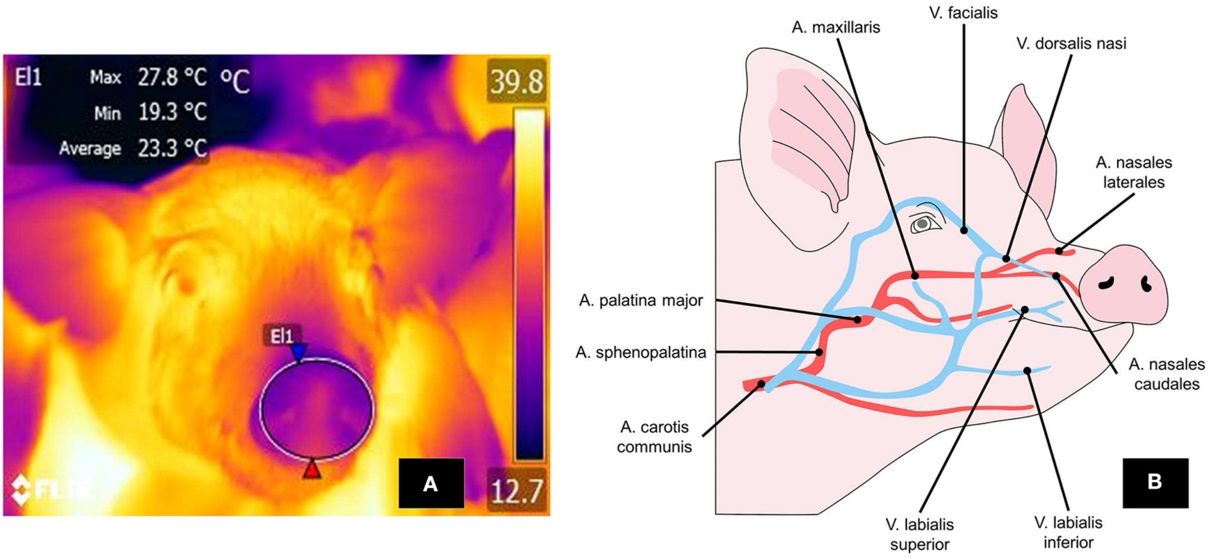

FIGURE 7
Thermal nasal window. **(A)** To mark the perinasal window, a large circle is drawn around the entire snout with the upper lip as its ventral limit. **(B)** The nasal region and vasomotor changes there depend on circulation through the lateral and caudal nasal arteries and the *dorsalis nasi* vein, a branch of the *facialis* vein. These structures respond with vasodilation or vasoconstriction, according to the stimulus perceived.

improves their thermoregulatory capacity and compensates for the limited availability of energy resources at birth. In older animals, food intake helps to maintain the temperature within the thermoneutrality zone which tends to be narrow. Therefore, during a state of hyperthermia, Therefore, the pig decreases feed intake (7, 43). These studies demonstrate the validity of IRT for determining thermal states in animals when significant temperature alterations occur. Results coincided with those mentioned in a scientific review, which stated that temperatures recorded in the ocular region reflect the circulatory control present in that area and its close relation to the ANS (8).

The physiological explanation of the temperature increase is based on greater cardiac activity in response to the catecholamine neurosecretion (adrenaline, noradrenaline) stimulating sympathetic nervous system activity (SNSi), affecting body temperature, a condition called stress-induced hyperthermia (88). Since this phenomenon induces a state of hyperthermia, IRT could serve to detect the increase. Pulido-Rodríguez et al. (116) evaluated the relation between temperatures in the ocular region (thermographic images taken while keeping a distance of 0.50–1 m) and cortisol levels in 66 piglets for 7 weeks post-weaning. They found that in the first 2 weeks, saliva cortisol levels and ocular temperatures presented a strong positive correlation ($r = 0.89$, $P < 0.05$) and concluded that higher temperatures in this region serve as a reliable indicator for detecting stress-induced hyperthermia and adrenal response.

These findings were confirmed by Machado et al. (117) in their study of 192 hogs transported for 170 km, where they recorded rectal and ocular temperatures (with a fixed distance of 0.50 m between the animal and the observer), saliva cortisol levels, respiratory rate, and lactate levels, while also comparing the influence of the upper and lower transport levels inside the vehicle. They observed that the average post-transport temperature in the animals at the upper level was higher than those below (upper = 39.9°C, lower = 38.1°C; P = 0.022). That finding coincided with higher lactate levels (upper = 61.63 mg/dL, lower = 58.26 mg/ dL; $P < 0.001$) to indicate that temperature readings provide information on both the thermal state of animals and their welfare levels (51, 114).

When considering hyperthermia, Weschenfelder et al. (118) evaluated 258 hogs prior to sacrifice to determine the effect of the pre-slaughter process and the influence of meat quality. Those authors measured ocular temperatures using IRT (keeping a distance of 0.25 m) and the pH of mass in the long dorsal, semi-membranous, and adductor muscles. Although observations showed that ocular temperatures correlated with plasma lactate levels ($r = 0.20$, $P = 0001$), with pH at 1-hour postmortem ($r = 0.18$, $P = 0.03$), and with pH of the semi-membranous muscle ($r = 0.20$, $P = 0.02$), the degree of correlation was weak. The authors concluded that the accuracy of IRT should consider elements such as dirt, hair, and humidity. Results coincided with those mentioned in a scientific review, which stated that temperatures recorded in the ocular region reflect the circulatory control present in that area and its close relation to the ANS (8).

The use of the ocular region to detect hyperthermia could also be used to identify other conditions, such as febrile states. Mota-Rojas et al.'s scientific review (104) observed that because IRT is used to detect anatomical regions with increased radiated heat, the ocular area could be utilized to recognize febrile states caused by infection. During infection, the physiological difference that causes the temperature increase during the fever must be the release of cytokines like interleukin-1, interleukin-6, and prostaglandin. Those substances are classified as pyrogenic molecules that stimulate the preoptic area of the hypothalamus to increase body heat (104).

The effects of febrile states were proven in a study of 124 Landrace x Yorkshire pigs diagnosed with *Actinobacillus sp.* infection. The researchers found that IRT taken at a distance of 0.50–1 m predicted body temperature increases of 0.80 and 0.35°C (119). That evidence was confirmed byLoughmiller et al. (120), who evaluated febrile responses in 28 pigs using IRT (keeping a distance of 2 m). In the six animals inoculated with *Actinobacillus pleuropneumoniae*, the authors observed a significant interaction between IRT temperature readings and inoculation with the pathogenic agent ($P < 0.001$), as temperatures were significantly higher than in the control animals (infected = 39.6 ± 0.3°C *vs.* non-infected = 32.6 ± 0.3°C, $P < 0.05$). These results demonstrate IRT's sensitivity for detecting febrile states and the need to analyze this thermal window for evaluating body temperature.

Febrile responses are not necessarily associated with pathological conditions in animals, as they can also occur as biomarkers of immune responses to vaccination (109). This was proven in a study of vaccinated pigs in which IRT (thermographic images of a group of pigs were taken keeping a distance of 2 m) detected a temperature increase of 1°C in the 3–8 h post-injection (121). Scientific evidence thus conclusively shows that the ocular region has useful clinical applications for treating sick animals. Other authors, however, argue in favor of using the lacrimal caruncle region because it is innervated by sympathetic fibers and responds to ANS activity and, therefore, could be a biomarker of stress (23, 24).

The thermal window of the lacrimal caruncle has been utilized in studies like one by Lonardi et al. (122) that evaluated 2500 Large White and Belgian Landrace pigs' eye temperature (keeping a distance of 0.8 m between camera and skin surface) under two conditions: castration and non-surgical handling. Those researchers observed that the temperature of the lacrimal caruncle was higher in the first group than in the one that received only handling at 3 h post-surgery (35.6 ± 0.08°C *vs.* 35.4 ± 0.09°C, $P < 0.05$). They also found that the temperature increase had a weak correlation with rectal temperatures ($r = 0.31$, $P < 0.01$), perhaps indicating that the ocular window is more specific for painful conditions or stress in swine. However, it has also been suggested that this window may be more helpful in identifying temperature decreases caused by increased sympathetic activity. This precise increase that triggers neurosecretion of catecholamines which causes vasoconstriction of the capillaries to reduce heat radiation, as has been reported in other species (18, 123). The behavior of this window continues to be studied, but its clinical utility is undeniable, as is the assistance it offers veterinarians. For this reason, it is necessary to gather additional data to efficiently demonstrate the information obtained from the ocular region in order to achieve a strong validation.

Another important factor is the thickness of the skin because it influences heat radiation. Moreover, the capacity to dissipate heat differs among anatomical sites like the ear, eye, back, and buttocks (108). This situation explains that thicker skin impedes dissipation, in contrast to thinner-skinned animals like the water buffalo (124–126) (pig: 30–140 µm vs. water buffalo: 50–115 µm) (24, 38). Zhang et al. (127) suggest that these variations are related to the reflection of radiant energy and emissivity, which vary at different anatomical sites, given that infrared cameras detect infrared radiation of 0.75–1.4 µm and thermal radiation of 8–15 µm (128). For example, the recommended emissivity values for pigs range from 0.94 to 0.98 (129, 130). However, the thoracic limbs and eyes values are 0.94 and 0.93, respectively. Therefore, the anatomical characteristics of the pig must be considered to achieve precise evaluations of surface temperatures in real-time. It is also essential to emphasize that other techniques must confirm IRT diagnoses. Thus, the best approach is to use IRT and other tools to optimize pig farm management.

Thermal window: Auricular

Another area that has been shown to satisfy the characteristics for consideration as a thermal window is the auricular region. Figure 6 shows the vasculature of the auricular pavilion, mediated by the caudal auricular artery (*auricularis caudalis*) divided into three regions: lateral (*lateralis*), intermediate (*intermedius*), and medial (*medialis*) (131). Innervation of this region depends on the auriculopalpebral nerve (*auriculopalpebralis*), a ramification of the facial nerve (*facialis*) (112).

Contrary to observations of the ocular window and its capacity to recognize hyperthermic states, the auricular area has been used to recognize hypothermia in piglets (7). In animals aged 1–13 days, the surface temperature of the auricular region presented a strong correlation ($r = 0.89$, $P < 0.01$) with rectal temperatures (132), so it may be indicative of the information provided by the pavilion. This theory was posited by Schmitt and O'Driscoll (133) to validate IRT for evaluating temperature in piglets. The authors assessed 67 animals by recording temperatures of the ear and back. Results showed that, in general, the temperature of the ear was lower in low-weight animals (1.5 kg = 35.2 ± 0.36°C *vs.* 1.74 kg = 36.5 ± 0.35°C, $P < 0.001$). This finding was similar to observations of low rectal temperatures in animals with severe growth delay (severe = 35.8 ± 0.46°C *vs.* mild = 37.2 ± 0.42°C, $P < 0.05$).

Some authors concur with this idea, according to Schmitt et al. (134), who evaluated the thermoregulatory capacity (taking thermographic images of the auricular window at a distance of 1 m) of two lines of piglets with differences in food consumption. They found that post-birth, the high-consumption animals had lower temperatures than those with low consumption (24.7 ± 0.37°C *vs.* 26.3 ± 0.36°C, $P < 0.005$). The explanation offered is that exposure to cold induces heat production by metabolic thermogenesis, which intensifies consumption of energy resources like BAT, while in neonates with scarce energy resources, food consumption increases to compensate forand regulate temperature (27). This explanation makes it possible to argue that the performance of thermoregulation in the first weeks of life, up to weaning –when the temperature is strongly influenced by factors like humidity and the animal's weight and average food consumption– corroborates the importance of monitoring this condition (7, 135). Vascularization and innervation of the auricular window responding to changes in ANS tone as an activation of the SNSi, as we saw in the section on the ocular window. This was posited in a study by Yañez et al. (136) based on an evaluation of 64 piglets weaned with or without social disruption who did or did not receive environmental enrichment. The study animals received environmental enrichment in suspended ropes, aromatized bottles, toys, and balls, while the control group did not. The authors found that temperatures in the auricular pavilion did not present changes under any of the treatments but that the temperature of the lacrimal caruncle was 1.7°C lower in the animals that did not receive enrichment but experienced disruption of the social order ($P < 0.05$; at a uniform distance of 1 m). In contrast, the animals without disruption and with environmental enrichment had higher lacrimal caruncle temperatures than controls ($P < 0.05$). From a comparative perspective, these results demonstrate that the meaning of the thermal response observed differed with the condition to which the animals were exposed and that areas like the ocular region, specifically the lacrimal caruncle, can help evaluate acute stress responses. In contrast, the ear region may indicate an animal's overall thermal state (137).

The results just reviewed contrast to those of Rocha et al. (138), who set out to validate anatomical sites like the ear, ocular region, neck, and rump (taking thermographic images at a distance of 1.50 and 2.6 m) in 120 pigs under two treatments, in this case, rough *vs*. gentle handling. They evaluated physiological parameters like heart and respiratory rate and body temperature, in addition to surface temperatures at the anatomical sites chosen for study. Observations included increased heart rate, body temperature, and saliva cortisol levels in the animals that received rough handling, with temperature increases in the ocular region and the ears of 7 ± 0.29°C and 5.86 ± 0.46°C, respectively. It is important to mention that those authors found moderate correlations for the regions of the eye and ear as saliva cortisol levels increased in the animals handled roughly ($r = 0.49$ and $r = 0.50$, respectively, $P < 0.001$). This study provides sufficient evidence to validate the lacrimal caruncle and ear region as areas where stress responses in pigs can be evaluated. Some authors, however, mention that readings from these areas can be affected by other factors, such as the distance at which they are taken, the specific region evaluated, humidity, and skin thickness, so these elements must be considered when choosing and evaluating anatomical sites (24, 132).

The auricular region, then, can be considered a thermal window due to its vascularization, which makes it especially sensitive to changes in environmental temperatures. It is a good site for detecting hypothermic states in neonates. Its innervation plays a vital role in changes in the diameter of the capillaries that can respond to catecholamine levels under stressful conditions, but it has the additional advantage of having been validated, like the ocular window (138).

Thermal window: Nasal

The nasal thermal window, located in the region of the same name, receives blood flow from the nasal artery, a ramification of the maxillary artery (*artery maxillaris*) (139) (Figure 7). This window has been used to evaluate the temperature of the nostrils because they can lose heat by evaporation through respiration (140).

Research on humans has analyzed the effects of stressful psychosocial and physical conditions on vasomotor control of the nose (141). The blood supply to the nose region is sensitive to activation of the SNSi, and, therefore, vasoconstriction of surface blood capillaries, translates into the reduced blood flow and less heat irradiating into the environment (136). However, interpretations of surface temperature readings from the nostrils must consider that respiration produces vapor. While the presence of this inspired vapor is undoubtedly a drawback regarding the usefulness of this thermal window, it can also be considered an advantage because it permits quantifying other parameters, such as respiratory rate. Ricci et al.'s (19) 7-day study of 26 pregnant sows collected data on the parameters of respiratory rate and rectal temperature together with IRT readings from the nasal window (from a distance of 1 and 1.5 m) in the morning and at night. The animals showed a moderate correlation between nasal temperature and respiratory rate ($r = 0.350$), meaning that as the temperature of the nasal region increases, the respiratory rate increases due to tachypnea as a thermoregulatory compensation mechanism, although there is no direct relationship between these two variables.

Similarly, Jorquera-Chavez et al. (142) conducted a pilot study with pigs infected with *Actinobacillus pleuropneumoniae*. They determined that the respiratory rate in the sick animals, evaluated by IRT of the nasal area, increased by an average of 10 breaths/min (rpm) compared to the healthy animals. This coincides with reports on bovines, where the use of this window has been explored as a non-invasive way to record respiratory function (24). However, this application requires additional study because free-moving pigs infected in a normal on-farm environment have not been tested (142). Furthermore, as was reported in a study of 24 male growing pigs exposed to heat stress (at 34°C), to date only rectal temperature has been associated efficiently to determine respiratory rate, with R2 values of 0.997 and 0.993 for acute and chronic heat stress, respectively (143). These findings reaffirm that said thermal window could be associated with the causal factors of heat stress.

Taking into account that rectal temperature is considered the gold standard for quantifying body temperature, Malmkvist et al. (144) analyzed the influence of the thermal environment (15°, 20°, 25°C) on sows during farrowing and lactation based on thermal responses in the head and body regions, as well as in rectal temperature. In their analysis of the data, the authors found that the temperature of the snout increased gradually in relation to the temperature of the holding room, as they recorded average values of 33°, 35°, and 36°C, respectively. They further reported that the rectal temperatures behaved similarly, showing influence by room temperature (38.0°, 38.7°, and 39.0°C, respectively). However, the nasal temperature of the sows maintained a weak correlation coefficient of 0.10 with rectal temperature, a value lower than those obtained for the ocular window (0.24) and udder (0.36). It is worth mentioning that the temperature increase of the snout was accompanied by tachypnoea, as rpm increased from 29 to 58 in rooms maintained at 15° and 25°C, respectively. Due to these findings, it is possible to affirm that the nasal window could be used to associate it with respiratory rate, not with rectal temperature, but it is subject to environmental variations and the effect of social stress factors (145).

These data provide a better understanding of the influence of the environment on the thermoregulation mechanisms of swine in different production stages. In the case of 16 guilts (primiparous sows), pregnant sows, lactating sows, and suckling piglets, IRT was evaluated in seven body regions, including the nose. In general, in all groups, this window showed the lowest temperature values, with an average of 33°C, in contrast to the temperatures registered in the ear root or the tail base (36.2° and 37.1°C, respectively). Their findings led the authors to suggest that IRT could serve to frequently monitor environmental temperatures or early disease states (146) since temperatures reflect the health status of animals (147).

Other thermal windows of the body

Pigs are endothermic animals that retain or dissipate heat depending on environmental and physiological conditions. They use metabolism for thermogenesis or vasomotor changes to perform heat exchange (148). Assessment of body temperature in pigs helps determine their health status non-invasively (127, 138). The ocular, nasal, and auricular zones are considered the main thermal windows, but they are not free of difficulties. In the case of the ocular window, the size of the orbital surface can affect temperature readings, and periocular structures like the eyelashes can occlude images. Likewise, animals tend to close their eyes when in agony, precluding the use of IRT. In the auricular zone, meanwhile, the conformation and position of the ear may restrict IRT's usefulness. Differences between breeds with erect or droopy ears can also cause temperature variations due toblood vessels' projection and hair's presence (149, 150).

The alternative proposed is to evaluate the temperature in broader regions like the back, flanks, thorax, or abdomen. A study of 99 Landrace x Yorkshire pigs by Feng et al. (119) found that shoulder, center-back, and rump temperatures (taken at a distance of 0.5 and 1 m) had an overall moderate to strong and positive correlation of 0.5-0.7 with rectal temperatures. Therefore, IRT can potentially evaluate the core temperature of animals. Similarly, a report on 91 newborn piglets captured back and flank temperatures at 11- time points between birth and 48 h postpartum. Due to the strong correlation ($r = 0.82$) with rectal temperatures found, the authors determined maximum surface temperature and rectal values, discovering that they were affected by time. At 25 and 30 min, respectively, those values decreased by 0.25 and 0.42°C. Moreover, they found that a maximum surface temperature of 30°C was indicative of rectal values <32°C (65). What these authors observed would confirm that other regions may be related to thermoregulation processes. For example, local temperature in the nostril is affected by other factors, while regions such as the shoulder or back are known as a pathway to losing heat, then body temperature influences its values (104).

The thermal response of the back can also be used to indicate the mental state of animals. In 46 pigs involved in agonistic interaction, back and ear temperatures decreased significantly, by 0.9°C, during a conspecific confrontation event, and this variation had a moderate negative correlation with higher lactate ($r = -0.49$) and glucose ($r = -0.32$) levels, where temperature decreases were accompanied by increases in both endocrine markers (149). The branches of the vertebral arteries that supply the transverse spinal muscles are the biological foundation of these changes, as the branches of the spinal nerves are susceptible to sympathetic stimulation during stress responses, in which they also participate (112).

Other regions that have been suggested as thermal windows? are the gluteal and vulvar areas. Scolari et al. (151) mention that these areas (monitored from a distance of 0.61 m) tend to change the temperature during estrus. Specifically, the vulvar region increased its temperature by 1.5°C 12 h before ovulation due to the hemodynamic changes that occur during estrus. This was confirmed by Simöes et al. (152), who found that vulvar-gluteal temperatures (monitored from a distance of 1 m) increased during proestrus and 25 h before estrus but decreased by 1.1 ± 0.9°C 6 h after estrus. These changes suggest the possibility of adopting this window to predict estrus and increase reproductive success. In addition, a high success rate of IRT in detecting estrus and ovulation in 80 sows was achieved by assessing these conditions in conjunction with ultrasonic sensors (153).

Regardless of the region used as a thermal window, it is important to consider that environmental, technical, and individual factors can cause variations in the results (154). In the case of environmental factors, Basak et al. (155) determined the relation of temperature and humidity inside pig barns to surface temperatures measured on the right and left flanks, forehead, and back. They found an association between the temperatures of the barn and the body. In addition to these two variables, they concluded that other elements –wind speed, air pressure, body weight, and food consumption– require models to determine their influence on IRT. Those findings are similar to the observations by Barreto et al. (156), who found a positive correlation between solar radiation, environmental humidity, and wind with body surface temperatures.

Regarding technical factors, the distance between the camera and the animal, the angle of view, and the resolution of the thermographic image are the main ones (154). Leizi et al. (157), after evaluating the influence of camera angle on body surface temperature readings, determined differences >2°C between angles of 74° *vs.* 76°. A technical report on six pigs published by Banhazi et al. (158) stated that perpendicular images taken at angles ≤60° minimized the error percentage of readings and made it possible to detect a thermal response called a cooling effect 10–15 min after wetting, even though the percentage of the wetting effect was registered only during the first minute. Strategies for minimizing the effect of the angle of view include using IRT with kinetic sensors, as this reduced temperature differences in a febrile swine model from 2°C without the sensor to 0.03–1.2°C with it (157).

On the other hand, the distance between the camera and the animal also shows ample variation from one study to another that can affect thermographic readings. Playá-Montmany and Tattersall (159) found a negative and moderate correlation between ocular temperatures ($r = -0.58$) and distance. They observed less temperature variation at a distance of 1.5 m than at 10–15 m. This can be attributed to the dynamics of radiation which tends to disperse toward other objects in the environment, causing a reduction in the values registered by IRT, which is why some authors recommend that thermograms should not be taken at a distance >1 meter (129). However, others recommend distances of 60 (130) or just 20 cm and maintaining a position perpendicular to the body (155). Given this variation, studies must be conducted to identify how distance affects temperature readings, and establish a standard.

Finally, within the individual factors, the presence of hair has been shown to cause temperature variations as large as 0.2°C (23), while a dark-colored layer can increase heat retention by impeding dissipation (11, 24). Pigs, however, have only fine hair that, on the one hand, impedes heat retention (160) but, on the other, leaves them susceptible to sudden temperature decreases, above all in the neonatal stage.

Future directions

Research into the use of IRT with swine seeks to confirm its applicability in new areas of study due to its information on physiological states and animal health. One possibility could be to implement IRT continuously to evaluate the temperature in real time on production units in both individual animals and entire herds and so determine the presence of lesions or infectious diseases (161, 162). Although these future applications would be the areas to be developed, it is necessary to establish the reliability of IRT at different body regions. For example, the ocular window has been shown to suffer less surface temperature variation than areas such as the back, where solar radiation, humidity, or dirt can alter the reading (107).

In this regard, IRT could aid in the early identification of sick animals that present clinical signs of fever (163). The presence of fever, and the vasomotor responses at the peripheral level t it generates, constitute an area of opportunity for applying IRT in farming systems since it can identify pathological processes based on changes in surface temperature (128). For example, Islam et al. (129) used IRT with piglets' head, body, and tail regions to detect signs of gastrointestinal infection caused by *Salmonella typhimurium* and *Escherichia coli*. They found increases in body surface temperatures at 24 h with a peak at 72 h (41° and 37.4°C, respectively; $p<0.005$). In relation to the respiratory disease caused by *Actinobacillus pleuropneuomoniae* in pigs, thermography detected changes in ocular and auricular temperatures (an increase of up to 1.8° and 8.1°C, respectively, in sick pigs) 4-6 h before the appearance of the first clinical signs (142), while at the thoracic level, IRT achieved 100% specificity and sensitivity (Cl 95%: 69–100%) as a method for orienting lung biopsies (110). Determining such temperature increases could also be applied in pigs sent for slaughter to identify those with systemic infections and then program quarantine measures for them (164). However, it is important to emphasize that its use is recommended in conjunction with other innovative methods, such as accelerometers, to evaluate the activity of animals (165). Other diagnostic tools are computerized tomography (166), radio-frequency identification, and machine-learning technologies that can estimate animals' productive parameters (161).

Likewise, IRT monitoring may help reduce economic losses for producers while simultaneously procuring animal welfare by allowing early detection of diseased individuals (162) or recognizing injured animals during transport and the association that this might have on meat quality due to acute stress or dehydration (8, 162). Applying IRT prior to slaughter, for example, makes it possible to estimate levels of well-being (118). In that period, pigs are susceptible to increases in body temperature due to transport and environmental temperatures inside the vehicle (167). IRT could be applied during transport to identify states of fatigue or hyperemia caused by travel, as Warriss et al. (168) demonstrated in their study of 28 pigs in which increases in ear temperatures (from 27.3 to 35.0°C) had a strong-to-moderate correlation with higher blood temperatures ($r = 0.71$, $p<0.001$) and creatine kinase ($r = 0.55$) and serum cortisol concentrations ($r = 0.50$). Because increases in body temperature and the activation of metabolic pathways can impact meat quality, IRT has been suggested as a way to determine animal welfare and predict pork quality (118), given that pre-slaughter stress involves a physiological response that alters thermoregulation and affects meat quality, as has been shown in cattle (169). In the case of pigs, an IRT study of the dorsal region from neck to rump of 500 pre-stunning animals successfully detected animals with defective carcasses. In pigs with skin surface temperatures above 32.2°C, 71% of 49 animals showed such defects as pale, soft, exudative meat (PSE, 6%) and dark, firm, dry meat (DFD, 22%) (130).

IRT may also make it possible to evaluate other key parameters, such as respiratory rates, based on changes in local thermal patterns in the nasal region. Indeed, this has been suggested to assess states of health continuously and non-invasively, and has been tested in cattle (24). The reproductive efficiency and fertility of breeding boars are affected by heat stress and its effects on spermatogenesis (170), a process that requires scrotal temperatures 4–6°C below the boar's body temperature to maintain semen quality and prevent infertility (171). Stravogianni et al. (172) evaluated this in five boars, reporting a strong, significant negative correlation ($r^2 > 0.5$) between increases in scrotal temperature measured by IRT and a rapid, progressive reduction of sperm motility and velocity. These applications suggest that IRT could help detect thermal changes that alter fertility in boars. However, additional information is required on the sensitivity of this tool in such assessments.

The mammary window of sows is another region that could yield more sensitive information on states of health, as Rosengart et al. (173) argued in their study of 513 postpartum hybrid Viktoria sows. After birth, the piglets produced were divided into three groups: healthy, sick, and suspicious, by measuring the surface temperature of the sows' mammary glands. The authors found that the temperature of this window increased by 1.1°C in sick animals compared to healthy ones ($P < 0.05$). This could indicate a discrepancy in the sensitivity of other windows compared to the mammary region, which might mean that this anatomical region is ideal for determining pathological states due to its particular characteristics (108). This same window might also be useful for complementing diagnoses of conditions like postpartum dysgalactia, which causes a temperature increase in the mammary gland. Studies have proven that IRT can detect this pathology with a sensitivity of 94.4% and a specificity of 89.5%, so it may be able to corroborate that the anatomo-morphological characteristics of this window make it effective for evaluating states of health (174).

Finally, the vulvar region of sows has been studied due to the possibility that certain circulatory changes in those animals may indicate of their physiological condition; that is, IRT could help identify the estrus cycle and support assisted reproduction (175). Some authors suggest that a temperature increase of 2°C in this region can be indicative of estrus, which can be associated with the number of navicular cells (176). However, no reports have yet determined the sensitivity and specificity that would lead to validating this window in sows.

IRT has promising applications for the continuous monitoring of animal health. However, it is necessary to recognize that future research must consider some parameters that still need to be discovered,, such as the cut-off points for identifying when an animal is outside its thermo neutrality zone, a field that has only been studied in large ruminants (24). Likewise, improving a sensitive automatic technique that can reduce the influence of intrinsic and extrinsic factors that alter heat radiation must be considered to reduce the underestimation of thermal changes at critical points during the pig production process.

Conclusions

Scientific evidence for the anatomical-physiological differences between piglets and adult hogs is clear. Because neonate piglets have immature thermoregulation systems and limited energy resources to compensate for ambient temperatures, their most important challenge is to prevent hypothermia during the drastic change from intrauterine to extrauterine conditions. In contrast, the adipose tissue deposits in adults can aid heat retention, though their scarce sweat glands impede dissipation. Hence, their greatest challenge is to avoid thermal stress. In light of this evidence, continuously evaluating these animals' thermal state is imperative.

Up to now, thermal windows like the armpit, back, shoulder, and snout are considered to have the greatest similarity to average rectal temperatures values, though the sites with reports of the best reliability and accessibility are the base of the ear, shoulder, and udder. The ocular and auricular thermal windows have emerged as viable options for evaluating thermal states in pigs and also for identifying stressful conditions since specific characteristics of this species make it possible to validate their effectiveness. The structure of the nasal window allows non-invasive evaluations of physiological parameters like respiratory rates, an obvious clinical application. On the other hand, the results obtained after evaluating the temperature of the dorsal, scrotal, mammary, and vulvar regions, suggest its efficacy in detecting defective carcasses and changes that alter fertility in boars, postpartum dysgalactia, and estrus, respectively. Scientific evidence for pigs, then, has demonstrated that the thermal windows analyzed herein aid in obtaining information on this species's general state of health.

Most studies have sought to validate thermal windows to ensure greater objectivity when using IRT with this species. However, to obtain accurate evaluations of the surface temperature, it must be considered that factors such as environmental temperature, the thickness of the hair or fur of each individual, and perfusion in the zones considered thermal windows cause considerable variations in the results.

Author contributions

All authors contributed to the conceptualization, writing, reading, and approval of the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Thermal stress-related physiological, behavioral, and serum biochemical responses in indigenous pigs adapted to Eastern Himalayan region

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Introduction: The current study was carried out to investigate the effect of micro-environmental variations on physiological, behavioral, and serum biochemical parameters of indigenous (Niang Megha), Hampshire, and crossbred (75% Hampshire X 25% Niang Megha).

Methods: Rectal temperature (T_R), skin surface temperature (T_{SS}), respiration rate (RR), and heart rate (HR) were recorded at 0,900 and 1,600 h weekly once for 2 months for each season in grower pigs of each genotype. CCTV video cameras were utilized to observe the behavioral changes. Five milliliters of blood samples was collected to estimate different biochemical parameters.

Results: Season affected ($p < 0.05$) all physiological parameters which generally increased during summer except T_R and RR of indigenous pig. $T_{R,}$ $T_{SS,}$ RR, and HR were significantly ($p < 0.05$) higher for Hampshire than for indigenous and crossbred in the summer season. The frequency and behavioral activities to heat loss or to conserve heat such as shivering and wallowing were lower except for physical activity that was higher at all times in indigenous pigs. Seasonal variations influenced metabolic activity and serum activity of alkaline phosphatase (ALP) and alanine transaminase (ALT), which rose in summer in all genotypes. Serum ALP and thyroxine (T_4) were significantly ($p < 0.05$) higher for indigenous pig in both the seasons. The insulin level was significantly ($p < 0.05$) higher in indigenous pigs with no significant difference between Hampshire and crossbred in summer whereas there was significant difference among the genotypes in winter. However, superoxide dismutase (SOD) showed no significant difference in the study. Indigenous pigs had the lowest serum cortisol concentrations, whereas Hampshire had the highest.

Conclusion: The current study's findings on several parameters of three different genotypes suggest that indigenous pigs in this region are more adaptable to the region's changing climatic conditions.

KEYWORDS

adaptation, behavior, indigenous, pig, thermal stress, response

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Introduction

The Eastern Himalayas are one of the most biologically rich areas on the Earth. In India, the Northeastern Hill (NEH) region represents the Eastern Himalayan region and has a total geographical area covering ∼2,62,379 sq. km. area and lies between 21°34′-29°50′ N latitudes and 87°32′-97°52′ E longitudes in the Eastern Himalayan hill region (1). This region is well-known for the diverse culture of human races and home to a large number of ethnic people (2), providing a diverse range of habitats to about 225 tribes in India, out of 450 in the country (3). Agriculture is the primary source of income for the majority of the rural population of this region (85%), who conducts mixed farming, with livestock accounting for 18% of the value of output from the agricultural sector (4). Moreover, the livestock sector plays a major role in the socioeconomic development in this region, and it is the major source of livelihood for 20 million people, especially women (5), also an important contributor to sustainable food security for many nations, particularly in low-income areas and marginal habitats that are unsuitable for crop production (6). Among all the livestock, pig husbandry is one of the popular livestock and total pig population is mainly dominated by local non-descript pigs (65–75%) in the region (7). Indigenous pig breeds bear unique features such as better heat tolerance, disease resistance, good maternal qualities, early sexual maturity (8), and good quality bristles (9) compared with exotic and crossbreds. The body coat color of indigenous pigs of this region is predominantly black in color and has long and dense hairs extending from wither to the hindquarter.

Climate change is a major worldwide challenge and leads to various environmental stresses that have an impact on the production of livestock (10). Animals can adapt to climatic stressors by different types of adaptive mechanisms including genetic or biological adaptation, phenotypic or physiological adaptation, acclimatization, and habituation (11). However, the response mechanisms that ensure survival are detrimental to performance and productivity (12). The physiological changes when exposed to environmental stress can be measured by the variation in respiration rate, rectal temperature, heart rate, and skin surface temperature (13). Increased respiration or panting increases airflow and evaporation of water from the lungs and hence releases additional heat (14). The physiological response of the animal to its internal and external environment is also reflected in the blood profiles. Changes in hemato-biochemical parameters are useful methods for determining the degree of stress caused by environmental and dietary factors (15) and adaptability to given environmental conditions of an animal (16). Metabolic hormones affecting thermogenesis can also be estimated as the physiological index of environmental adaptation of an animal (17). Similarly, pigs exhibit natural behavioral responses to thermal stress, which include huddling together and shivering when cold, seeking shade, wallowing in water, and changing from diurnal to nocturnal feeding times when heat stressed during the day (18). Pigs have a thick subcutaneous adipose tissue layer and fewer sweat glands; therefore, pigs control their body temperature by behavioral thermoregulation instead of sweating (19). To enhance heat dissipation, they increase direct contact with cool surfaces (20), modifying their lying position, increased excretion, and wallowing in their excreta (21). According to anecdotal evidence, indigenous pigs of this region are better adapted to harsh climatic conditions and subsistence farming, but there is little scientific evidence to back up the statement. Indigenous breeds are more thermotolerant than crossbred and purebred animals in terms of the adaptation potential of the livestock species (22). Indigenous breeds that have evolved in tropical and subtropical regions have a higher adaptive capacity to such stress than exotic breeds. The South African indigenous Windsnyer pigs had better thermoregulatory mechanisms than the large white pigs of the temperate region in the semiarid climate of South Africa (23). Indian breeds of cattle such as *Bos indicus* perform well as compared to exotic cattle such as *Bos taurus* under stressful tropical environments (24). These are some examples of unique adaptive characteristics of the indigenous breeds, which evolved in stressful tropical environments enabling them to survive adverse environments. Therefore, it was hypothesized that indigenous pigs (Niang Megha) have better thermoregulatory mechanisms under changing climatic conditions of this region than exotic or crossbred pigs.

The objective of this study was set out to investigate the physiological, behavioral, and serum biochemical changes in response to thermal stress challenges in indigenous, crossbred, and exotic breeds of pigs.

Materials and methods

Location of the study

The present study was carried out in a pig farm of ICAR Research Complex for NEH Region, Umiam, Meghalaya. The study site is located at 25° 41′ 21″ N latitude and 91° 55′ 25″ E longitude with an altitude of 1,010 m above the mean sea level which falls in humid subtropical high rainfall area and receives rain in the range from 2,239 mm to 2,953 mm annually. In this region, the hottest months are usually July and August, while the coldest months are December and January.

Meteorological measurements

The meteorological data were collected from the Division of System Research and Engineering (DSRE) of the institute during the experimental months. The temperature humidity index (THI) was calculated for each season using the formula, THI = 0.8T+ (RHT-14.4)/100 + 46.4 (25). The thermoneutral zone for a pig is between 18 and 20°C (26). The THI value $\leq$75 means no stress, 75 to 78 stressful, and $\geq$78 extreme stress (27). The THI value of 79–83 is considered danger, and exceeding this causes severe stress and death (28).

Experimental animals and experimental design

Two experiments were conducted separately for summer (July–August) and winter (December–January) to study the effect of heat stress (summer) and cold stress (winter) on three genotypes of pigs, namely, indigenous (Niang Megha), Hampshire, and crossbred (having 75% Hampshire and 25% Niang Megha inheritance). Niang Megha is a registered indigenous pig breed of Meghalaya in India (accession no.: INDIA_PIG_1300_NIANGMEGHA_09002), with a small body size at maturity, having dense and long hairs and known for being better adapted to its native climatic conditions. Crossbred pig used in the present study is a crossbred pig variety called "Lumsniang" developed in the ICAR Research Complex for the Northeastern Hill Region, Meghalaya, for better adaptability and performance in the hill ecosystem of the Eastern Himalaya region. Six grower pigs (5–6 months) from each genetic group were selected for each experiment. The experimental animals were maintained in the pen system of housing and fed with balanced concentrate mesh feed two times daily and drinking water was provided *ad libitum* throughout the period. The experimental pigs were maintained under the standard and uniform managemental conditions. The animals were dewormed on a routine basis and regularly vaccinated. The welfare of the pigs was protected, and the study was approved by the Institutional Animal Ethics Committee (IAEC).

Physiological responses to summer and winter

Physiological parameters including rectal temperature (T_R), skin surface temperature (T_{SS}), heart rate (HR), and respiration rate (RR) were recorded at 0,900 and 1,600 h weekly once for 2 months for each season, and the mean values were considered. Rectal temperature was recorded by inserting a clinical digital thermometer into the rectum of the pigs with proper restraining to avoid stress. The measurement for skin surface temperature was taken at the lumbar region of the pig using an infrared thermal imager Testo 875i (Testo India Pvt. Ltd., Pune, India). The emissivity of 0.95 was taken as the standard emissivity of the pig body surface in the study. Heart rate was determined by counting the number of heartbeats per minute using a stethoscope. Respiration rate was taken when the pig was at the resting phase by visual observation of flank movement to detect breaths per minute. All the parameters were taken by two trained personnel for all the experimental animals to avoid variation between individuals.

Behavioral response to summer and winter

Behavioral mechanisms associated with heat and cold stress were observed in summer and winter, respectively. The pigs were monitored for different lying positions, such as huddling, shivering, standing, wallowing, and physical activity, by using CCTV video cameras. A total of six CCTV video cameras were used for this study. The thermoregulatory behavior patterns of each genetic group were recorded for 60 days in both the seasons. The duration (min) and frequency (%) for each activity during 24 h (from 6.00 a.m. until 6.00 a.m. of the next day) for 8 days were taken weekly once (for 8 weeks) in each season from pre-recorded videos for each animal.

Serum biochemical changes in response to summer and winter

Blood samples were collected from all the experimental animals separately at 15-day interval during two experimental seasons. Five milliliters of blood samples was aseptically collected before feeding in the morning by venipuncture of the anterior vena cava using sterilized plastic disposable syringes from each animal. The collected samples were immediately transferred to a sterile serum separator vacutainer tube (Becton Dickinson, Franklin, USA), allowed to clot, and then centrifuged at 3,000 rpm for 15 min at room temperature. During blood collection, pigs were handled very carefully to avoid handling stress, and the experimental animals were restrained in the ventro-dorsal position. The collected sera samples were stored at −20°C until further analysis. To assess the metabolic and serum biochemical changes in grower pigs in response to heat stress (summer) and cold stress (winter), commercially available kits were used to analyze the following parameters: alkaline phosphatase (ALP), insulin, thyroxine (T4), superoxide dismutase (SOD), acid phosphatase, alanine transaminase (ALT), and cortisol. All the parameters were estimated as per the manufacturer's instruction.

Statistical analysis

The data on physiological, behavioral, and serum biochemical parameters in different genetic groups and between the seasons were analyzed by comparing the means through multivariate analysis of variance (ANOVA). The data on behavioral activities were first transformed using log 10 to normalize it before analysis. The mean differences of different genetic groups for different parameters and between the seasons were tested for statistical significance (at a 5% level of significance) by Duncan's multiple range test (DMRT) using SPSS version 22.0 statistical software.

Results

Meteorological measurements

The maximum and minimum temperature, relative humidity, and temperature humidity index for the study period are presented in Table 1. The present study recorded a maximum THI of 93–96 and a minimum THI of 73–79 during the summer months. Similarly, a maximum THI of 83–87 and a minimum THI of 53–63 were recorded during winter. The highest temperature, relative humidity, and THI were recorded in the months of June (temp. 29.3°C, RH 86%, THI 95.89) and July (temp. 29.70°C, RH 88.90 %, THI 96.47) during the study period. The lowest temperature, relative humidity, and THI were recorded in January (temp. 5.50°C, RH 44.6%, THI 53.11) and February (temp. 8°C, RH 40.8%, THI 55.92).

Physiological measurements

Physiological measurements of different genetic groups during summer and winter are presented in Table 2. In the summer season, indigenous pigs had significantly lower ($p < 0.05$) T_R with no significant difference between Hampshire and crossbreed pigs. Skin surface temperature was significantly higher ($p < 0.05$) in Hampshire with no significant difference between indigenous and crossbred pigs. There were significant differences in RR and HR among the genetic groups. Indigenous pigs had significantly lower ($p < 0.05$) RR and HR followed by crossbred pigs and the highest was recorded in Hampshire.

In the winter season, there were no significant differences in T_R and T_{SS} among the genetic groups. Although RR was significantly higher ($p < 0.05$) in Hampshire, but had no significant ($p < 0.05$) difference between indigenous and crossbred pigs. Heart rate had a significant ($p < 0.05$) difference among the genetic groups. Hampshire had significantly higher ($p < 0.05$) HR followed by indigenous, and the lowest was recorded in crossbred pigs.

Behavioral mechanisms response to summer and winter

Different behavioral mechanisms were observed among the genetic groups when exposed to heat and cold stresses in the experimental animals (Table 3). Hampshire showed significantly ($p < 0.05$) higher duration and frequency of lateral and half-lateral lying positions in both the seasons followed by crossbred and indigenous pigs, while the duration and frequency of sternal lying were significantly ($p < 0.05$) higher in indigenous pigs followed by crossbred and Hampshire pigs. Similarly, indigenous pigs showed higher duration and frequency of standing and physical activity in both the seasons. Wallowing behavior was observed only in the summer season, while huddling and shivering behavior were observed in the winter season in all the genetic groups. Wallowing and shivering thermogenesis behavior were observed significantly ($p < 0.05$) highest in Hampshire followed by crossbred pigs during heat and cold stresses, respectively, whereas huddling behavior was observed significantly ($p < 0.05$) highest in indigenous pigs followed by crossbred pigs during cold stress.

Serum biochemical response to summer and winter

The serum biochemical responses to heat and cold stresses are presented in Table 4. The result revealed that during heat stress, the serum concentrations of alkaline phosphatase (ALP) and thyroxine (T_4) were significantly ($p < 0.05$) higher in indigenous pigs followed by crossbred and Hampshire. Among different genetic groups, the serum insulin level was significantly ($p < 0.05$) higher in indigenous pigs; however, there was no significant difference between crossbred and Hampshire. The serum concentrations of alanine transaminase (ALT) differed significantly ($p < 0.05$) among different genetic groups. It was significantly ($p < 0.05$) higher in crossbred followed by Hampshire and indigenous pigs. The activity of superoxide dismutase (SOD) and acid phosphatase showed no significant difference among the genetic groups during heat stress. The cortisol level was significantly ($p < 0.05$) lowest in indigenous pigs followed by crossbred and highest in Hampshire during heat stress.

Similarly, during cold stress the serum concentrations of alkaline phosphatase (ALP) and thyroxine (T_4) were significantly ($p < 0.05$) higher in indigenous pigs followed by crossbred and Hampshire. The serum insulin levels and ALT concentrations were significantly ($p < 0.05$) different among the genetic groups. The insulin levels were recorded highest in Hampshire followed by crossbred and indigenous pigs; however, the ALT concentrations were recorded highest in crossbred pigs followed by Hampshire and indigenous pigs. No significant

TABLE 1 Maximum and minimum temperature, relative humidity, and THI recorded during experimental period.

Season	Months	Temperature (°C)		R.H (%)		THI	
		Max	Min	Max	Min	Max	Min
Summer	July	29.70	21.40	88.90	76.00	96.42	79.64
	August	28.00	20.90	90.50	77.30	94.00	79.13
Winter	December	22.90	8.60	86.40	54.70	84.36	57.84
	January	22.10	5.50	85.90	44.60	82.92	53.11

R.H, relative humidity; THI, temperature humidity index.

TABLE 2 Means ± SE for physiological parameters of indigenous, Hampshire, and crossbred pigs during the study period.

Physiological parameters	Seasons	Indigenous ($n = 6$)	Hampshire ($n = 6$)	Crossbred ($n = 6$)
Rectal temperature (°C)	Summer	38.32 ± 0.06^a	39.39 ± 0.02^b	39.10 ± 0.05^b
	Winter	38.04 ± 0.07^a	38.59 ± 0.1^a	38.49 ± 0.06^a
Skin surface temperature (°C)	Summer	28.49 ± 0.03^a	30.51 ± 0.3^b	28.35 ± 0.03^a
	Winter	21.66 ± 0.04^a	21.58 ± 0.5^a	21.64 ± 0.06^a
Respiratory rate (breaths/min)	Summer	22.42 ± 0.13^a	34.42 ± 0.06^b	27.67 ± 0.11^c
	Winter	17.17 ± 0.9^a	20.17 ± 0.09^b	17.83 ± 0.17^a
Heart rate (bpm)	Summer	62.75 ± 0.17^a	76.58 ± 0.15^b	64.58 ± 0.18^c
	Winter	55.42 ± 0.08^a	63.25 ± 0.08^b	52.75 ± 0.12^c

Values are the average of measurements taken at different times (0,900 and 1,600 h for 4 days) of the day during each season. $^{a-c}$Values in the same row with different superscripts differ significantly ($p < 0.05$).

difference in SOD and acid phosphatase was observed among the genetic groups. The cortisol level was significantly ($p < 0.05$) higher in Hampshire with no significant difference between indigenous and crossbred pigs.

Discussion

It is important to know that pigs are susceptible to heat and cold stresses. The present study revealed that the microclimate conditions of the present study location cause heat stress and cold stress in pigs during the summer and winter seasons, respectively. The maximum and minimum THI values during the summer season were above the established normal THI values of 75 or less for pigs (27). The THI values observed in the current study were those that have previously been reported to cause extreme stress in pigs and may even be fatal (27, 28). The primary response of animals under thermal stress is an increase in respiration rate, rectal temperature, and heart rate (29). Rectal temperature is a delayed indicator of heat stress only responding when the temperature is over 27°C or the THI is >80 (30, 31). In the present study, Hampshire and crossbred pigs had higher T_R during heat stress than indigenous pigs. Given that an increase in T_R is an indication of heat stress, higher T_R in Hampshire and crossbred pigs may indicate that they are more susceptible to heat stress. This is confirmed by the previous study, where pigs exposed to heat stress had a higher rectal temperature (32). While lower TR in indigenous pigs suggest that indigenous pigs might have a better thermoregulatory mechanism to dissipate heat from the body. T_R for indigenous and exotic pigs under heat stress, however, did not differ according to some studies (23, 33). During heat stress, Hampshire pigs were found to have higher T_{SS} than indigenous and crossbred pigs, which may indicate that they are more susceptible to heat stress. Elevated T_{SS} with an increase in ambient temperature was reported in pigs (23). Furthermore, exposure of ruminants to high environmental temperature also increased skin temperature (34), documented in Nguni and Boran cattle breeds (35) and Osmanabadi goats (36). This higher T_{SS} might be directly attributable to the vasodilatation of the skin capillary bed, which would enhance blood now to the cutaneous blood vessels, allowing more efficient heat transfer to the surroundings and sensible heat loss (36). The fact that indigenous and crossbred pigs had lower T_{SS} may be related to their long hair and high hair density, which serve as insulation and reduce heat loss, as demonstrated by Silanikove (30). Stress triggers the hypothalamus, which enhances respiratory activity to speed up heat escape from the body through respiratory evaporation (37). In animals, there is a correlation between respiration rate and the surrounding temperature and microenvironments (28). Thus,

TABLE 3 Means ± SE for behavioral parameters of indigenous, Hampshire, and crossbred pigs in response to heat and cold stress observed under a CCTV video camera during the experimental period.

Behavioral parameters	Season	Duration (minutes/day)			Frequency/day (%)		
		Indigenous ($n = 6$)	Hampshire ($n = 6$)	Crossbred ($n = 6$)	Indigenous ($n = 6$)	Hampshire ($n = 6$)	Crossbred ($n = 6$)
Lateral lying	Summer	204.02 ± 1.99^{a}	613.69 ± 1.58^{b}	453.69 ± 0.67^{c}	14.17 ± 0.14^{a}	42.62 ± 0.11^{b}	31.51 ± 0.05^{c}
	Winter	284.96 ± 0.57^{a}	396.69 ± 1.43^{b}	353.54 ± 0.51^{c}	19.79 ± 0.04^{a}	27.55 ± 0.1^{b}	24.55 ± 0.04^{c}
Sternal lying	Summer	364.38 ± 2.08^{a}	105.38 ± 1.52^{b}	215.19 ± 1.24^{c}	23.30 ± 0.14^{a}	7.32 ± 0.11^{b}	14.94 ± 0.09^{c}
	Winter	152.54 ± 0.57^{a}	101.42 ± 1.45^{b}	147.06 ± 0.85^{a}	10.59 ± 0.04^{a}	7.04 ± 0.1^{b}	10.21 ± 0.06^{a}
Half lateral lying	Summer	81.83 ± 1.82^{a}	310.63 ± 0.00^{b}	202.35 ± 1.35^{c}	5.68 ± 0.13^{a}	21.57 ± 0.27^{b}	14.05 ± 0.09^{c}
	Winter	77.38 ± 0.62^{a}	119.88 ± 1.04^{b}	111.42 ± 0.43^{c}	5.37 ± 0.04^{a}	8.32 ± 0.07^{b}	7.74 ± 0.03^{c}
Huddling	Summer	-	-	-	-	-	-
	Winter	404.52 ± 0.64^{a}	380.73 ± 0.97^{b}	401.42 ± 0.92^{c}	28.09 ± 0.04^{a}	26.44 ± 0.07^{b}	27.88 ± 0.06^{c}
Shivering	Summer	-	-	-	-	-	-
	Winter	79.69 ± 0.44^{a}	146.27 ± 1.31^{b}	120.79 ± 0.93^{c}	5.53 ± 0.30^{a}	10.16 ± 0.09^{b}	14.19 ± 5.41^{c}
Standing	Summer	411.35 ± 4.92^{a}	92.19 ± 3.24^{b}	205.23 ± 1.25^{c}	28.57 ± 0.34^{a}	6.40 ± 0.23^{b}	14.25 ± 0.09^{c}
	Winter	99.88 ± 2.02^{a}	55.15 ± 1.76^{b}	60.94 ± 1.30^{c}	6.94 ± 0.14^{a}	3.83 ± 0.12^{b}	7.43 ± 3.01^{c}
Wallowing	Summer	105.79 ± 1.59^{a}	180.44 ± 2.38^{b}	140.25 ± 1.33^{c}	7.35 ± 0.11^{a}	12.53 ± 0.17^{b}	9.74 ± 0.09^{c}
	Winter	-	-	-	-	-	-
Physical activity	Summer	272.63 ± 1.64^{a}	137.69 ± 1.02^{b}	223.29 ± 0.38^{c}	18.93 ± 0.11^{a}	9.56 ± 0.07^{b}	15.51 ± 0.03^{c}
	Winter	341.04 ± 1.14^{a}	239.88 ± 0.66^{b}	244.83 ± 2.16^{c}	23.68 ± 0.08^{a}	16.66 ± 0.05^{b}	17 ± 0.15^{c}

$^{(a-c)}$Values in the same row with different superscripts differ significantly ($p < 0.05$).

when animals are exposed to high ambient temperatures, they have an increased rate of respiration and perspiration (31). However, it was well-documented that porcine sweat glands are non-functional (19). Respiration rate is the first sign of heat stress and can be affected by temperatures as low as 21.3°C (31) or a THI of 73 (30). In the present study, RR of indigenous and crossbred pigs was within the normal range, that is, 15–30 breaths per minute as reported by Silanikove (30); however, it was higher than the normal range during heat stress in Hampshire, suggesting the susceptibility to heat stress. Among the genetic groups, higher RR in Hampshire and crossbred pigs during heat stress in the study could be the mechanism to dissipate more heat from the body by evaporating to the surrounding. Our finding of lower respiratory rate of indigenous pigs when exposed to heat stress corroborates with the finding of Moyo (38) in indigenous Windsnyer pigs of South Africa, which might be due to better adaptability of indigenous pigs to the agroclimatic condition of the region. An increase in RR was also reported in pigs exposed to high heat load (23, 39) and various cattle breeds (40). Heart rate is a stress marker that can be changed in response to thermal stress. Hampshire had higher HR than indigenous and crossbred pigs during heat stress, and this could be an attempt to dissipate excess heat to its surroundings by increasing blood flow to its peripheral tissues. A similar observation was earlier observed and documented by Madzimure et al. (23) who reported higher HR in large white than in indigenous Windsnyer pig in South Africa. Similar findings were also reported in sheep breeds reared in the Indian semiarid regions (41) and in other farm animals (36).

In the winter season, the temperature reaches a minimum of 5.5°C in the study location, which is below the lower limit of the thermoneutral zone (18–20°C) for a pig (26). Although pigs are susceptible to cold stress, there was not much variation in the physiological parameters during cold stress among the genetic groups. Pigs have a thick subcutaneous adipose tissue layer, which is attributed to maintain their normal physiological parameters during cold stress. Previous research has shown that when pigs are exposed to low temperature, they may increase heat production through muscular shivering thermogenesis (42), conserve heat through changing posture to reduce the body surface exposed to cold (43), build nests, huddling together, and select favorable microhabitats (42, 44). Similarly, on exposure to cold temperature, increased physical activity, huddling, and shivering were observed in the present study. Increased physical activity and huddling were observed highest in indigenous pigs followed by crossbred and Hampshire, while shivering was observed highest in Hampshire followed by crossbred pigs. Similarly, huddling together in cold temperature was observed in indigenous pigs (Windsnyer and Kolbroek) of South Africa (38). Increased physical activity was reported in large white (38) and crossbred (large white x Pietrain) pigs (45), which is contrary to the present study observation where it is highest in indigenous pigs.

Less time in standing and sitting and more time in lying postures in comparison with control during the cooling phase after the pigs are exposed to high temperature are reported in cooled sow (46). Similarly, more time in lying postures was observed during heat stress than cold stress and the highest lying behavior was observed in Hampshire followed by crossbred in the entire present study. Heat-stressed sows have been observed to show reduced standing posture (47) and consistently increase lying postures, especially lying laterally (48). Similarly, less standing time and more laterally lying posture were observed during heat stress, and the highest was observed in Hampshire followed by crossbred in the present study. Lying laterally appears to be a strategical posture when pigs suffer from heat stress, which is due to the skin surface in contact with the floor, which is greater than any other posture, enhancing heat loss through conduction (48). Reduced overall activity is one of the behavioral strategies used to help animals cope with heat stress. Physical activity was significantly reduced in heat stress in all the genotypes. Among the genetic groups, physical activity was observed highest in indigenous followed by crossbred than in Hampshire in both the seasons. Wallowing and standing behavior is one of the adaptive behavioral mechanisms to dissipate heat and reduce heat stress. When the heat load increased, animals spent more time standing to maximize heat dissipation by increasing the surface area of the skin exposed to air or wind flow (49). Standing behavior was observed highest in indigenous pigs, whereas wallowing behavior was highest in Hampshire. Pigs wallow for a variety of reasons, including cooling and protection from solar radiation and preventing attacks from insects (50). Wallowing allows pigs to lose heat more effectively during hot weather than sweating as well as during cold conditions; mud acts as insulation, allowing them to keep their body warm (14). However, wallowing behavior was not observed in all groups during the cold season in the present study as they are kept in an intensive system of rearing. Similar to previous findings, in this present study as well-different behavioral responses to conserve heat or dissipate heat during the cold and hot seasons were observed. In this study, indigenous pigs exhibited increased physical activity to generate body heat and huddled together in an attempt to conserve heat. On the contrary, Hampshire pigs attempt to increase heat production by shivering thermogenesis. Similarly, indigenous pigs showed the increased standing time to dissipate heat, while Hampshire and crossbred pigs presented a higher time of wallowing and reduced physical activity to reduce heat production. The fact that Hampshire and crossbred pigs spent more time in lying and wallowing could indicate that they were attempting to cool down to a comfortable temperature through evaporative cooling. However, physical activity was significantly higher and wallowing behavior was significantly lower in indigenous pigs followed by crossbred pigs. These behavioral responses indicate better

TABLE 4 Means ± SE of indigenous, Hampshire, and crossbred pigs for serum biochemical parameters during the study period.

Parameters	Seasons	Indigenous ($n = 6$)	Hampshire ($n = 6$)	Crossbred ($n = 6$)
Alkaline phosphatase (ng/ml)	Summer	8.41 ± 0.12^a	3.56 ± 0.01^b	5.41 ± 0.03^c
	Winter	6.46 ± 0.03^a	1.66 ± 0.05^b	3.48 ± 0.06^c
Alanine transaminase (U/l)	Summer	22.16 ± 0.03^a	28.26 ± 0.04^b	38.23 ± 0.13^c
	Winter	13.72 ± 0.03^a	17.88 ± 0.4^b	19.47 ± 0.09^c
Insulin (µIU/ml)	Summer	7.01 ± 0.11^a	4.18 ± 0.1^b	4.8 ± 0.04^b
	Winter	5.48 ± 0.03^a	12.48 ± 0.05^b	7.42 ± 0.03^c
T_4 (ng/ml)	Summer	38.64 ± 0.27^a	5.52 ± 0.06^b	18.08 ± 0.04^c
	Winter	45.64 ± 0.25^a	11.64 ± 0.04^b	26.64 ± 0.11^c
SOD (U/l)	Summer	1.91 ± 0.05	1.73 ± 0.03	1.86 ± 0.06
	Winter	1.83 ± 0.60	1.54 ± 0.01	1.79 ± 0.03
Acid phosphatase (µmol/min/ml)	Summer	0.042 ± 0.00	0.12 ± 0.01	0.049 ± 0.00
	Winter	0.07 ± 0.05	0.09 ± 0.00	0.026 ± 0.01
Cortisol (ng/ml)	Summer	28.5 ± 0.15^a	56.8 ± 0.07^b	40.2 ± 0.02^c
	Winter	27.64 ± 0.01^a	47.8 ± 0.02^b	28.3 ± 0.24^a

$^{(a-c)}$Values in the same row with different superscripts differ significantly ($p < 0.05$).

homeostasis and adaptability of indigenous and crossbred pigs than Hampshire.

This study demonstrates that most of the metabolic and serum biochemical activities were influenced by thermal stress. Some metabolic enzymes increase their activity when exposed to high ambient temperatures. Among other enzymes, alanine aminotransferase (ALT) is one of the important metabolic enzymes that increased its activity when exposed to stress as frequently reported by several researchers. The increased activity of these enzymes was reported in goats (51), sheep (52), and pig (53, 54) during exposure to heat stress. A similar finding was observed in the present study. According to Banerjee et al. (55), an increase in the activity of this enzyme is related to the higher adaptive capability of the animals to cope with heat stress. It was also reported that ALT activity increases during stress (56), such as increased after severe exercise in humans (57) and restraint in rats (58). The enzyme activity of ALT was observed highest in Hampshire followed by crossbred pigs, suggesting that Hampshire pigs are more susceptible to stress than indigenous and crossbred pigs of this region. Acid phosphatase (ACP) and alkaline phosphatase (ALP) are two key enzymes involved in animal metabolism. The levels of these enzymes are generally low in heat-stressed animals, which could be attributed to a metabolic shift in the animals (51). Several investigations have reported a decrease in ALP activity in heat-stressed animals (53, 59), including pigs (60), which is contradictory to our present finding. Why ALP activity is higher during summer than winter in the present study is not known. It is thought that reduced ALP activity is attributed to reduce the functioning of the liver during heat stress exposure (61). The present observation revealed that the activity of serum ALP was highest in indigenous followed by crossbred pigs and lowest in Hampshire during the study periods. However, acid phosphatase did not differ significantly among genotypes in the present study. The above observations could suggest that indigenous pigs and crossbred are more resilient and have better adaptability to different climatic conditions than Hampshire.

The blood insulin concentration directly reflects the energy status of the animal to sustain production under extreme environmental conditions (62). An increase in basal and stimulated circulating insulin has been reported in a variety of species (63), including pigs (64), during heat stress. Ironically, Sejian et al. (17) reported a significant reduction in the insulin level after thermal exposure in goats. Similarly, we observed a higher level of insulin in indigenous pigs, which is inconsistent with the earlier findings (63, 64) and lower level of insulin in Hampshire and crossbred pigs (17) during heat stress. This difference in the level of insulin could be due to their nutritional status. A reduction in the level of insulin in Hampshire and crossbred pigs could be due to heat stress and resulted in reduced feed intake, as reported by Sejian et al. (65), and there was a significant reduction in the insulin level when thermal stress was combined with restricted feeding. Our findings also showed that exposure to a cold environment significantly reduced the level of insulin in indigenous pigs, but significantly increased in Hampshire and crossbred pigs. In rats, cold exposure decreased insulin secretion (66), stimulating the insulin signaling pathways in the brown adipose tissues for utilizing energy for thermogenesis (67). However, the increased level of insulin in Hampshire and crossbred pigs when exposed to a cold environment could be due to increased feed intake and elevated glucose concentration in the body, which stimulates the release of insulin.

The thyroid gland is extremely sensitive to temperature changes in the environment (68). Proper thyroid gland function and thyroid hormone activity are thought to be essential for domestic animals to maintain productive performance (69). Increased secretion of thyroid hormone increases body metabolism and hence heat production (28). Various researchers have reported a decrease in thyroid hormone (T_4 and T_3) blood concentrations in diverse species when exposed to heat stress (70, 71) including pigs (28). The serum concentrations of T_4 and T_3 were higher in cold stress than in heat stress conditions (52). The previous findings support the finding of the present study in that the serum concentration of T_4 was shown to be significantly lower during heat stress. Reduced concentrations of circulating T_3 and T_4 have been reported in heifers (72), sheep (73), and goat (69), suggestive of an attempt to reduce metabolic rate and thus metabolic heat production. Overall, during the study periods, indigenous pigs demonstrated a significantly higher T_4 serum concentration than either of the other genotypes. The lower level of T_4 in Hampshire and crossbred pigs might indicate that they are more prone to cold and heat stresses than that in indigenous pigs. When an animal is exposed to heat, reduced food intake and metabolism slow down, which leads to negative energy balance and results in hypofunction of the thyroid gland (74). Consequently, Sejian et al. (17) reported a decrease in the level of thyroid hormones (T_3 and T_4) in stressed animals. In the present study, higher thyroid hormone (T_4) secretion may be indicative of the superior adaptive capability of indigenous pigs to the different climatic conditions of this region.

Antioxidants, such as SOD and glutathione peroxidase, can efficiently eliminate reactive oxygen species (ROS) from the intracellular environment by detoxifying them (75). During hot and cold climate, there is an increase in the rate of reactive oxygen species (ROS) production leading to the activation of antioxidant enzymes to scavenge the ROS (76). Some researchers have also observed higher activity of SOD as a marker of oxidative stress in various species during hot ambient temperature (77). However, lower activity of SOD during heat stress pig was documented by Yang et al. (78). Kataria and Kataria (76) reported significantly higher activity of SOD during hot and cold environments as compared to moderate environment. However, in the present study, the enzymatic activity of SOD did not show a significant alteration among the genotypes during thermal stress.

The detection of cortisol is one of the most widely used methods to assess stress in animals. It has proven to be a major stress hormone and a reliable indicator of stress (79). An increase in circulating cortisol concentrations caused by the activation of the hypothalamic–pituitary–adrenal axis is one of the most frequent and general responses of an animal to stressful situations (30). An increase in serum cortisol levels is related to the increase in adrenocortical activity, a characteristic linked to the activation of the autonomic nervous system by stress (80). Therefore, higher serum cortisol concentrations in Hampshire during the study periods suggest that they are more vulnerable to heat and cold stresses. It was also observed that there was a significant increase in serum cortisol concentrations during summer in crossbred pigs, suggestive of heat stress. Similarly, higher cortisol level in heat-stressed pig was reported from arid tracts in Rajasthan, India (81). However, indigenous pigs of this region maintained the serum cortisol concentrations during thermal stress. The findings indicate that the climatic conditions of this region are stressful to Hampshire and crossbred pigs than indigenous pigs of this region.

Conclusion

The results suggest that indigenous pigs had better adaptability to heat and cold stresses than Hampshire and crossbred pigs. The superior tolerance to thermal stress of indigenous pigs was associated with an ability to maintain their physiological and behavioral activities. Behavioral activities to heat loss or conserve heat such as shivering and wallowing were lower in indigenous pigs, but higher physical activity during thermal stress. The better adaptability is also related to higher metabolic activity, which is shown by higher T_4 activity and lower serum cortisol concentrations. Also, the better adaptability of indigenous pigs might be due to their unique anatomical features such as small body size, short legs, and long and dense bristles. Further detailed studies need to be carried out for a better understanding of the thermoregulatory mechanism of indigenous pigs and identifying the gene responsible for the resilient traits and developed adaptation signature that will be useful for the mainstreaming in the breeding program under changing climatic conditions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Ethics Committee (IAEC), ICAR Research Complex for NEH Region, Umiam, Meghalaya, India.

Author contributions

Conception, design of study, and interpretation were done by KG. Data collection and analysis were performed by CG and NSS. The manuscript was drafted by CG, NSS, and KG. Critical revision of the manuscript was done by NMS. All authors contributed to the manuscript revision and have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Economically sustainable shade design for feedlot cattle

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Provision of shade reduces radiant heat load on feedlot cattle, thus reducing demand of water and energy for thermoregulation. While the positive effects of shade on animal welfare are widely known, the literature lacks data on the magnitude of its economic impacts. In this study, we propose the concept of novel shade design to prove that a correctly oriented and dimensioned roof structure, which optimizes shade to be displaced within the pens, motivates cattle to seek shade, protect them from short-wave solar radiation, and is resilient to counteract weather adverse conditions. The beneficial outcome is improvement in animal welfare and productive performance, as well as increments on financial return and sustainability. To attest these benefits, eight hundred *B. indicus* × *Bos taurus* bulls were randomly assigned in pens with or without shade from a galvanized steel-roof structure. Performance data (e.g., dry matter intake, body weight gain, feed efficiency and hot carcass weight) and heat stress indicators (e.g., subcutaneous temperature, body-surface temperature, respiratory rate and water intake) were assessed along the study period. The economic outcomes derived from shade implementation were determined using the net present value. Meteorological variables were also monitored every 1 min, and grouped in a thermal comfort index for feedlot cattle, the *InComfort Index* (InCI). The shade structure efficiently reduced radiant heat load on cattle in pens with shade. According to the classification of the InCI, during very hot days (InCI > 0.6; around noon with mean solar radiation above 800 W m^{-2} and mean air temperature above 33°C), greater proportion (80%) of animals in shaded pens were using shade. Under such circumstances, cattle in shade had water intake reduced by 3.4 L per animal, body temperature was lower by 5°C, subcutaneous temperature was lower by 1°C and respiration rate was lower by 10 breaths min^{-1} compared to animals in pens without shade ($P = 0.0001$). Although dry matter intake was similar ($P = 0.6805$), cattle in pens with shade had higher average daily gain reflected in a heavier hot carcass weight (8 kg animal^{-1}; $P = 0.0002$). Considering an initial investment of $90 per animal to build a structure that lasts 15 years, the expected payback time is four finishing cycles (~110 days per cycle). In conclusion, this study confirms that the proposed novel shade design is economically profitable, improves performance, and enhances animal welfare.

KEYWORDS

animal welfare, heat load, cattle, shading, profitability, sustainable intensification

1. Introduction

Artificial shade is in the forefront of environmental modification to mitigate the negative impacts of heat stress and to improve welfare of beef cattle, particularly in tropical environments where animals face high levels of short-wave solar radiation (1, 2). The benefits of shade for animals come from reducing radiant heat gain (3, 4), body heat storage (5), evaporative cooling through panting and sweating (6–9) and by increasing the frequency of beneficial behaviors (e.g., lying down, ruminating, and playing (10–12).

Although the positive wellbeing and behavioral effects of providing shade for animals are unquestionable, their economic benefits are still inconclusive. Some studies have found positive outcomes on animal performance and economic gains (13, 14), others did not (10, 15). These economic uncertainties could perhaps be explained by the various shade structures and experimental designs tested in previous investigations. For example, use of 30 vs. 80%-blockage shade cloth, experiments with different animal categories (heifers, steers, and bulls), and different levels of heat load experienced by cattle (16). Some experiences of Brazilian beef producers were reported using shade cloth, mostly due to its lower cost of implementation over other shade materials (15, 17). However, limitation of this type of shade infrastructure lies on poor durability and life-span (18), especially if installed at locations with heavy windy and rainy conditions (19). The uncertainty on the economic return of shade implementation may therefore explain why beef producers are still reluctant on to the idea of providing artificial shades, e.g., $<20\%$ of feedlot in Brazil and US provide artificial shades (20–22).

In this study, we propose a novel concept of shade that could be used in tropical areas. It is a design that combines rectangular pens with a shade structure mounted in a north-south orientation. This design makes shade to be projected within the pens, motivates shading-seeking behavior of cattle, and efficiently protects them against short-wave solar radiation, while improves animal welfare, increases productivity performance, and offers an interesting 1–2 year payback time. To attest these benefits, 800 steers were randomly assigned in shaded and unshaded pens. The animals and the environment were monitored to (1) describe the thermal environment experienced by the animals, (2) determine the impact of shading on heat stress indicators, (3) assess the impacts of shading on dry matter intake, water intake, average daily gain, and hot carcass weight, and (4) provide analyses concerning economic outcomes of the shading structure. To the best of our knowledge, this is the first robust study to show the economic benefits of shade in feedlot cattle under commercial conditions of Brazil, where close to seven million cattle are kept in feedlots without shade (20). This issue is of great importance because Brazil is located in a tropical region where solar irradiance throughout the year is high and relatively constant, a climatic factor that poses great challenge to the welfare of livestock (4).

2. Material and methods

2.1. Location and design of the shading structure

The study was performed from November 2019 to March 2020 at the experimental facilities of a commercial feedlot in Altair, São Paulo, Brazil (Agro-Pastoril Paschoal Campanelli Research Center, 20°S latitude, altitude of 557 m) (Figure 1). Overall, climate classification according to Köppen-Geiger is B^2, with precipitation rate above 1200 mm $year^{-1}$ occurring at summer months (From November to February), and dry winters (From May to July). The novel concept of shade design used in this study comes from a shade structure that optimizes maximum availability and displacement of shade within pens, blockage against solar radiation, resilience to weather adverse conditions, and ease of daily management operations. We proposed rectangular pens with 15 × 50 m (width × length) instead of square pens, and the shade structure installed on the north-south fence line. This design favors a moving shade from east to west within the pen, and motivates shade-seeking cattle to follow the shade, which results in reduced wet areas because of congregation of animals (Figure 2). Additional benefit of this design is that the shade structure on the fence line eliminates barriers to the equipment used to clean out the pens.

The shade structure was made from galvanized coated aluminum/zinc/silicon (55% × 43.5% × 1.5%) steel-roof structure (Galvalume; Companhia Siderúrgica Nacional, CSN). The anchors were made of steel instead of wood because the cost of steel is the same as that of wood in Brazil. The frame assembly was made of tubular beams which is easier to assemble (Figure 3). Pillars anchored with polyvinyl chloride (PVC) and concrete provide protection against corrosion. The feedlot environment is very corrosive because of high concentration of urine and feces. The height of the roof was 5 m and consisted of parallel tiers mounted 10 cm apart tied with double steel cables. This design reduces tension and gives more flexibility to the shade structure during windy weather. Moreover, the shade area that is lost by the openings is minimal.

2.2. Experimental design

All animal procedures were approved by the Animal Ethics Committee of the São Paulo State University (Protocol number 016339/19). A total of 800 *B. indicus* × *Bos taurus,* predominantly Nellore bulls were used in the study. Approximately 2 weeks before the beginning of the study, the animals were treated with anthelmintic medication (1 mL 20 kg BW of 10% fenbendazole, MSD Saúde Animal, São Paulo, Brazil), immunized against bovine respiratory disease (1 mL 45 kg of BW; MSD Saúde Animal, São Paulo, Brazil), and clostridia (5 ml $animal^{-1}$; Valée S/A Produtos Veterinários, Montes Claros, Brazil).

Sixteen soil-surfaced pens were used, eight of them had the novel shade design that provided 3.0 m^2 of shade floor area/animal. The 800 bulls were equally divided between pens with shade, and pens without shade (50 animals pen^{-1}). Each bull was provided with 15 m^2 space, 0.30 cm of concrete bunk space, and 3.0 × 0.8 × 0.25 m (length × width × height) water trough. The bulls were assigned in blocks by their fasted initial body weight ($BW_i \pm$ SEM) (Block 1 = 305 ± 0.10 kg; block 2 = 337.80 ± 0.10 kg; block 3 = 375.20 ± 0.01 kg; and block 4 = 413 ± 0.10 kg of BW_i), and weekly harvested (one block per week) in the order of heavier to lighter block. Furthermore, bulls were phenotypically characterized by a trained observer on the basis of muscularity, structure, body length, hip and wither height. They were then grouped into five different phenotypes: Nellore, Black Angus x Nellore, crossbred (Zebu breeds, mostly Nellore), crossbred (European dairy breeds), and crossbred (European beef breeds).

FIGURE 1
Experimental facilities of the commercial cattle feedlot in Altair, São Paulo, Brazil (20° 31′ 25″ S; 49° 03′ 32″ W).

2.3. Nutritional management

Prior to the experiment, bulls were purchased from nine different locations (average transportation distance of 480 km) and allocated in three 16 ha pastures of *Cynodon ssp.*, equipped with feeding bunk. During this phase, all animals received pre-experimental (maintenance) diet (Table 1), for at least 15 days, in order to reestablish physiological and ruminal conditions. During the experimental period, bulls were fed with adaptation (days 1 to 14), growing (days 15 to 35) and finishing diets (block 4 = days 36 to 109; block 3 = days 36 to 114, block 2: days 36 to 119; and block 1: days 36 to 124), formulated to meet or exceed an average daily gain (ADG, kg day^{-1}) of 1.5 kg (Table 2) (24). Bulls were fed twice daily (08:30h and 15:00h), and visual bunk score calls were daily recorded at 06:45h (25). Diet dry matter adjustments of ingredients were then performed on a daily basis using a Koster Moisture Tester (Model D, Koster Crop Tester Inc. Medina Ohio, USA). Feed refusals of every pen were daily collected and weighted before the first meal. Treatment composite samples of ration, refusals and feedstuffs were daily and weekly collected (respectively) and then frozen at −20°C to determine dry matter intake (DMI, kg animal^{-1} day^{-1}) and for chemical analyses. All the samples from diets were dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were grounded with a Wiley-type mill (1 mm screen, MA-680, Marconi Ltda, Piracicaba, São Paulo, Brazil) and analyzed for ash (method 924.05) (26), NDF (27), CP (28) and EE (method 920.85) (29).

2.2.1. Water intake and performance data

Water was freely available. Each water trough was equipped with hydrometers to determine daily water intake (DWI; m^3 animal^{-1} day^{-1}). Hydrometer readings were taken every morning. The average daily water intake (DWI, m^3 animal^{-1} day^{-1}), dry matter intake (DMI, kg animal^{-1} day^{-1}), average daily gain (ADG, kg animal^{-1} day^{-1}), and conversion (FC, feed: gain ratio) were determined for each pen. The DWI was determined by measuring water flow to the water troughs as follows:

$$DWI_i = \frac{(H_2O)_{ij}}{N_{ij}}$$

Where, H_2O is the amount of water intake in the i^{th} ordinal day of the experiment in the j^{th} pen ($j = 1,\ldots,16$) and N is the number of animals in the i^{th} ordinal day of the experiment in the j^{th} pen. The DMI was determined based on the difference of the offered and refused feed, as follows:

$$DMI_i = \frac{(f_o - f_r)_{ij}}{N_{ij}}$$

Where, f_o is the amount of dry matter offered (kg day^{-1}), while f_r is the amount of dry matter refused collected daily at the feed bunk. The ADG were calculated based on fasted initial body weight (BW_i, kg animal^{-1}; 16 h of feed and water withdrawal) and fasted final body weight (BW_f, kg animal^{-1}, 16 h of feed and water withdrawal) as follows,

$$\text{ADG} = \frac{\text{BW}_f - \text{BW}_i}{n}$$

Where, n is the total number of days on feeding. The BW_i and BW_f were obtained using a scale that was regularly calibrated with

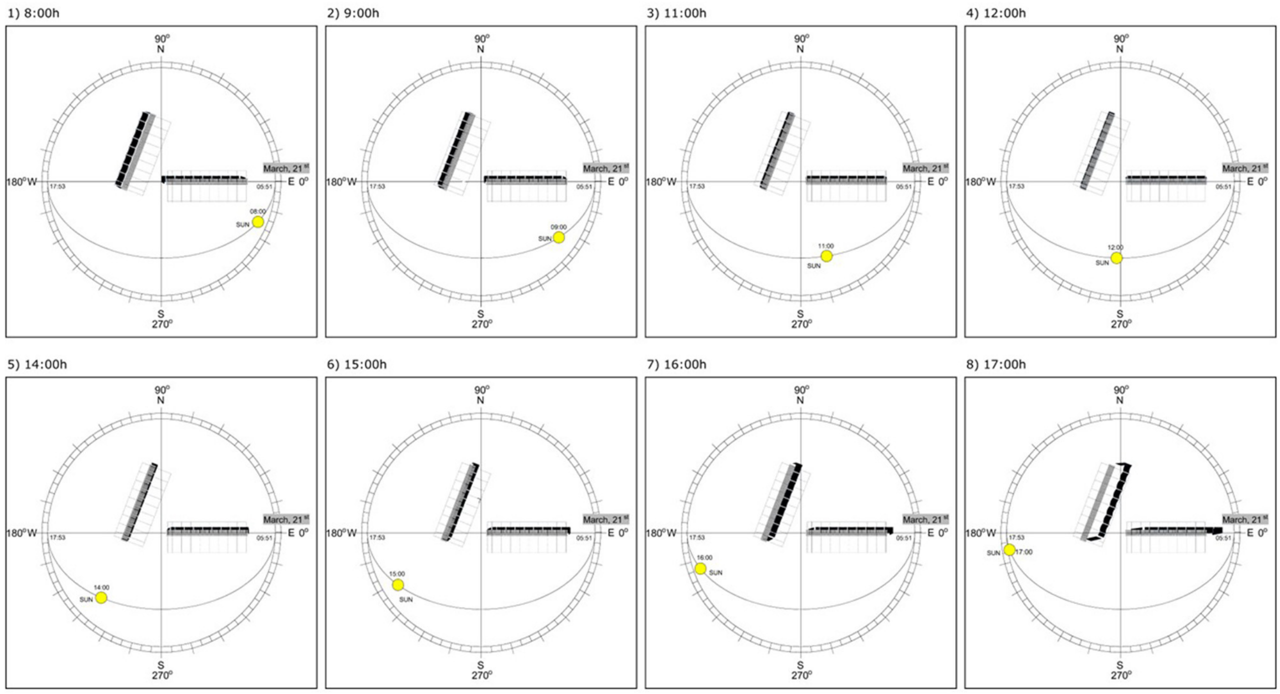

FIGURE 2
Layout of the shade displacement (from 08:00 to 17:00 h) of the shade structure at 18° of North-South orientation. The shade displacement is also simulated for East-West orientation. These simulations were made considering the latitude of the experimental site, and for the day 120 (Julian day, march, 21) of the year (2022). The space filled in black represents shade projected.

450 kg standard-weight. The feed conversion (FC) was calculated as follows,

$$\mathrm{FC} = \frac{DMI}{\mathrm{ADG}}$$

After reaching their expected BW_f, the animals were transported (330 km) to a commercial packing plant, and hot carcass weight (HCW, kg animal^{-1}) was obtained after complete evisceration and remotion of kidney, pelvic and heart fat of the carcass.

2.2.2. Meteorological data

Solar irradiance (R_S, W m^{-2}; CMP-22, Kipp and Zonen, Delft, Netherlands; spectral range = 0.3–3.6 μm), ultraviolet solar radiation (U_V, W m^{-2}; spectral range = 0.28–0.4 μm), air temperature (T_A,°C; range = −40 to +70, accuracy ± 0.1°C), black-globe temperature in the sun (T_{Gsun},°C; accuracy ± 0.1°C), relative humidity (R_H, %; accuracy ± 3 %), wind speed (W_S, m s^{-1}; accuracy ± 0.44, m s^{-1}) and daily precipitation (P, mm h^{-1}), were all continuously recorded every minute with a portable weather station (WS-18 model 110, Nova Lynk, Auburn, CA, USA) placed near (∼50 m) the pens with shade and without shade. Temperature sensors were also placed inside the pens and water troughs to better characterize the microclimate experienced by the bullocks in the shaded and unshaded pens. These measurements were recorded every 5 min.

A set of six black-globe devices were placed in two shaded pens (Figure 4), and three black globes were placed in three unshaded pens, positioned two meters above the ground surface. Miniature data loggers (i-bottom DS1925L, Maxim Integrated, Sao Jose, US; size = 0.60 × 1.70 cm, height × diameter; accuracy ± 0.5°C) were inserted inside globes for measuring black-globe temperature. The black-globe devices in pens with shade were placed underneath the roof ($T_{Gshade1,}$°C) and exposed to clear sky ($T_{Gshade2,}$ $T_{Gshade3,}$°C). Three temperature sensors (i-buttom) were previously waxed (Sasol wax, GmbH D-20457) and placed inside the water troughs to obtain water temperature (°C). The ground surface temperature underneath the shade and in the full sun were periodically measured with an infrared thermal camera (FLIR SC660; Wilsonville, USA; temperature range = −40°C to 1500°C; spectral range = 7.5 to 13 μm; adjustable emissivity range = 0.1 to 1; resolution ± 0.04°C; accuracy ± 1°C).

2.2.3. Thermal stress indicators

Subcutaneous temperature (T_{SC},°C), respiratory rate (R_R, breaths min^{-1}), and body surface temperature (T_S,°C) were measured both in bulls housed in shaded and unshaded pens. Behavior of bulls in the shaded pens were also observed. Ten days before the experimental period, miniature implantable bio-loggers (i-buttom DS1922L, Maxim Integrated, Sao Jose, US; size = 0.60 × 1.70 cm, height × diameter; accuracy ± 0.06°C) were surgically implanted in forty animals, twenty in the shaded pens, and the other twenty in the pens without shade. Before the implantation, all the loggers were calibrated at 2°C increments between 30 and 42°C in a thermally insulated box against a highly-accurate thermocouple (Type K; temperature range = −40 to 1300°C; accuracy ± 0.2°C). Logging and storage of T_{SC} data was set to measure every 2 min during the experimental period.

The R_R and T_S were recorded every 30 min from 08:00 to 17:00 h, on 80 animals (40 in the shaded and another 40 in the unshaded

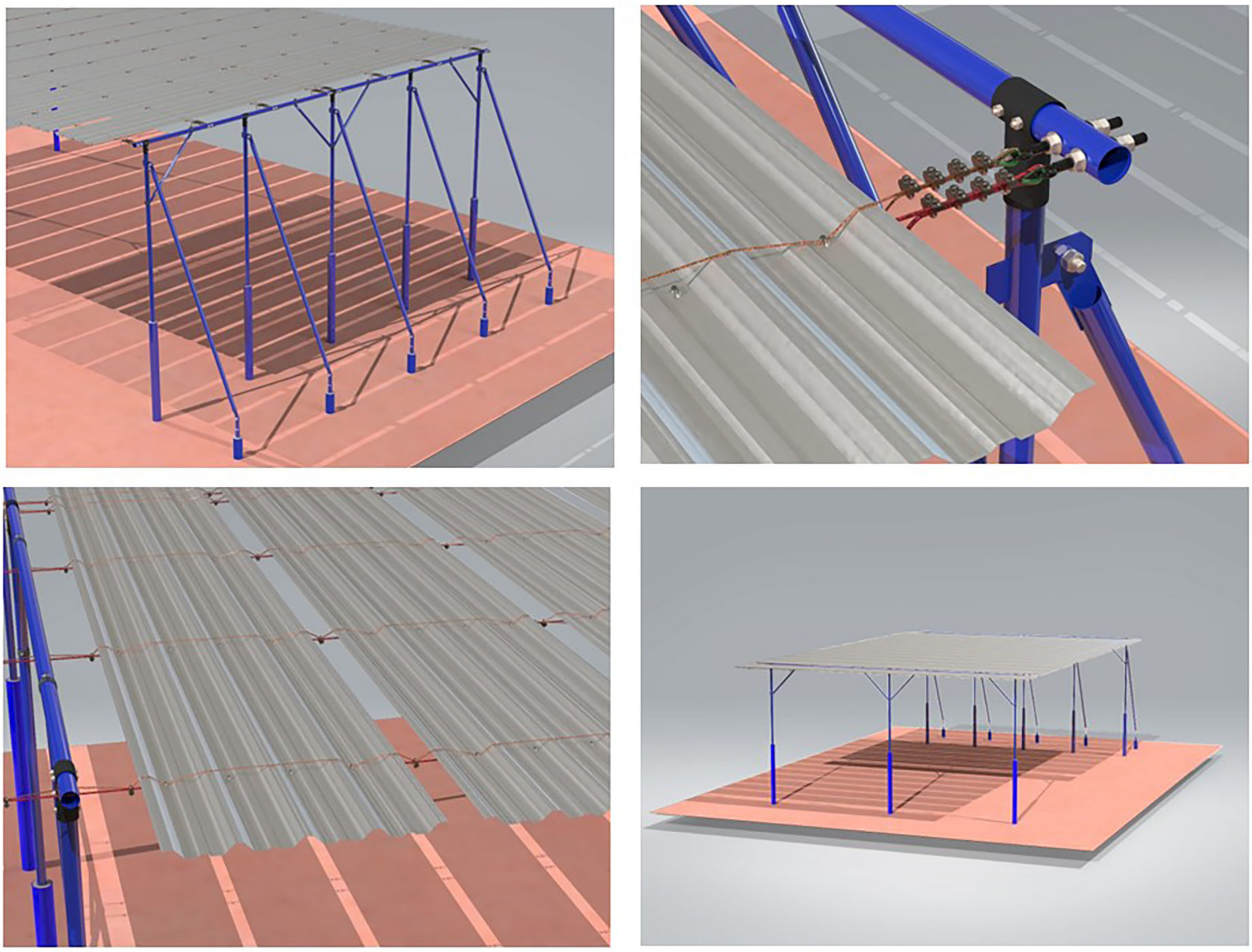

FIGURE 3
Principal components of the shade structure.

pens) during 20 days. Respiration rate was observed by five observers positioned outside the pens (~10 m apart from the animals) by visually counting the flank movements of the bulls. Body surface temperature was measured using an infrared thermal camera (FLIR SC660; emissivity = 0.98) scanning the dorsal right side of the animals at a distance of 3.0 m. For each bull, the T_S was considered as a mean of the scanned dorsal right side, from the scapular to rump region. Images were analyzed using FLIR Thermal Studio. Number of bulls using shade or at feed bunk was periodically monitored by using direct, focal and instantaneous samplings every 15 min, from 08:00 to 17:00 h. A total of 1500 observations from 250 animals were scanned along 20 days during adaptation, growing and finishing phase. Shade use was recorded whenever the head or one of the hooves of an animal was underneath the shade area. If not, it was considered as exposed to solar radiation. Feed bunk use was recorded whenever an animal was at standing position close (~0.5 m) to the feed bunk.

TABLE 1 Values used in the economic analyses of the shade structure.

Variables (Running costs)	Treatments (pens)	
	Shaded	Unshaded
Carcass value, R$ 15 kg^{-1}	55.00	55.00
Cost of shade implemented, R$ animal^{-1}	90.00	0
Capital cost per feedlot cycle, %	5	5
Funrural tax, %	2.1	2.1
Earned income tax, %	10	10
Animal transport guide fees, R$ animal^{-1}	2.60	2.60
Benefits		
Hot carcass weight, kg animal^{-1}	326.51	317.97
Carcass value at slaughter, R$ animal^{-1}	55.00	55.00
Lifespan of the shade structure, years	15	-

All monetary values are in USD$.

2.3. Economic outcomes

To determine the economic outcomes and the payback time of the proposed shade design, we calculated the net present value (NPV) as

$$NPV = \left[\frac{CF_1}{(1+r)^1} + \frac{CF_2}{(1+r)^2} + \frac{CF_1}{(1+r)^3} + \frac{CF_n}{(1+r)^n}\right] - \Pi_0$$

TABLE 2 Ingredients and nutrients of feed diets used in the shaded and unshaded pens.

Feed ingredient (%, DM basis)	Diets			
	Pre-exp	Adaptation	Growing	Finishing
High moisture corn	6.85	8.50	10.65	14.95
Citrus pulp	9.86	27.40	35.80	36.45
Cottonseed meal	-	8.90	8.70	10.30
Soybean meal	15.07	15.07	12.60	9.35
Sugar cane bagasse	20.55	5.50	5.80	3.75
Sugar cane silage	30.82	-	-	-
Corn silage	-	19.20	13.00	11.20
Protected fat[b]	-	-	2.30	3.10
Urea	0.91	0.90	0.95	1.00
Molasses	13.70	12.35	7.85	7.50
Tracemineral supplement[a]	3.15	2.30	2.40	2.45
Nutrients, %				
Dry matter	45.00	62.00	68.00	69.00
Crude protein	12.80	16.00	14.60	13.70
Degradable intake protein (% of crude protein)	79.00	80.00	77.00	79.00
Non-fibrous carbohydrates	42.00	47.00	48.00	49.00
Total digestible nutrients	75.00	86.00	85.00	88.00
peNDF[d]	21.00	19.00	17.00	16.00
Ca	0.93	1.25	1.45	1.47
P	0.42	0.45	0.40	0.40
NEm, Mcal/kg[c]	1.49	1.85	1.85	1.90
NEg, Mcal/kg[c]	0.90	1.25	1.20	1.30

[a] Calcium 165.0 g/kg, phosphorus 23.0 g/kg, cobalt 25.0 mg/kg, copper 420.0 mg/kg, sodium 40.0 g/kg, sulfur 14.0 g/kg, iodine 25.0 mg/kg, magnesium 15.0 g/kg, manganese 810.0 mg/kg, selenium 15.0 mg/kg, zinc 1,500.0 mg/kg, iron 0 mg/kg, vitamin A 72,000 IU/kg, vitamin D3 14,370 IU/kg, vitamin E 500 IU/kg, monensin 714.0 mg/kg, virginiamycin 714.0 mg/kg.

[b] Calcium soap, approximately 86% of fatty acids and 14% of calcium.

[c] Estimated by L.R.NS.

[d] Fraction of the fiber that stimulates chewing activity and maintains a healthy rumen environment by combining the chemical and physical properties of feeds (23).

Where, Π_0 is the initial investment of the shade structure ($), $FC_{(n)}$ is net cash flow for a given period of days; r is the cost of the capital, and n is the number of days of each feedlot cycle (∼110 days). We estimated the initial investment of the artificial shade structure in USD$ 90.00 animal^{-1} (USD$ 30.00 per m^2 of projected shade, assuming 3 m^2 animal^{-1}).

2.4. Statistical analyses

2.4.1. Heat load experienced by feedlot cattle

Principal component analyses (30–34) were used to observe dissimilarities of the days of feeding for the meteorological conditions (T_A, H_R, R_S, U, W_S and T_G) experienced by the animals. Principal components were obtained by computing eigenvalues (λ_i) and its respective eigenvectors $e_i' = [e_{i1}\ e_{i2}\ e_{i3}]$ of the data correlation matrix. Bi-dimensional representation of the multidimensional set was created by using scores of the first $(PCA_{1j} = e_{11}T_A + e_{12}H_R + e_{13}R_S + e_{14}U + e_{15}W_S + e_{16}T_G)$, and second principal component $(PCA_{2j} = e_{21}T_A + e_{22}H_R + e_{23}R_S + e_{24}U + e_{25}W_S + e_{26}T_G)$. All principal components were used in order to develop an environmental index, the *InComfort Index* (InCI) based on the Membership Function Value Analysis:

$$\mathrm{InCI} = \sum_{i=1}^{n} \left[\mathrm{R}\left(\lambda_i\right) \mathrm{W}\left(\mathrm{e_i}\right)\right]$$

Where, n is the number of principal components and InCI is the weighted membership value calculated with principal components for each day linked with its meteorological condition and level of heat stress experienced by the animals. The $R(\lambda_i)$ is given by

$$\mathrm{R}\left(\lambda_i\right) = \frac{\lambda_i - \lambda_{i(\min)}}{\lambda_{i(\max)} - \lambda_{i(\min)}}$$

Where, λ_i is the value of i^{th} principal component, $\lambda_{i(\min)}$ and $\lambda_{i(\max)}$ are the maximum and minimum values of i^{th} principal component, respectively. The $W(e_i)$ is given by

$$\mathrm{W}\left(\mathrm{e_i}\right) = \mathrm{e_i} / \sum_{i=1}^{n} \mathrm{e_i}$$

Where, $W(e_i)$ is the weight of the i^{th} principal component among all the principal components selected for evaluating level of heat stress experienced by animals on i^{th} day, and e_i is the contribution rate of the i^{th} principal component. Based on daily water intake and respiratory rate, the InCI were grouped into four classes: rainy days, when the mean is $0 \leq \mathrm{InCI} \leq 1$, and precipitation rate above 20 mm day^{-1}; cloudy days, when the mean is $0 \leq \mathrm{InCI} \leq 0.4$; hot days, when $0.4 < \mathrm{InCI} \leq 0.6$; and very hot days, when the mean is $0.6 < \mathrm{InCI} \leq 1$.

2.4.2. Confirmatory models

In this study, we investigated the effects of the following independent variables on cattle performance and heat stress responses: shade vs. no shade, weight block, coat color (black vs. light-colored cattle), time of day, heat load experienced during the days of feeding and association between them. Confirmatory models were then fitted by applying conventional statistical techniques

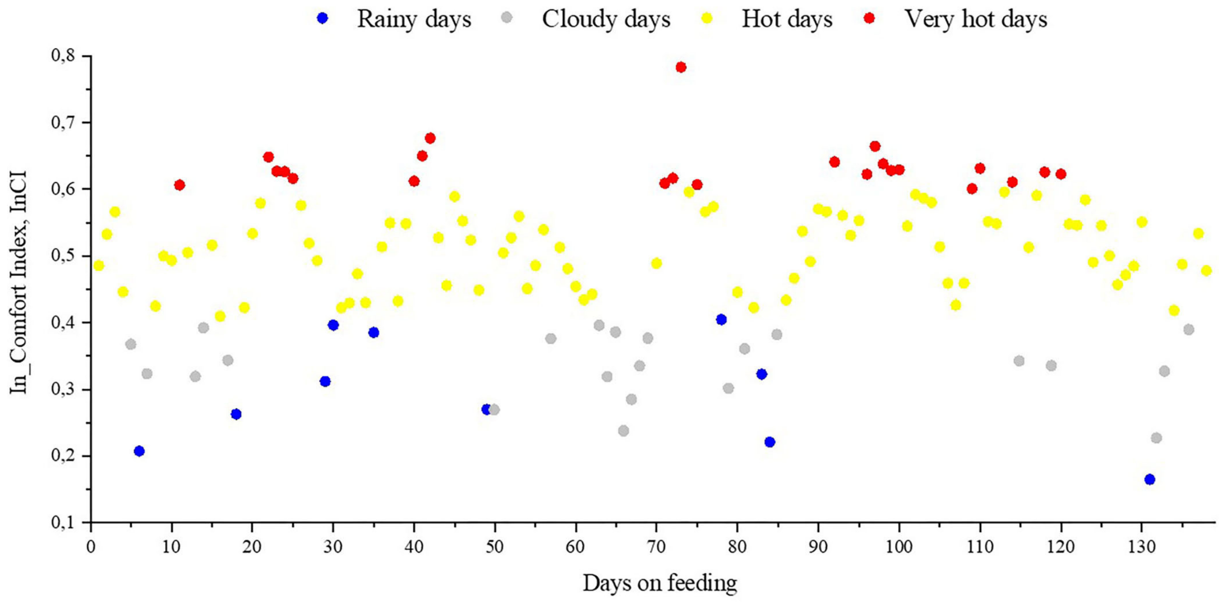

FIGURE 4
Daily mean values of the InComfort Index (InCI) over the days on feeding. Rainy days ($0 \leq \text{InCI} \geq 1$, and precipitation rate above 20 mm d^{-1}); cloudy days ($0 \leq \text{InCI} \geq 0.4$); hot days ($0.4 \leq \text{InCI} \geq 0.6$); very hot days ($0.6 \leq \text{InCI} \geq 1$). There were 8 days classified as rainy, 24 as cloudy, 83 as hot, and 23 as very hot over the days on feeding. Refer to Table 3 and Figure 5 for details of meteorological conditions experience by feedlot cattle in each class of days.

TABLE 3 Meteorological variables (mean, min, and max) during the study period according to environmental index (InComfort, InCI).

Meteorological variables	Classes of InCI[†]			
	Rainy days (*n* = 8)	Cloudy days (*n* = 24)	Hot days (*n* = 83)	Very hot days (*n* = 23)
Air temperature,°C	23.80 (18.0–37.5)	24.5 (14.0–37.7)	26.0 (15.7–38.5)	28.0 (18.0–41.0)
*Black globe temperature,°C	27.5 (16.8–48.2)	26.0 (12.3–48.3)	28.8 (14.5–53.5)	31.5 (16.8–55.0)
Relative humidity, %	80.0 (35–100)	75.5 (23.0–97.0)	70.0 (21.0–98.0)	66.0 (25.0–98)
Solar irradiance, W m^{-2}	265.75 (0–1100)	264.60 (0–1090)	488 (0–1199)	635 (0–1250)
Daily precipitation, mm d^{-1}	43 (21–106)	2.12 (0–14)	1.76 (0–18)	2.05 (0–4.5)
Wind speed, m s^{-1}	2.0 (0–15)	3.0 (0–14)	3.0 (0–15)	3.0 (0–10)
Water temperature,°C	25 (23–30)	26 (22.8–28)	28 (23–30)	29 (22.9–31)

*Black globe temperature taken in the shade; [†] Rainy days ($0 \leq \text{InCI} \geq 1$, and precipitation rate above 20 mm d^{-1}); cloudy days ($0 \leq \text{InCI} \geq 0.4$); hot days ($0.4 \leq \text{InCI} \geq 0.6$); very hot days ($0.6 \leq \text{InCI} \geq 1$).

through mixed model based on Generalized Least Squares (GLS) using the Statistical Analysis System [SAS Institute, Version 8; (35)]. Because of the repeated nature of the data (e.g., days on feeding and time of day), the covariance structure of the model must be chosen carefully (36). Different covariance structures were tested (compound symmetry, first-order auto regression, Toeplitz, first-order ante-dependence and others) and the best covariance structure was chosen based on the Akaike's information criterion (AIC), AIC corrected (AICC), and Bayesian information criterion (BIC). To choose the best fitted models that predict dry matter intake (a), daily water intake (b) average daily gain, and (c) hot carcass weight (d) the following independent variables were considered:

$$\begin{aligned} Y_{ijkl} &= \mu + S_i + B_j + SB_{ij} + H_k + SH_{ik} + SBH_{ijk} + D_l(SBH)_{ijk} \\ &\quad + \varepsilon_{ijklm} \qquad [a,\ b] \\ Y_{ijkl} &= \mu + S_i + SP_{ij} + B_k + SB_{ik} + C_l(SB)_{ij} + \varepsilon_{ijklm} \qquad [c,\ d] \end{aligned}$$

Where, Y_{ijkl} is the *n*th measurement of the dependent variable; S is the fixed term of the *i*th shade treatment (S = shade and unshaded pens); B is the fixed effect of the *j*th class of initial fasted body weight [B = block 1 (305 kg), block 2 (337.80 kg), block 3 (375.20 kg), block 4 (413 kg)]; SB is the interaction between the *i*th shade treatment and *j*th class of body weight; H is the fixed effect of the *k*th class of the InComfort index (H = rainy, cloudy, hot and very hot days); SH is the interaction between the *j*th shade treatment and *k*th class of the InComfort index; SBH is the interaction between the *i*th shade treatment, *j*th class of body weight and *k*th class of the InComfort index; and D is the fixed effect of the *l*th days on feeding (D = 1,...,124 days) within the interaction between *i*th shade treatment, *j*th class of body weight and *k*th class of the InComfort index. Models c and d had SP as interaction between *i*th shade treatment and *j*th phenotypic trait of cattle, and C is the fixed effect of the *l*th corral pen within the interaction between *i*th shade treatment and *k*th class of body weight shade treatment. The μ is the parametric mean and

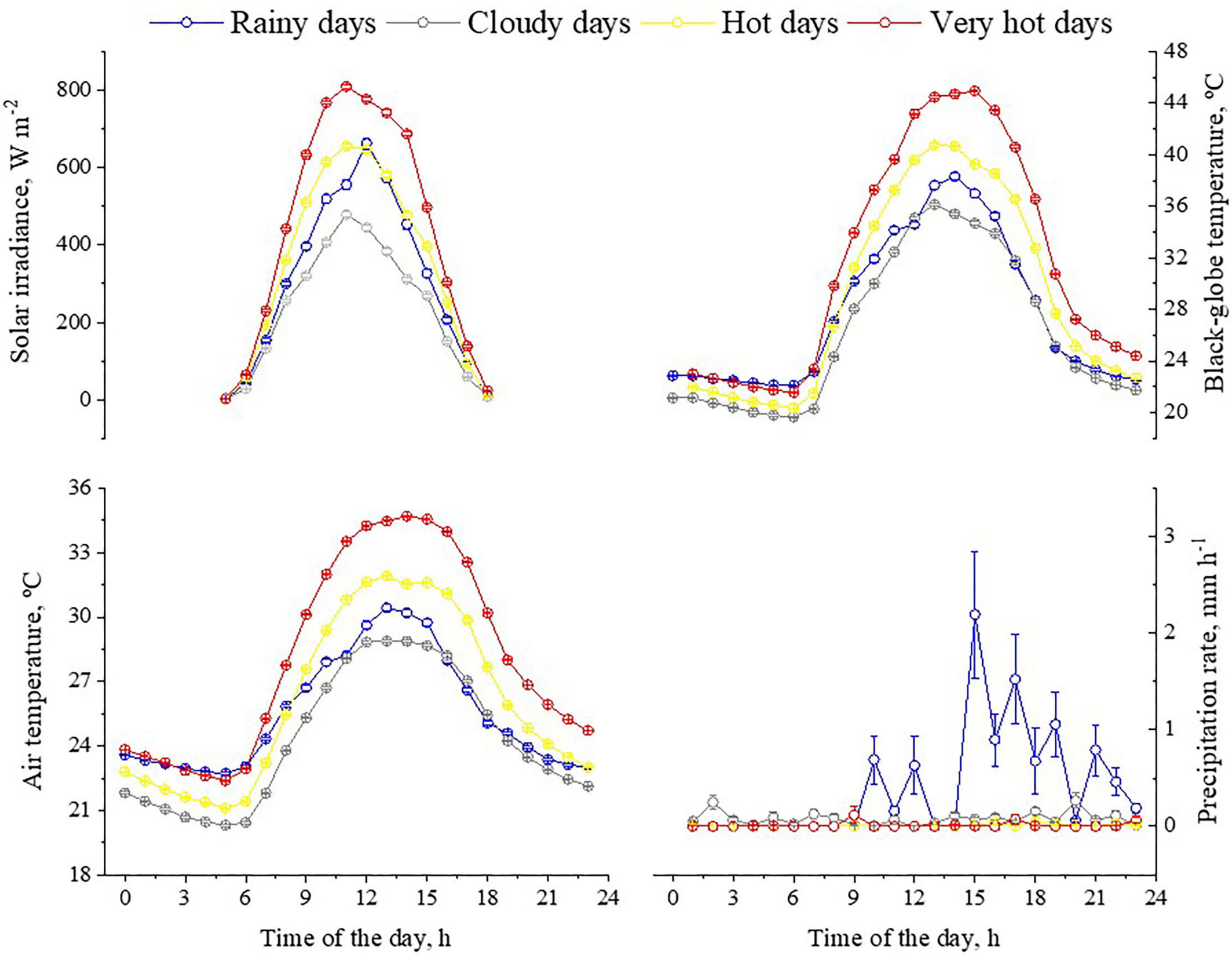

FIGURE 5

Least square (±SEM) means of the meteorological variables measured during the experimental period in accordance to the classes of the InComfort Index (InCI). Classes of the InCI: Rainy days (0 ≤ InCI ≥ 1, and precipitation rate above 20 mm d^{-1}); cloudy days (0 ≤ InCI ≥ 0.4); hot days (0.4 ≤ InCI ≥ 0.6); very hot days (0.6 ≤ InCI ≥ 1).

$\varepsilon_{\mathrm{ijklm}}$ is the residual term. For subcutaneous temperature (e), body surface (f), and respiratory rate (g) the best fitted models were:

$$\begin{aligned} Y_{ijklm} &= \mu + S_{\mathrm{i}} + C_{\mathrm{j}} + SC_{\mathrm{ij}} + H_{\mathrm{k}} + D_{\mathrm{l}}(H)_{\mathrm{k}} + T_{\mathrm{m}} \\ &\quad + SCHT_{\mathrm{ijkm}} + \varepsilon_{\mathrm{ijklm}} && [e] \\ Y_{ijk} &= \mu + S_{\mathrm{i}} + C_{\mathrm{j}} + SCT_{\mathrm{ijk}} + \varepsilon_{\mathrm{ijkl}} && [f, g, h] \end{aligned}$$

Where, S is the fixed term of the *i*th shade treatment; C is the fixed effect of the *j*th coat color (C = Dark and light colored cattle); SC is the interaction between the *i*th shade treatment and *j*th coat color; H is the fixed effect of the *k*th class of the InComfort index; D is the fixed effect of the *l*th days on feeding within *k*th class of the thermal comfort; T is the fixed effect of the *m*th time of the day (T = 1,..., 24 h); and SCHT is the interaction between *i*th shade treatment, *j*th coat color, *k*th class of the InComfort index and *m*th time of the day. For models f, g, and h, SCT is the interaction between *i*th shade treatment, *j*th coat color and *k*th classes of solar irradiance. $\varepsilon_{\mathrm{ijklm}}$ and $\varepsilon_{\mathrm{ijkl}}$ are residual terms. Behavioral data were analyzed using nonparametric regression analyses through the Generalized Additive Models (GAM Procedure), by fitting air temperature, black-globe temperature, solar irradiance, and wind speed as independent variables.

3. Results and discussion

We confirmed the hypothesis that the proposed novel shade design not only benefits cattle comfort, but is also economically sustainable. Three main findings support this hypothesis: First, based on the radiant temperatures taken within the shade and full sun, the structure with shade efficiently reduced radiant heat load on the animals, especially on very hot days (Figure 7). During these days heat stress indicators were significantly reduced compared to bulls without access to shade. Overall, the values were 5°C lower in body-surface temperature, 1°C lower in subcutaneous temperature, 10 breaths $\min^{-1}$ lower for respiration rate, and 3.4 L animal^{-1} lower for water intake (Figures 6, 7, 9, 10; $P = 0.0001$). Even though dry matter intake was similar ($P = 0.6805$) for the bulls in shade and no shade (Table 4), those in pen with shade presented better feed conversion ($P = 0.0004$) and

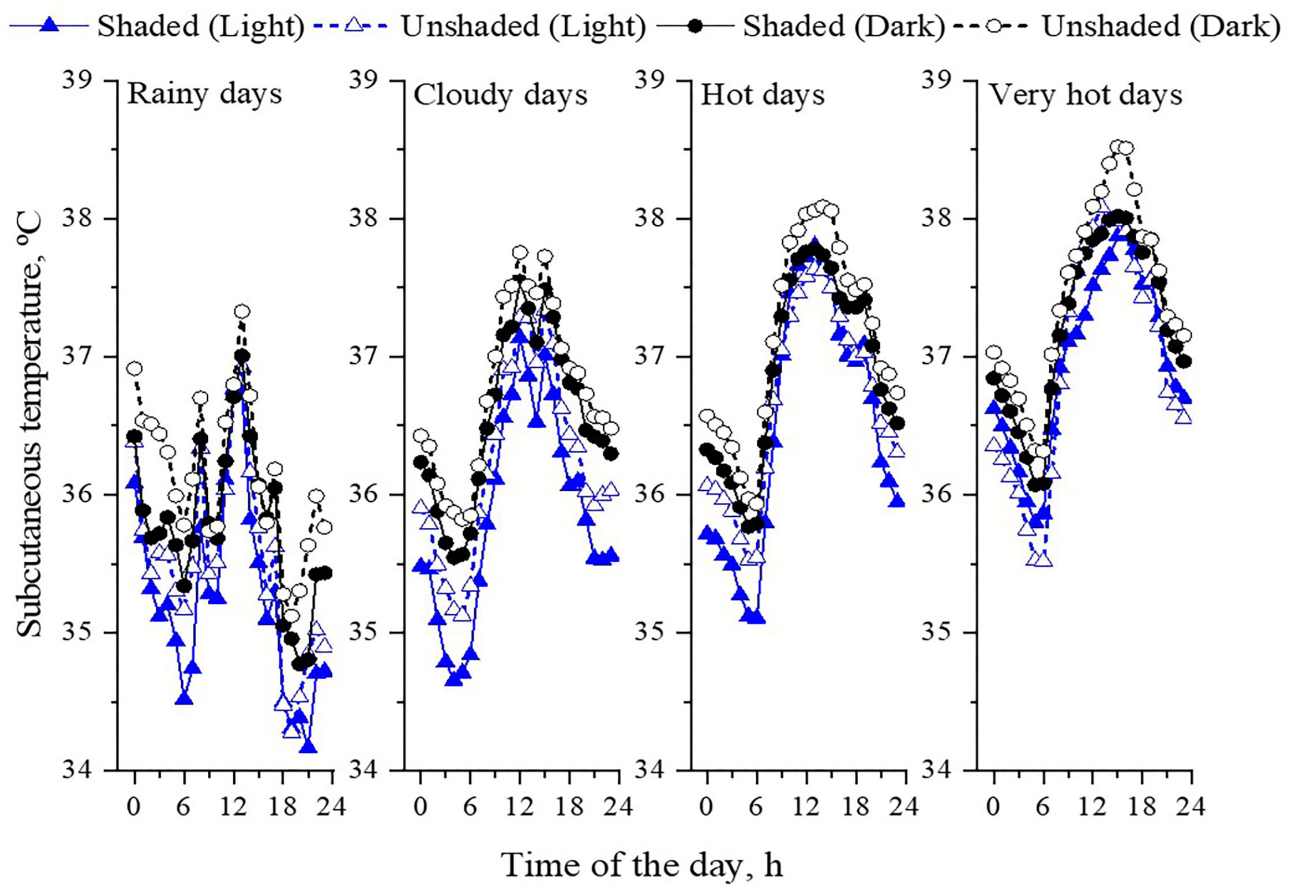

FIGURE 6
Daily pattern of subcutaneous temperature in light (predominantly Nellore) and black colored (*Bos indicus* x *Bos taurus*), shaded and unshaded bulls in accordance to the classes of the InComfort Index (InCI). Classes of the InCI: Rainy days (0 ≤ InCI ≥ 1, and precipitation rate above 20 mm d^{-1}); cloudy days (0 ≤ InCI ≥ 0.4); hot days (0.4 ≤ InCI ≥ 0.6); very hot days (0.6 ≤ InCI ≥ 1).

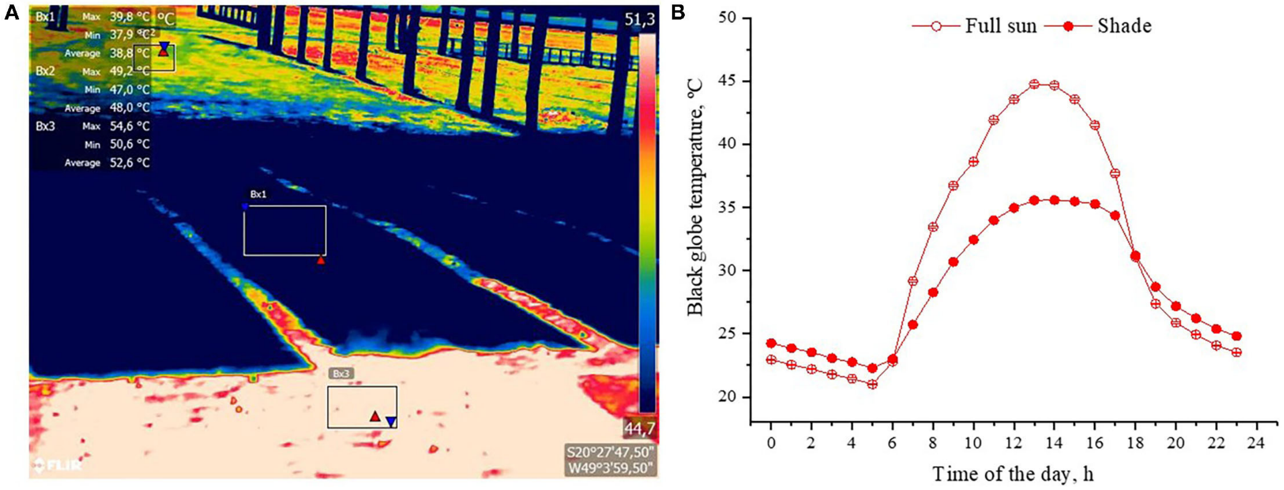

FIGURE 7
Radiant heat load experienced by feedlot cattle; **(A)** thermogram of a shaded and unshaded ground surface. Meteorological conditions that this thermal image was captured were: Air temperature = 35°C; solar irradiance = 850 W m^{-2}; **(B)** Least square means (±SEM) of the black globe temperature taken in full sun and shade in days classified as very hot (0.6 ≤ InCI ≥ 1).

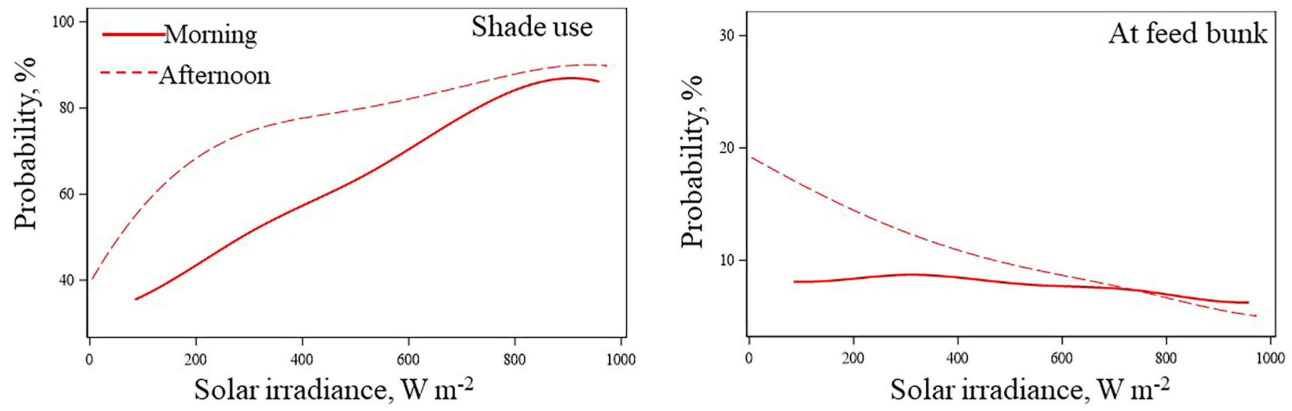

FIGURE 8
Estimated probabilities as a function of solar irradiance for bulls to be in shade or at the feed bunk.

heavier hot carcass weight ($P = 0.0002$; Figure 11). The increment on hot carcass weight of the bulls in pens with shade was sufficient reason to make the proposed shade design economically viable. The payback time is expected to be within four feeding cycles (Figure 12).

The meteorological conditions experienced by the feedlot cattle were characterized by a single variable, an environmental index for beef cattle (InComfort Index, InCI). The variable accounted for the combined effects of solar irradiance, black-globe and air temperature, humidity, wind speed, and precipitation rate. Feeding days were grouped into four classes (Table 3; Figures 4, 5). During the experimental period, the bullocks were exposed for 100 days to hot and very hot conditions (e.g., 23 very hot days and 83 hot days). During these days, from 10:00 to 15:00h, mean solar radiation exceeded 600 W m^{-2}, black-globe temperature was above 40°C, and air temperature was above 30°C. In addition to hot conditions, bulls were exposed to 23 cloudy days, during which the mean solar irradiance remained below 600 W m^{-2}, and mean air temperature was below 30°C. Bulls were also exposed to eight rainy days, during which the mean precipitation rate ranged from 0 to 0.25 mm h^{-1}. Based on our previous study with similar animals, feedlot cattle were outside their zone of least thermoregulation during the days classified as hot and very hot (8, 37–40). Indeed, bulls stored more heat during hot and very hot days compared to the cooler days (i.e., rainy and cloudy days), especially the animals with dark hair coat and with no access to shade (Figure 6).

Our study well attested that shade alleviated heat stress responses of cattle, mostly due to the abatement of radiant heat gain through two main sources: From direct and diffuse short-wave solar radiation, where on hottest times of hot and very hot days, by seeking shade, cattle could avoid levels of solar irradiance as much as 1200 W m^{-2} (Table 3). Animals with dark hair coat absorb almost three times more solar radiation than those with light hair (4, 5, 41). This difference in solar load explains why the body surface temperature of dark bulls is higher compared to light-haired cattle (Figure 9). Animals in shade also received less long-wave radiation emitted from the shaded surface. During the hottest time of the very hot days, the radiant temperature of the shaded ground surface was 15°C lower than the unshaded areas, a difference that represents 100 W m^{-2} less long-wave radiation being emitted from the ground surface (Figure 7). Bulls in pens with shade also experience less heat gain by conduction when lying down on shaded surfaces.

Maximum shade utilization of cattle occurred when levels of solar irradiance exceeded 800 W m^{-2}, either in the morning or afternoon (Figure 8; probability higher than 80%), a level of thermal radiation that also coincided with the fewer probabilities for bulls to be at feed bunk. Bulls in shade had their respiratory rate reduced and overall were less peripherally vasodilated than bulls in pens without shade as heat load increased (Figures 6, 9). The reduction was higher for cattle with dark hair coat. Similarly, Lees et al. (42) attested that both *B. taurus* dark hair coat (Black Angus) and *B. indicus* light hair coat cattle (Brahman) sought shade as the solar load increased, and panted less when they were in the shade. Considering that increments on respiratory rate and peripheral vasodilatation are positively correlated with mass and heat transfer through respiratory and cutaneous surface (8, 43), bulls in shade trigger less heat dissipation to maintain thermal equilibrium compared to bullocks with no access to shade.

When cattle are exposed to hot conditions and are unable to access shade, they can sustain high rates of evaporative water loss to maintain their thermal equilibrium, provided that body fluids can be replaced timeously (44), a physiological adjustment that increases requirements for water turnover. In this study, it was observed that bulls in pens with and without shade increased water intake during hotter days (Figure 10). However, bulls in pens without shade presented greater water intake than bullocks in shade (36.1 vs. 34.9 ± 0.19 L animal d^{-1}). The increase was greater during the very hot days (40.73 vs. 37.30 ± 0.47; Figure 10). (45) also reported greater water intake for *B. taurus* cattle housed in pens without shade compared to cattle in shade (53 vs. 49 L $animal^{-1}$ d^{-1}), particularly when exposed to hot days. In this study, using mean difference of 3.43 L $animal^{-1}$ d^{-1} for cattle in pens without shade vs. cattle in pens with shade during 23 very hot days, and extrapolating to 100 days in a year with similar meteorological conditions and for 20 000 animals, the use of shade would potentially save 6860 m^3 of clean water. The saving emphasizes the effectiveness of using shade to alleviate heat stress of cattle and reduce water consumption.

Use of the proposed novel shade design increased efficiency in dry matter intake. Both groups of cattle in shade and no shade ate less during hotter and rainy days. Bulls may decrease intake during

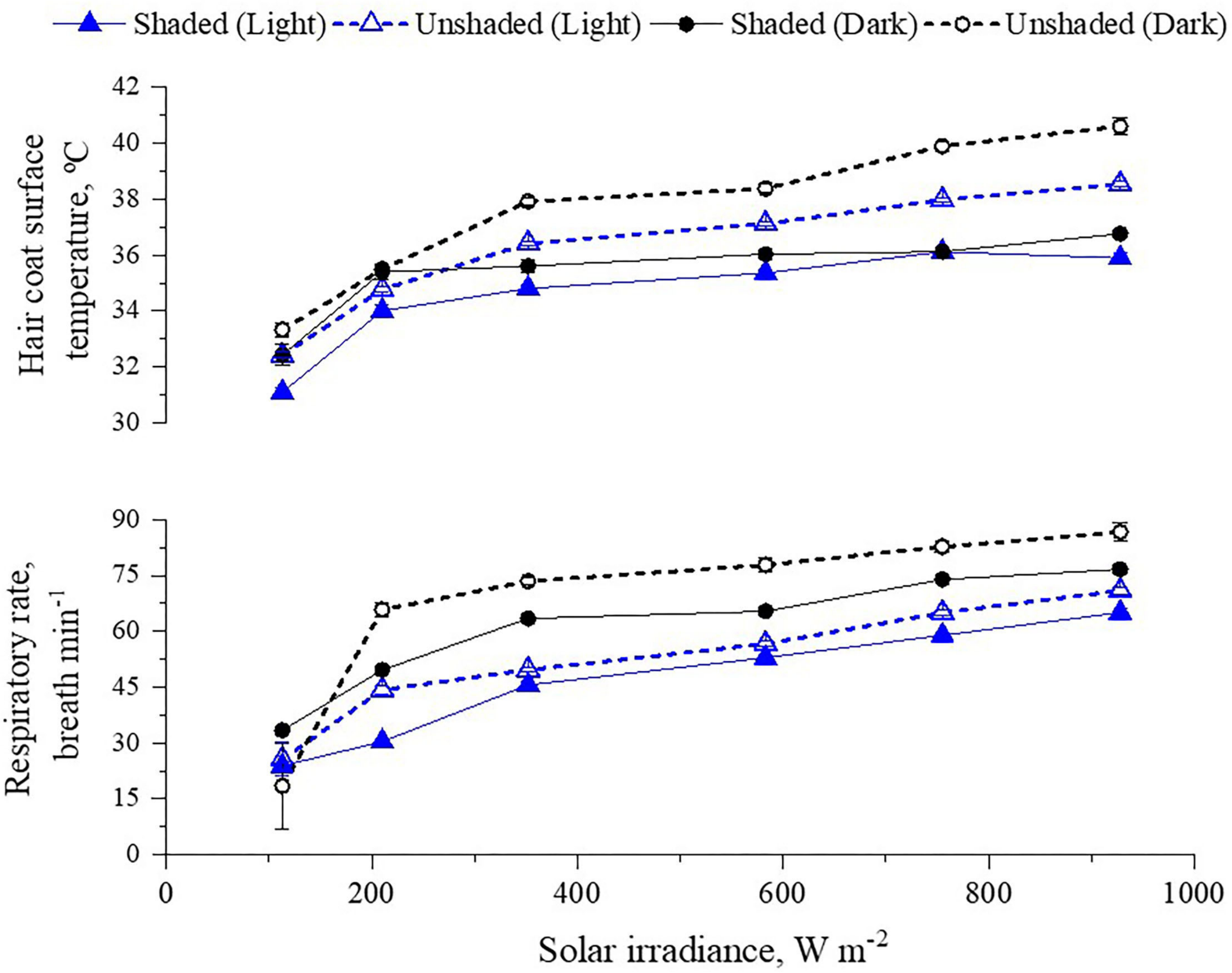

FIGURE 9
Least square means (±SEM) of hair coat surface temperature and respiratory rate of shaded and unshaded light- and dark-haired cattle according to the classes of solar irradiance.

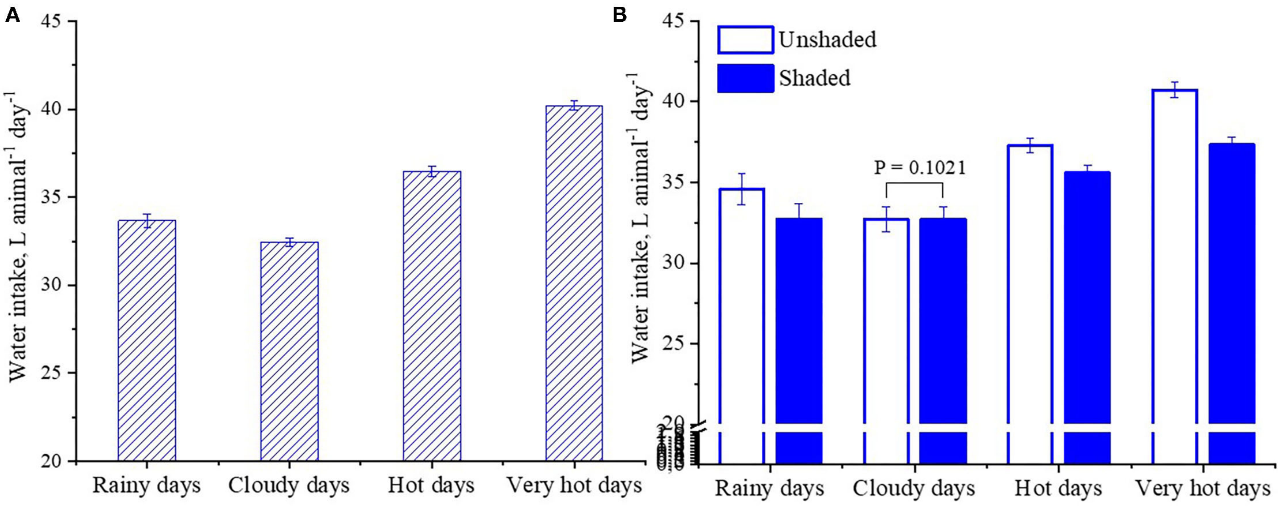

FIGURE 10
Least square means (±SEM) of daily water intake of feedlot cattle according to the classes of the InComfort Index (InCI). Classes of the InCI: Rainy days (0 ≤ InCI ≥ 1, and precipitation rate above 20 mm d^{-1}); cloudy days (0 ≤ InCI ≥ 0.4); hot days (0.4 ≤ InCI ≥ 0.6); very hot days (0.6 ≤ InCI ≥ 1). **(A)** Fixed effect of the InCI. **(B)** Effects of shade treatments within the InCI.

hotter days as a tentative mechanism to reduce heat generation by metabolism (2). During rainy days, we speculate that cattle consume less due to an overall reduction in activity and due to soaked feed in the bunks. The dry matter intake was not statistically different between cattle with or without shade availability (Table 4), an average of 11.45 and 11.51 ± 0.17 kg animal^{-1} d^{-1}, for shaded and unshaded cattle, respectively. However, cattle in pens with shade presented better feed conversion ($P = 0.0389$), which was nearly improved by 4.5 % (6.83 vs. 7.15 ± 0.10) as well as increased average daily gain ($P = 0.0004$) by 5% (1.657 vs. 1565 ± 0.01 kg animal^{-1} d^{-1}) compared to cattle housed in pens without shade (Table 4). This result shows that use of shade may reduce energy requirements for maintenance of feedlot cattle in tropical conditions.

The higher average daily gain of bulls in pens with shade reflected in heavier ($P = 0.0002$) hot carcass weight than those kept in pens without shade (327.02 ± 1.32 vs. 319.12 ± 1.27 kg; Figure 11). Phenotypes of cattle characterized as *B. indicus* Nellore, mean difference for hot carcass weight between cattle in shade and no shade pens was close to 5 kg, while those grouped as crossbred Angus x Nellore, the difference was nearly 15 kg, which suggests that less heat tolerant breeds of cattle can benefit more with heat alleviating strategies. Physiological, metabolic, and behavioral mechanisms of combating heat stress may affect gain efficiency of growing cattle (46). For example, when exposed to high heat load, more blood flows toward the skin to increase heat loss (47), a

TABLE 4 Performance data (least square means ±SEM) of shaded and unshaded cattle.

Item	Treatments (Pens)		SEM	*P*-value
	Unshaded	Shaded		
Initial body weight, kg	358.78	358.88	0.6895	0.9146
Final body weight, kg	562.96	574.55	2.1034	0.0001
Average daily gain, kg d^{-1}	1.565	1.657	0.0182	0.0004
Dry matter intake, kg d^{-1}	11.43	11.51	0.1730	0.6805
Dry matter intake, %	2.36	2.33	0.0383	0.6204
Feed conversion	7.15	6.83	0.1036	0.0389
Hot carcass weight, kg	317.97	326.51	1.5917	0.0002
Dressing percentage	56.62	56.87	0.1091	0.1110

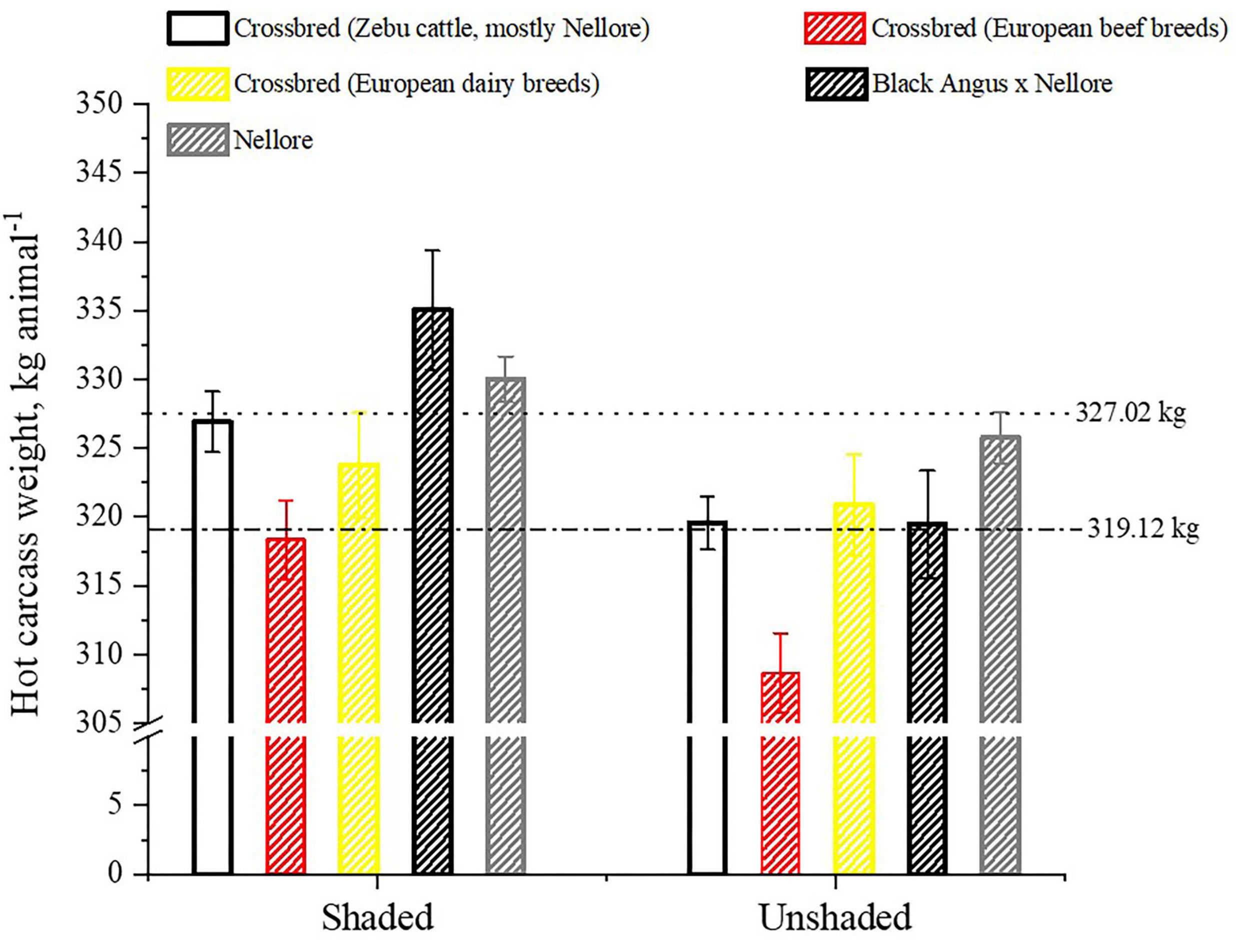

FIGURE 11
Least square means (±SEM) of hot carcass weight (HCW, kg) of cattle housed in pens with shade and without shade. Dotted line is the least square mean of HCW for the shaded pens; dashed line is the least square mean of HCW for the unshaded pens.

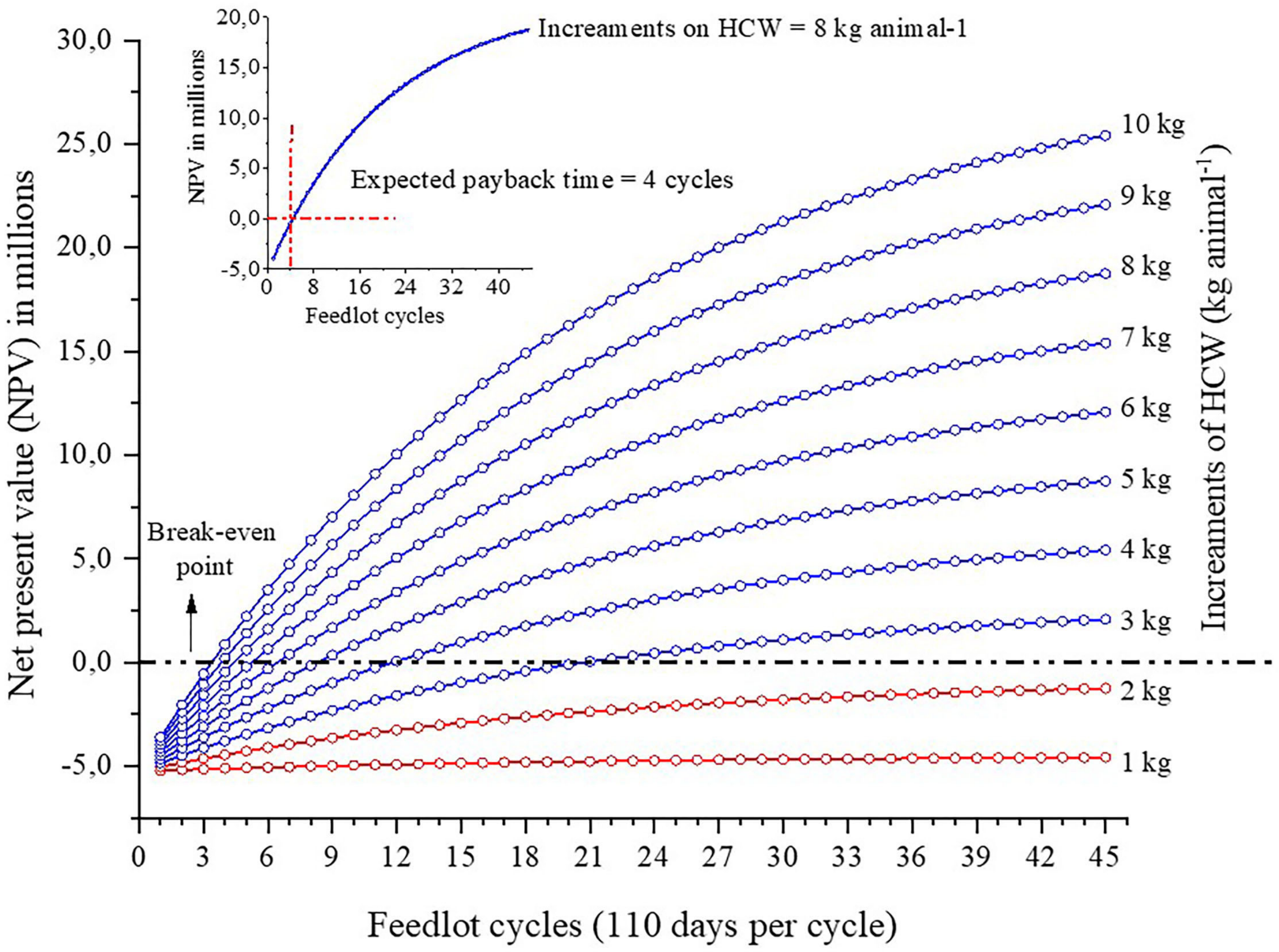

FIGURE 12
Net present value as a function of number of feedlot cycles for different increments in hot carcass weight. Carcass value at slaughter was taken in November 16, 2020. Dashed line delimits the break-even point; red dotted line represents level of increment for HCW that break-even point is not attained.

physiological mechanism that can compromise nutrient absorption and post-absorptive metabolism (48). In this study, a higher level of peripheral vasodilatation may have contributed for the lower growth performance for bullocks in the pens without shade compared to those in pens with shade. In a recent meta-analytical study, Edwards-Callaway et al. (16) also reported that gain efficiency of cattle housed in pens with shade improved by 3.4%, by reflecting mean increments of hot carcass weight of 6 kg. Our study is the first to confirm that use of shade improves gain efficiency even for more heat tolerant breeds of cattle such as *B. indicus* Nellore.

The economic feasibility of this study was done on the basis of net present value. The increment in hot carcass weight resulted in a payback time within the first four feedlot cycles (Figure 12). Considering that feedlots may run three feeding cycles per year (or at least two), the payback time for this shade structure, which has an expected lifespan of 15 years, is <2 years. Also, the economic analysis shows that the break-even point is not reached if increments on hot carcass weight of finished cattle are lower than 2.5 kg animal^{-1}. Similarly, during the summer months in Australia, Sullivan et al. (13) reported gain efficiency of Black Angus heifers in pens with shade (using 80%-blockage shade-cloth), increments of AU$ 60 animal^{-1} on carcass value. It is important to note, however, that use of shade cloth will not fully protect animals from direct and diffuse short-wave solar radiation, and have poor durability and life-span (18), especially if installed at locations where heavy wind and rainy conditions exist (19). The shade structure design which was installed in 2019 have been highly resilient to heavy rain (>50 mm hr^{-1}) and wind (80 km hr^{-1}).

The concept of this novel shade design proposed herein challenges the conventional east-west orientation design normally used for shade structures in tropical environments. The later design projects a "static" shade under the roof structure, and consequently becomes causes for cattle congregation, soil compression and mud formation. To alleviate these problems, we propose a combination of rectangular pens with a roof structure-oriented north-south that makes the shade to displace along the east-west direction along the pen (Figure 13). The outcome of using this design motivated cattle to seek shade, resulted in reduced heat stress indicators, decreased water intake and improved feed efficiency. We believe that the economic gains demonstrated from using this novel shade design will promote its use in feedlot operations in tropical climates. More robust economic outcomes however can be obtained through analyses of at least two feedlot cycles in a year, e.g., by taking into account period of the year with more days of moderate cold. At the latitude of the present study (20°S), cold days are likely to occur between May and July. Furthermore, further investigations are also needed

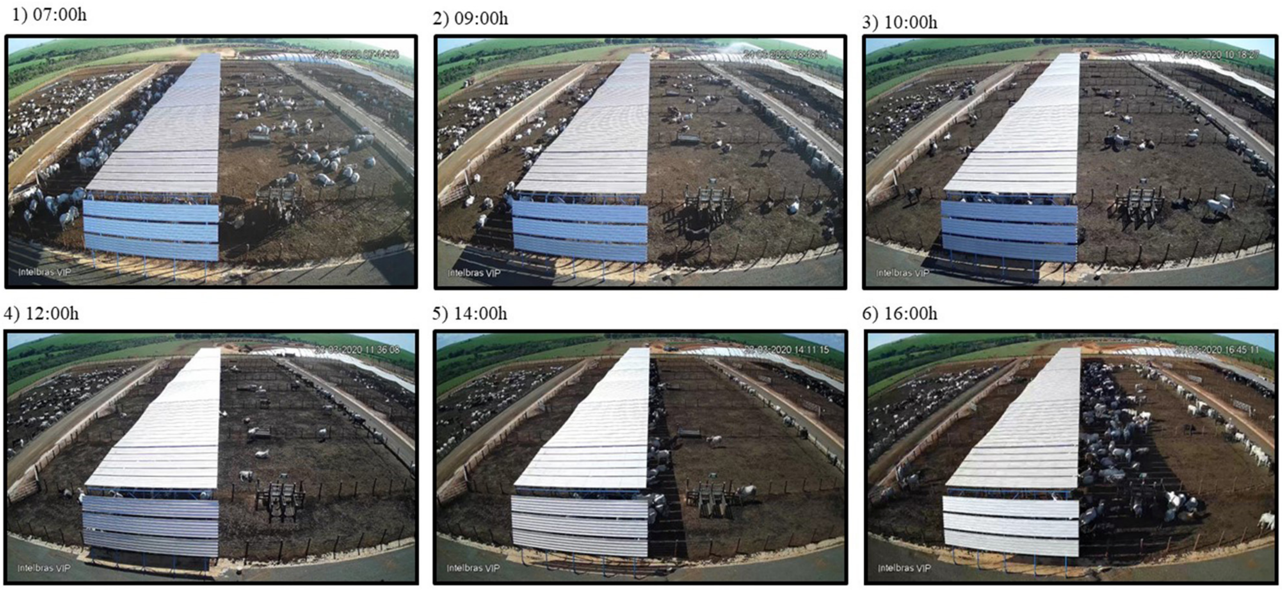

FIGURE 13
Displacement of shade projection from east to west (20° 31′ 25″ S; 49° 03′ 32″ W) at different hours of the day. (March, 20, 2020). Bulls seem to move in the pen following the displacement of the shade. Image source: Courtesy of Agro-Pastoril Paschoal-Campanelli.

to attest if benefits observed in this study can be replicated in other latitudes.

4. Conclusions

The following conclusions can be drawn from this study which involved 800 feedlot cattle randomly assigned in pens with and without shade access to determine the effectiveness and economic viability of shade use:

(1) The proposed novel shade design combines a roof structure with a north-south orientation, rectangular instead of square pens (length at least three times higher than width), and roof height of at least five meters. This design allows almost 100% of the pen area to be in shade throughout the day.
(2) Cattle in pens with shade presented higher average daily gain, reflecting in heavier hot carcass weight (8 kg per animal) compared to cattle with no access to shade.
(3) The novel shade design effectively buffers the negative impacts of high radiant heat load on cattle and resists adverse weather impacts as high as 50 mm hr^{-1} precipitation combined with >80 km hr^{-1} wind speed.
(4) The expected payback period for the novel shade design was within four finishing cycles (~110 days per cycle), assuming an initial investment of USD$90 per animal to build the structure that lasts 15 years.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

All protocols performed in the study were approved by the IACUC of the São Paulo State University, Brazil.

Author contributions

Conceptualization: AM, RP, GM, and VC. Methodology, validation, and data collection: AM, RP, and GM. Formal analysis: AM and GM. Writing original draft–manuscript: VF. Original draft-figures: AM, GM, and VF. Writing–review and editing: AM, HM, KG, and RP. Visualization: AM, KG, RP, GM, HM, MC, and VF. Supervision: AM and RP. Funding acquisition: AM, VC, and RP. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The effect of birth weight and time of day on the thermal response of newborn water buffalo calves

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During the 1st days of life, water buffalo calves, especially those with low birth weight, are susceptible to hypothermic mortality due to scarce energy reserves provided by fats. This means that monitoring the thermal state of newborns is essential. The objectives of the present study were to apply infrared thermography (IRT) in 109 buffalo calves to detect differences in the surface temperatures of six thermal windows –lacrimal gland, lacrimal caruncle, periocular region, nostrils, ear canal, pelvic limbs–, and determine their association to birth weight during the first 6 days of life. The calves were divided into four categories according to their weight (Q_1, 37.8–41.25 kg; Q_2, 41.3–46.3 kg; Q_3, 46.4–56.3 kg; Q_4, 56.4–60.3 kg). The thermographic images were recorded in the morning and afternoon. Results showed that the animals in Q_4 registered the highest temperatures in all the thermal windows, and that these were higher in the afternoon ($p < 0.0001$). When considering the thermal windows, those located in the facial region recorded the highest temperatures; in contrast, the temperatures at the pelvic limbs remained below the average values of the other windows (33.41 and 33.76°C in the morning and afternoon, respectively). According to these results, the birth weight of water buffaloes is a factor that alters their thermoregulation during the 1st days of life, a condition that can be partially compensated by colostrum intake to promote development of an efficient thermoregulatory mechanism in water buffalo calves.

KEYWORDS

body temperature, infrared thermography, newborns, thermoregulation, hypothermia

1. Introduction

At birth, water buffalo calves face extrauterine temperatures that can be 10–15°C below their core temperature (1). During this critical period, maintaining body temperature is essential for survival (2). However, to achieve efficient thermoregulation, animals require energy reserves or energy production that depend on the characteristics of individuals at birth (3) and the modulations of this parameter are closely related to stability of cellular, muscular, nervous endocrine, renal, cardiovascular and respiratory functions (4), and ensured by mechanisms of thermolysis and thermogenesis (5). Not only dams' weight at parturition (6), but also birthweight is a risk factor related to neonatal mortality, especially in neonates (7) born with weights below the average range (38.2 ± 0.2 kg in buffalo calves) (8), as this exposes them to hypothermia due to a high specific surface area and reduced thermogenic capacity (9, 10). In addition, birth weight is associated with the capacity for adequate growth and development of buffaloes throughout their productive life (11). Calves with lower weight may also show a decrease in colostrum consumption compared to animals with medium to high weight. The colostrum is the main source of energy for newborn ruminants (12), and reduced energy resources affect non-shivering thermogenesis, which is the main mechanism for heat production in these neonates (3, 13). Certain morphological characteristics of the water buffalo, i.e., scarce hair, thick epidermis, high melanin concentration, and the small number and large size of sweat glands, all favor the development of thermoregulatory mechanisms that differ from those commonly observed in conventional cattle (*Bos taurus* and *Bos indicus*) (14). Other elements that influence this response are environmental temperatures and the weather, as windy and rainy conditions at the beginning of spring or winter impact the thermoregulation process (15, 16).

Given these effects, evaluating the temperature of newborns offers a way to identify state of hypothermia and prevent the consequences of hypothermia (3). Assessing the surface temperature of animals by means of infrared thermography (IRT) has been shown to be an effective tool to non-invasively determine the peripheral thermoregulatory response of species such as puppies (6), Holstein Friesian calves (17), and piglets (5, 18). However, applications of this technique in water buffaloes are limited, and existing studies have not established the relationship between time of the day, and weight on the thermoregulatory mechanisms of this species. Therefore, the objectives of the present study were: (i) to evaluate, by means of IRT, surface temperatures in six thermal windows of calves from birth to 6 days of life; (ii) to establish the relationship between the birth weight of water buffaloes and their thermoregulatory capacity during the 1st days of life; and (iii) to evaluate the effect of the time of day on changes in the microcirculation of calves.

2. Materials and methods

2.1. Location

This study was conducted in a buffalo production unit in south-eastern Mexico, at an elevation of 10 m above sea level. The zone is characterized by a subtropical wet climate or Cfa, according to the Koppen-Geiger classification, with an average temperature of 27°C and annual rainfall of 2,900 mm. The experimental periods were September to November 2020 and September to November 2021. The ambient temperature during the evaluation period had average maximum and minimum values of 36°C and 23°C. Maximum and minimum percentages of relative humidity fluctuated from 81 to 94%, respectively.

2.2. Study population

To evaluate calves from birth (day 0), 123 dam water buffaloes (*Bubalus bubalis*) of the Buffalypso breed, close to calving, were monitored. One week before calving, the animals were moved to a 5,000 m^2 maternity paddock (pasture) with a capacity of 20 buffaloes per group. The females were fed forage native to the region (*Paspalum fasciculatum* and *Hymenachne amplexicaulis*) with 50 g of mineral supplement. At birth, the calves were distributed into four groups according to quartiles (18–21). In total, 109 calves were included in this study. Fourteen female buffaloes and their calves were excluded because their agonistic temperament made it impossible to approach the calves to obtain the required measurements.

Immediately after the first intake of colostrum and formation of the mother-calf bond (observed as licking and ingestion of amniotic fluids and placental membranes by the dams) (7), the calves were weighed using a 100-kg Outmate Digital Crane Scale® (Data Weighing Systems, USA) with a readout accuracy of 0.1 lb/0.05 kg-grams. Newborns were then divided into four experimental groups according to birth weight in each quartile. The first group (Q_1) included 25% of the calves with the lowest values recorded; quartile two (Q_2) included the lowest 25% of the mean; quartile three (Q_3) included 25% of values above the mean; and quartile four (Q_4) included 25% of the highest values. In this way, the distribution in quartiles was: Q_1 (37.8–41.2 kg, $n = 25$ calves); Q_2 (41.3–46.3 kg, $n = 29$ calves); Q_3 (46.4–56.3 kg, $n = 29$ calves); and Q_4 (56.4–60.3 kg, $n = 26$ calves).

2.3. Infrared thermography

Radiometric images were taken with a thermal camera model FLIR® Thermal TM E80 (FLIR Systems, USA), with a resolution of 320 × 240 pixels, thermal sensitivity of <0.045°C, precision ± 2°C or 2% and an emissivity of 0.95. Each radiometric capture was performed at 1–2 m from the calf, focusing on three regions: lateral facial, frontal facial, and left or right latero- lateral. Six thermal windows were assessed: lacrimal gland, lacrimal caruncle, periocular region, nostrils, ear canal, and pelvic limbs (Figure 1). The measurement of surface temperatures in these windows was carried out for a period of 6 days, from the day of birth (day 0) to the 5th day postpartum (day 5).

Thermographic monitoring was performed twice: from 8:00 a.m. to 9:00 a.m. and from 4:00 p.m. to 5:00 p.m. in the central zone of Mexico. The ambient temperature during the September-November period ranged from 23 to 27°C in the morning

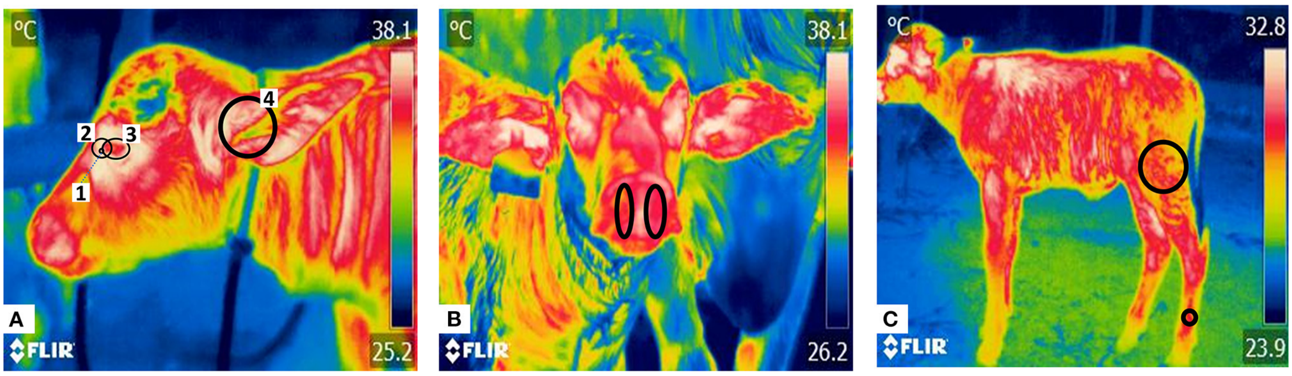

FIGURE 1
Thermal windows in newborn water buffaloes. Thermal windows captured in three radiometric images (lateral facial, frontal facial, and latero-lateral). **(A)** The lateral facial view shows four thermal windows: (1) the lacrimal gland, delimited by a circle in the medial quadrant of the ocular orbit; (2) the lacrimal caruncle, delimited by a circle around the lacrimal gland, from the medial region of the eye toward the rostral area of the palpebral commissure; (3) the periocular region, outlining the upper and lower eyelids in an ellipse; and (4) the auditory canal, marked with a circle covering the entire acoustic meatus. **(B)** Frontal facial region with delimitation of the nostrils. The temperature irradiated from this area was registered by drawing an ellipse based on each nostril's upper and lower end. **(C)** Latero-lateral region, where two thermal windows can be observed. The pelvic limb is delimited by two circles: one placed at the insertion of the *M. biceps femoris* and the second at the distal end, 10 cm above the hooves, located in the *superficial digital flexor muscle*. The average values of these measures were obtained.

(8:00-—9:00 a.m.) to 32–36°C (4:00 to 5:00 p.m.) in the afternoon. Relative humidity fluctuated from 81 to 93% in the morning to 82–94% in the afternoon. During the thermographic monitoring, neonates and their dams remained in the maternity paddocks all the time. The thermal imaging sequence was performed in the paddock. Buffalo calves were not moved or handled from their birthplace, so the thermal evaluation was carried out directly on the grass. Thermal images were taken in the following sequence: the camera placed in front of the animal to take the frontal facial region, then to the side to obtain the lateral facial view. Subsequently, the left latero-lateral and the right latero-lateral regions were captured on each side of the animal.

All radiometric images were stored in JPG format for later analysis using FLIR Tools Systems, USA® software to set points in the proposed thermal windows to obtain maximum, minimum, and average temperatures from each calf on each sampling day during the mornings and afternoons.

2.4. Statistical analysis

The GraphPad Prism statistical package (ver. 9.4.0) was used to obtain descriptive statistics for the weight of the animals (Q_1, Q_2, Q_3, Q_4), time of day (morning, afternoon), thermal window, and age of the animals (days 0, 1, 2, 3, 4, 5). The Shapiro-Wilk test was performed to analyze the normality of the data. The weight and age of the calves and the time of day were considered as independent variables, whereas the temperature of the thermal windows was considered a dependent variable. To evaluate the effects of these variables, analysis of variance (ANOVA) was used in a mixed linear model:

$$Y_{\mathrm{ijkl}} = \mu + W_{\mathrm{i}} + T_{\mathrm{j}} + A_{\mathrm{k}} + W.T.\mathrm{Aijk} + \beta\mathrm{l} + e_{\mathrm{ijk}}$$

where:
Y = response variable (temperature of the thermal windows).
W_i = effect of the weight of the animals (Q_1, Q_2, Q_3, Q_4).
T_j = effect of time of day (morning, afternoon).
A_k = effect of the animals' age (days 0, 1, 2, 3, 4, 5).
β = random effect (per animal).
μ = population mean.
e = residue.

Correlations between the time of day and thermal window temperatures on day 0 were obtained using Spearman correlation. Tukey's *post-hoc* test was adopted to evaluate the differences between means. In all significance level of $p < 0.05$ was set.

2.5. Ethics statement

The animals monitored in this study were not touched or stressed, as infrared thermography is a non-invasive technique; therefore, the protocol did not require approval from an Ethics Committee. During the study, handling of the animals was carried out under the guidelines of the Official Mexican Standard, NOM-062-ZOO-1999, which stipulates the technical specifications for the production, care, and ethical use of animals.

3. Results

A total of 23,544 recordings of maximum, minimum, and average surface temperatures were obtained from newborn water buffaloes during the first 6 days of life. In general, significant differences were observed among the six thermal windows due to the weight and time of day. These differences are described as follows.

3.1. Effect of weight on dermic microcirculation changes in newborn buffaloes during the 1st days of life

Table 1 lists the thermal responses of the pelvic limb region. On the morning of day 0, the surface temperature was 1.49°C higher in Q_3 and Q_4 than in Q_1 and Q_2 ($p < 0.05$). A similar pattern was observed on the following days, were the lowest temperatures occurred on day 0 but increased by 4.53°C in Q_1 and 6.43°C in Q_4 on day 1. On the afternoon of day 0, the lowest temperatures were observed on this day, but they showed significant increases ($p < 0.05$) of 4.35°C and 6.43 °C in Q_1 and Q_4, respectively, on day 1. The surface temperatures of the pelvic limb in Q_2 were 0.74, 0.94, and 1.19°C lower than those in Q_1, Q_3, and Q_4, respectively ($p< 0.05$). This response was observed in the following days since heavier calves (Q_3 and Q_4) maintained higher temperatures until the end of the evaluation period.

Regarding the surface temperature of the lacrimal caruncle (Table 2), in the morning, significant differences ($p < 0.05$) were observed among the quartiles and over time. On the morning of day 0, Q_4 was found to be 0.9°C higher than Q_2 ($p < 0.05$), and it can be observed that Q_4 did not show significant variations in the mornings of the days evaluated. On day 1, 2, and 3, buffalo calves from Q_3 and Q_4 showed the highest temperatures when compared to Q_1 and Q_2 ($p < 0.05$). On days 4 and 5, the temperatures of Q_1, Q_3, and Q_4 were significantly higher than those of Q_2 ($p < 0.05$). Readings from the afternoon of day 1 showed that Q_1, Q_3, and Q_4 presented a temperature 0.7°C higher than those of Q_2 ($p < 0.05$). Interestingly, at day 5, calves from Q_2 had lower lacrimal caruncle temperature than the rest of the groups, although a periodically increasing was reported during the 6 days ($p < 0.05$).

The surface temperature readings from the periocular region showed differences among quartiles and over time (Table 3). Regarding the difference among the four weight groups, in the morning of day 0, temperatures in Q_3 and Q_4 were 1.6°C higher than in Q_1 and Q_2 ($p < 0.05$). Although a progressive increase in temperature was recorded in all groups [e.g., the temperature of Q_1 on the morning of day 5 increased 0.6°C compared to day 3 ($p < 0.05$)], on day 5, Q_1, Q_3, and Q_4 were 0.7°C higher than Q_2 ($p < 0.05$). This response was also observed during the afternoon of day 5, when Q_1 and Q_4 were 0.6°C higher than Q_2 ($p < 0.05$) while Q_3 and Q_4 recorded the highest values during all the evaluation days ($p < 0.05$). It is important to mention here that in contrast to Q_1, Q_4 only showed a decrease of 0.91°C in the morning and afternoon of day 1 but then remained without any significant variation until the end of the evaluation period.

Regarding the thermal response of the auditory canal in the water buffalo calves (Table 4), during the morning of the six evaluation days Q_3 and Q_4 had higher temperature than Q_1 and Q_2, ($p < 0.05$). The same response was observed in the afternoon, and only on day 5, the temperatures of Q_1 and Q_4 were 0.3°C higher than those obtained in Q_2 and Q_3 ($p < 0.05$). The series of readings taken over time indicated that Q_1 did not present significant differences until day 2, when an increase of 1.91°C was found. In contrast, Q_4 showed a significant decrease of 1.65°C. on day 1, followed by a significant increase of 0.96°C on day 2. Q_1 and Q_4 only presented significant variations toward the end of the afternoon evaluations, but not during the morning study period, when Q_1 tended to show a decrease on day three, with a difference of 1.06°C with respect to day 2. Later, the temperature began to increase, by 0.94 and 0.99°C on days 4 and 5, respectively.

The surface temperature in the nostrils, as shown in Table 5, had the lowest values during the morning of day 0 in Q_1 and Q_2 ($p < 0.05$). Consecutively, on day 5, Q_3 and Q_4 were 1.4°C higher than Q_1 and Q_2. When considering afternoon temperatures, they had a similar pattern between groups. On day 0, the temperatures of Q_3 and Q_4 were 0.8°C higher than Q_1 and 1.4°C higher than Q_2. This response was maintained during the rest of the days, and when comparing events, observations showed that Q_1, Q_3, and Q_4 had significantly higher morning and afternoon temperatures on day 0 than on day 1 ($p < 0.05$). The temperature on day 1 was between 0.9 and 1°C higher than on days 2, 3, 4, and 5 ($p < 0.05$). Regarding Q_2, readings showed that in both the morning and afternoon of day 0 the temperatures were 1.1°C higher than on day 1 ($p < 0.05$), and that on the same day there was a difference of 1°C compared to day 3 ($p < 0.05$).

The lacrimal gland showed significant differences on the morning of day 0, where Q_3 and Q_4 were 0.7°C higher than Q_1 and 0.2°C higher than Q_2, respectively ($p < 0.05$; Table 6). During the rest of the days, temperatures of calves belonging to Q_3 and Q_4 were higher than Q_1 and Q_2 ($p < 0.05$). On the afternoon of days 0 and 5, the temperatures of Q_1, Q_3, and Q_4 were significantly higher than those in Q_2 ($p < 0.05$), while on day 2, Q_3 and Q_4 had temperatures 0.7°C higher than those of Q_1 and Q_2 ($p < 0.05$).

3.2. Thermal variation according to body region

In all groups, the lacrimal gland and lacrimal caruncle had the highest temperatures. In Q_2, the lacrimal caruncle had significantly higher temperatures, with a difference of 0.16°C compared to the lacrimal gland. Similarly, the Q_3 and Q_4 newborns had temperature differences of 0.2–0.11°C between these two areas (Table 7). With respect to the periocular region, all the study animals had high temperatures in the thermal window, except those in Q_4, where no significant differences were found with the auditory canal (36.601 ± 0.02 vs. 36.491 ± 0.03). The windows with the lowest temperatures were the nostrils and the pelvic limbs. In these zones, the buffaloes in Q_1 and Q_2 showed differences of up to 1.47°C.

Regarding the comparison among weights, the lacrimal gland in Q_1 had the lowest temperature, with a difference of up to 0.62°C compared to Q_4. The lacrimal caruncle in Q_1 and Q_2 did not show significant differences within the groups, while there were differences in Q_3 and Q_4, who had the highest temperatures (37.132 ± 0.02 and 37.331 ± 0.02, respectively). The periocular region, auditory canal, nostrils, and pelvic limbs in Q_2 had the lowest temperatures, with significant differences of 1.07, 1.95, 1.74, and 2.67°C, respectively, compared to Q_4.

3.3. Effect of the time of day on calf thermoregulation during the 1st days of life

The temperatures of the lacrimal gland, lacrimal caruncle, periocular region, auditory canal, nostrils, and pelvic limb were

TABLE 1 Mean pelvic limb surface temperature ± standard error (SE) in newborn water buffaloes ($n = 109$) at different birth weights and daytimes (morning/afternoon) during the first 6 days of life.

Day	Q_1		Q_2		Q_3		Q_4	
	$n = 25$		$n = 29$		$n = 29$		$n = 26$	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
0	$28.97 \pm 0.07^{3,a*}$	$29.62 \pm 0.07^{3,a}$	$28.44 \pm 0.08^{3,b*}$	$28.88 \pm 0.08^{3,b*}$	$29.62 \pm 0.06^{3,c*}$	$29.82 \pm 0.06^{3,a}$	$29.93 \pm 0.08^{2,d,*}$	$30.07 \pm 0.08^{3,a}$
1	$33.32 \pm 0.11^{1,c*}$	$33.97 \pm 0.11^{1,c}$	$33.13 \pm 0.13^{1,c*}$	$33.57 \pm 0.14^{1,2,c*}$	$35.96 \pm 0.09^{1,b*}$	$36.16 \pm 0.09^{1,b}$	$36.36 \pm 0.07^{1,a*}$	$36.50 \pm 0.07^{1,a}$
2	$33.44 \pm 0.14^{1,b*}$	$34.10 \pm 0.14^{1,b}$	$32.90 \pm 0.14^{1,c*}$	$33.34 \pm 0.15^{1,c*}$	$35.38 \pm 0.19^{1,2,a}$	$35.58 \pm 0.19^{1,2,a}$	$35.88 \pm 0.11^{1,a*}$	$36.03 \pm 0.11^{1,2,a}$
3	$32.20 \pm 0.17^{2,b*}$	$32.86 \pm 0.17^{2,b}$	$31.91 \pm 0.14^{2,b*}$	$32.34 \pm 0.14^{2,b}$	$35.05 \pm 0.13^{2,a*}$	$35.25 \pm 0.13^{2,a}$	$35.40 \pm 0.07^{1,a*}$	$35.55 \pm 0.07^{2,a}$
4	$33.35 \pm 0.11^{1,b}$	$34.00 \pm 0.11^{1,b}$	$32.93 \pm 0.11^{1,b}$	$33.37 \pm 0.11^{1,c}$	$34.93 \pm 0.15^{2,a}$	$34.93 \pm 0.15^{2,a}$	$35.13 \pm 0.15^{1,a}$	$35.38 \pm 0.13^{2,a}$
5	$33.23 \pm 0.13^{1,b*}$	$33.88 \pm 0.13^{1,b}$	$32.69 \pm 0.13^{1,2,b*}$	$33.13 \pm 0.13^{1,2,c}$	$35.46 \pm 0.17^{1,2,a*}$	$35.66 \pm 0.17^{1,a}$	$35.94 \pm 0.14^{1,a*}$	$36.08 \pm 0.15^{1,2,a}$

Linear mixed model analysis with a Tuckey *post-hoc* test using the GraphPad program (VER. 9.4.0).

n, number of calves; Weight of calves according to category: Q_1, 37.8–41.2 kg; Q_2, 41.3–46.3 kg; Q_3, 46.4–56.3 kg; Q_4, 56.4–60.3 kg. Mean ± standard error.

[a,b,c,d] Different lowercase indicate a statistical difference between groups.

[1,2,3] Different numbers indicate statistically significant differences between days in the same weight group.

*Indicates a significant statistical difference in temperature due to the effect of time of day.

TABLE 2 Mean lacrimal caruncle surface temperature ± standard error (SE) in 109 newborn water buffaloes at different birth weights and daytimes (morning/afternoon) during the first 6 days of life.

Day	Q_1		Q_2		Q_3		Q_4	
	$n = 25$		$n = 29$		$n = 29$		$n = 26$	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
0	$36.75 \pm 0.12^{1,2,a,*}$	$37.40 \pm 0.12^{1,2,a}$	$36.23 \pm 0.11^{1,2,b*}$	$36.67 \pm 0.12^{1,2,a}$	$37.0 \pm 0.09^{1,2,a,b*}$	$37.20 \pm 0.09^{1,a}$	$37.17 \pm 0.06^{1,a*}$	$37.31 \pm 0.06^{1,a}$
1	$36.49 \pm 0.05^{2,b*}$	$37.14 \pm 0.05^{2,a}$	$35.99 \pm 0.09^{2,c*}$	$36.42 \pm 0.09^{2,b}$	$36.96 \pm 0.06^{2,a*}$	$37.16 \pm 0.06^{1,a}$	$37.09 \pm 0.08^{1,a*}$	$37.23 \pm 0.08^{1,a}$
2	$36.72 \pm 0.18^{1,2,a*}$	$37.38 \pm 0.18^{1,2,a,b}$	$36.18 \pm 0.17^{1,2,a*}$	$36.62 \pm 0.18^{1,2,a,b}$	$37.02 \pm 0.12^{1,a*}$	$37.22 \pm 0.12^{1,a}$	$37.38 \pm 0.09^{1,a*}$	$37.52 \pm 0.09^{1,a,b}$
3	$36.23 \pm 0.10^{2,b*}$	$36.88 \pm 0.11^{2,a,b}$	$35.87 \pm 0.09^{2,b*}$	$36.31 \pm 0.10^{2,b}$	$37.14 \pm 0.06^{1,a*}$	$37.34 \pm 0.06^{1,a}$	$37.36 \pm 0.06^{1,a*}$	$37.50 \pm 0.06^{1,a}$
4	$36.78 \pm 0.11^{1,a,b*}$	$37.43 \pm 0.11^{1,2,a}$	$36.27 \pm 0.11^{1,2,b*}$	$36.71 \pm 0.11^{1,2,a}$	$37.04 \pm 0.06^{1,2,a*}$	$37.24 \pm 0.06^{1,a,b}$	$37.26 \pm 0.05^{1,a*}$	$37.40 \pm 0.05^{1,a,b}$
5	$36.94 \pm 0.06^{1,a*}$	$37.60 \pm 0.06^{1,a}$	$36.53 \pm 0.08^{1,b*}$	$36.97 \pm 0.09^{1,b}$	$37.02 \pm 0.04^{1,2,a*}$	$37.22 \pm 0.04^{1,a,b}$	$37.28 \pm 0.04^{1,a*}$	$37.43 \pm 0.03^{1,a,b}$

Linear mixed model analysis with a Tuckey *post-hoc* test using the GraphPad program (VER. 9.4.0).

n, number of calves; Weight of calves according to category: Q_1, 37.8–41.2 kg; Q_2, 41.3–46.3 kg; Q_3, 46.4–56.3 kg; Q_4, 56.4–60.3 kg. Mean ± standard error.

[a,b] Different lowercase indicate a statistical difference between groups.

[1,2] Different numbers indicate statistically significant differences between days in the same weight group.

*Indicates a significant statistical difference in temperature due to the effect of time of day.

TABLE 3 Mean periocular surface temperature ± standard error (SE) in 109 newborn water buffaloes at different birth weights and daytimes (morning/afternoon) during the first 6 days of life.

Day	Q_1		Q_2		Q_3		Q_4	
	$n = 25$		$n = 29$		$n = 29$		$n = 26$	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
0	$35.63 \pm 0.06^{1,2,b*}$	$36.28 \pm 0.06^{1,2,b}$	$35.26 \pm 0.07^{1,b*}$	$35.70 \pm 0.07^{1,c}$	$37.01 \pm 0.11^{1,a*}$	$37.21 \pm 0.11^{1,a}$	$37.21 \pm 0.07^{1,a*}$	$37.35 \pm 0.08^{1,a}$
1	$35.66 \pm 0.04^{1,2,b*}$	$36.31 \pm 0.04^{1,2,a}$	$35.20 \pm 0.06^{1,c*}$	$35.64 \pm 0.06^{1,b}$	$36.10 \pm 0.03^{2,a*}$	$36.30 \pm 0.03^{2,a}$	$36.30 \pm 0.04^{2,a*}$	$36.44 \pm 0.04^{2,a}$
2	$35.90 \pm 0.16^{1,2,a,b*}$	$36.55 \pm 0.16^{1,2,a}$	$35.29 \pm 0.16^{1,b*}$	$35.73 \pm 0.17^{1,b}$	$36.13 \pm 0.10^{2,a*}$	$36.32 \pm 0.10^{2,a,b}$	$36.30 \pm 0.08^{2,a*}$	$36.44 \pm 0.08^{2,a}$
3	$35.40 \pm 0.09^{2,b*}$	$36.06 \pm 0.10^{2,b,c}$	$35.12 \pm 0.10^{1,b*}$	$35.56 \pm 0.10^{1,c}$	$36.38 \pm 0.08^{2,a*}$	$36.58 \pm 0.08^{2,a,b}$	$36.52 \pm 0.06^{2,a*}$	$36.66 \pm 0.07^{2,a}$
4	$35.90 \pm 0.07^{1,2,a,b*}$	$36.55 \pm 0.07^{1,2,a,b}$	$35.47 \pm 0.08^{1,b*}$	$35.90 \pm 0.09^{1,b}$	$36.35 \pm 0.10^{2,a*}$	$36.55 \pm 0.10^{2,a}$	$36.40 \pm 0.10^{2,a*}$	$36.54 \pm 0.10^{2,a,b}$
5	$36.06 \pm 0.10^{1,a,b*}$	$36.71 \pm 0.10^{1,a}$	$35.54 \pm 0.11^{1,b*}$	$35.98 \pm 0.11^{1,b}$	$36.22 \pm 0.08^{2,a*}$	$36.42 \pm 0.08^{2,a,b}$	$36.42 \pm 0.05^{2,a*}$	$36.56 \pm 0.05^{2,a}$

Linear mixed model analysis with a Tuckey *post-hoc* test using the GraphPad program (VER. 9.4.0).

n, number of calves; Weight of calves according to category: Q_1, 37.8–41.2 kg; Q_2, 41.3–46.3 kg; Q_3, 46.4–56.3 kg; Q_4, 56.4–60.3 kg. Mean ± standard error.

[a,b] Different lowercase indicate a statistical difference between groups.

[1,2] Different numbers indicate statistically significant differences between days in the same weight group.

*Indicates a significant statistical difference in temperature due to the effect of time of day.

TABLE 4 Mean auditory canal surface temperature ± standard error (SE) in 109 newborn water buffaloes at different birth weights and daytimes (morning/afternoon) during the first 6 days of life.

Day	Q_1		Q_2		Q_3		Q_4	
	$n = 25$		$n = 29$		$n = 29$		$n = 26$	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
0	$34.03 \pm 0.19^{2,3,b*}$	$34.68 \pm 0.19^{2,3,b}$	$33.62 \pm 0.21^{2,3,b*}$	$34.06 \pm 0.21^{2,3,b}$	$36.58 \pm 0.22^{1,2,a*}$	$36.78 \pm 0.22^{1,2,a}$	$37.07 \pm 0.07^{1,a*}$	$37.21 \pm 0.07^{1,a}$
1	$33.43 \pm 0.05^{3,b*}$	$34.08 \pm 0.06^{3,b}$	$32.96 \pm 0.09^{3,c*}$	$33.39 \pm 0.10^{3,b}$	$35.29 \pm 0.11^{4,a*}$	$35.49 \pm 0.11^{4,a}$	$35.42 \pm 0.13^{4,a*}$	$35.56 \pm 0.14^{4,a}$
2	$35.34 \pm 0.34^{1,a,b*}$	$35.99 \pm 0.34^{1,2,a,b}$	$34.45 \pm 0.36^{1,2,b*}$	$34.89 \pm 0.36^{1,2,b}$	$35.93 \pm 0.20^{3,a,b*}$	$36.13 \pm 0.20^{3,a,b}$	$36.38 \pm 0.10^{2,3,a*}$	$36.52 \pm 0.09^{2,3,a}$
3	$34.28 \pm 0.27^{2,3,b*}$	$34.94 \pm 0.27^{2,3,b}$	$34.13 \pm 0.23^{2,b*}$	$34.57 \pm 0.22^{2,b}$	$36.61 \pm 0.06^{1,a*}$	$36.81 \pm 0.06^{1,a}$	$36.79 \pm 0.05^{1,2,a*}$	$36.93 \pm 0.05^{1,2,a}$
4	$35.22 \pm 0.26^{1,2,b*}$	$35.88 \pm 0.26^{1,2,a,b}$	$34.92 \pm 0.21^{1,2,b*}$	$35.36 \pm 0.22^{1,2,b}$	$36.29 \pm 0.06^{2,3,a*}$	$36.49 \pm 0.06^{2,3,a}$	$36.49 \pm 0.06^{2,3,a*}$	$36.63 \pm 0.05^{2,3,a}$
5	$36.21 \pm 0.09^{1,a*}$	$36.86 \pm 0.09^{1,a}$	$35.68 \pm 0.11^{1,b*}$	$36.12 \pm 0.11^{1,b}$	$36.26 \pm 0.06^{2,3,a}$	$36.46 \pm 0.06^{2,3,b*}$	$36.37 \pm 0.06^{3,a*}$	$36.51 \pm 0.07^{3,a}$

Linear mixed model analysis with a Tuckey *post-hoc* test using the GraphPad program (VER. 9.4.0).

n, number of calves; Weight of calves according to category: Q_1, 37.8–41.2 kg; Q_2, 41.3–46.3 kg; Q_3, 46.4–56.3 kg; Q_4, 56.4–60.3 kg. Mean ± standard error.

[a,b] Different lowercase indicate a statistical difference between groups.

[1,2,3] Different numbers indicate statistically significant differences between days in the same weight group.

*Indicates a significant statistical difference in temperature due to the effect of time of day.

TABLE 5 Mean nostrils surface temperature ± standard error (SE) in 109 newborn water buffaloes at different birth weights and daytimes (morning/afternoon) during the first 6 days of life.

Day	Q_1		Q_2		Q_3		Q_4	
	$n = 25$		$n = 29$		$n = 29$		$n = 26$	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
0	$35.33 \pm 0.07^{1,b*}$	$35.98 \pm 0.07^{1,b}$	$34.89 \pm 0.06^{1,c*}$	$35.32 \pm 0.06^{1,c}$	$36.57 \pm 0.07^{1,a*}$	$36.77 \pm 0.07^{1,a}$	$36.75 \pm 0.07^{1,a*}$	$36.89 \pm 0.08^{1,a}$
1	$34.35 \pm 0.12^{2,c*}$	$35.00 \pm 0.12^{2,b}$	$33.79 \pm 0.11^{2,c*}$	$34.23 \pm 0.11^{2,c}$	$35.88 \pm 0.11^{2,b*}$	$36.08 \pm 0.11^{2,a}$	$36.24 \pm 0.05^{2,a*}$	$36.38 \pm 0.05^{2,a}$
2	$33.54 \pm 0.11^{3,b*}$	$34.19 \pm 0.11^{3,b}$	$32.90 \pm 0.23^{2,3,b*}$	$33.33 \pm 0.23^{2,3,b}$	$34.56 \pm 0.19^{3,a*}$	$34.76^{3,a} \pm 0.19$	$35.06 \pm 0.13^{3,a*}$	$35.20 \pm 0.14^{3,a}$
3	$33.24 \pm 0.12^{3,b*}$	$33.90 \pm 0.13^{3,b}$	$32.77 \pm 0.13^{3,b*}$	$33.21 \pm 0.13^{3,c}$	$34.27 \pm 0.12^{3,a*}$	$34.47 \pm 0.12^{3,a,b}$	$34.65 \pm 0.06^{3,4,a*}$	$34.79 \pm 0.07^{3,4,a}$
4	$33.58 \pm 0.12^{3,b,c*}$	$34.23 \pm 0.12^{3,a}$	$33.27 \pm 0.12^{2,3,c*}$	$33.70 \pm 0.13^{2,3,a}$	$34.26 \pm 0.12^{3,a,b*}$	$34.46 \pm 0.12^{3,a}$	$34.54 \pm 0.11^{4,a*}$	$34.68 \pm 0.11^{4,a}$
5	$33.30 \pm 0.12^{3,b*}$	$33.95 \pm 0.12^{3,b}$	$33.02 \pm 0.12^{2,3,b*}$	$33.46 \pm 0.12^{2,3,b}$	$34.27 \pm 0.11^{3,a*}$	$34.47 \pm 0.11^{3,a}$	$34.74 \pm 0.08^{3,4,a*}$	$34.88 \pm 0.08^{3,4,a}$

Linear mixed model analysis with a Tuckey *post-hoc* test using the GraphPad program (VER. 9.4.0).

n, number of calves; Weight of calves according to category: Q_1, 37.8–41.2 kg; Q_2, 41.3–46.3 kg; Q_3, 46.4–56.3 kg; Q_4, 56.4–60.3 kg. Mean ± standard error.

[a,b,c] Different lowercase indicate a statistical difference between groups.

[1,2,3] Different numbers indicate statistically significant differences between days in the same weight group.

*Indicates a significant statistical difference in temperature due to the effect of time of day.

0.3°C higher in the afternoon than the morning ($p < 0.05$; Figure 2). The lacrimal gland and lacrimal caruncle presented significantly higher values than the periocular region, auditory canal, nostrils, and pelvic limb, in both the morning and afternoon ($p < 0.05$). Similarly, the temperatures of the periocular region, auditory canal, nostrils, and pelvic limb differed ($p < 0.05$). Notably, the lowest temperatures were recorded in the pelvic limb (Figure 2).

There was a positive correlation of 0.99 between the surface temperatures of these thermal windows and the time of day ($p < 0.0001$; Table 8).

4. Discussion

4.1. Effect of weight on the thermoregulation of buffalo calves

In this study, the heavier animals in Q_3 and Q_4–weight >46 kg—maintained higher surface temperatures than the lighter ones in Q_1 and Q_2 ($p < 0.05$). These results show a relationship between birth weight and the thermoregulation of these calves (1), a finding that has been reported for other species, such as pigs and sheep (22, 23). This could be due to a possible difference in the disposition of energy resources, such as brown adipose tissue and glucose, between a low birth weight or a high birth weight animal. In other words, when a newborn has a low birth weight, its response to compensate for hypothermia will be more limited compared to an animal with a higher weight.

Due to the exposure to cold in mammals, a coordinated response is exerted with the Central Nervous System that produces the activation of the sympathetic nervous system, which promotes the neurosecretion of catecholamines (24). Consequently, a response at the vasomotor level is observed with peripheral vasoconstriction to reduce heat loss. In addition to this, a metabolic response is generated due to the interaction with norepinephrine that produces the breakdown of triglycerides in adipocytes that leads to intracellular activation of cAMP and protein kinase A, for the mitochondrial combustion

TABLE 6 Mean lacrimal gland surface temperature ± standard error (SE) in 109 newborn water buffaloes at different birth weights and daytimes (morning/afternoon) during the first 6 days of life.

Day	Q_1		Q_2		Q_3		Q_4	
	$n = 25$		$n = 29$		$n = 29$		$n = 26$	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
0	$36.86 \pm 0.09^{1,b*}$	$37.51 \pm 0.09^{1,a}$	$36.33 \pm 0.10^{1,2,c*}$	$36.77 \pm 0.10^{1,2,b}$	$37.28 \pm 0.08^{1.a*}$	$37.48 \pm 0.08^{1,a}$	$37.40 \pm 0.07^{1,a*}$	$37.54 \pm 0.07^{1,a}$
1	$36.05 \pm 0.09^{2,b*}$	$36.70 \pm 0.09^{2,b}$	$35.62 \pm 0.11^{3,b*}$	$36.06 \pm 0.12^{3,c}$	$37.17 \pm 0.07^{1,a*}$	$37.37 \pm 0.07^{1,a}$	$37.22 \pm 0.10^{1,a*}$	$37.35 \pm 0.10^{1,a,b}$
2	$36.50 \pm 0.20^{1,2,b*}$	$37.15 \pm 0.20^{1,2,b}$	$35.96 \pm 0.21^{1,2,3,b*}$	$36.40 \pm 0.22^{2,3,b}$	$37.13 \pm 0.12^{1,a*}$	$37.33 \pm 0.12^{1,a}$	$37.34 \pm 0.09^{1,a*}$	$37.48 \pm 0.08^{1,a}$
3	$35.84 \pm 0.22^{3,b*}$	$36.50 \pm 0.22^{2,b,c}$	$35.83 \pm 0.17^{2,3,b*}$	$36.27 \pm 0.17^{2,3,c}$	$37.25 \pm 0.07^{1,a*}$	$37.45 \pm 0.07^{1,a,b}$	$37.47 \pm 0.04^{1,a*,}$	$37.62 \pm 0.05^{1,a}$
4	$36.77 \pm 0.10^{1,b,c*}$	$37.42 \pm 0.10^{1,a,b}$	$36.42 \pm 0.10^{1,2,c*}$	$36.86 \pm 0.10^{1,2,b}$	$37.29 \pm 0.05^{1,a,b*}$	$37.49 \pm 0.05^{1,a}$	$37.42 \pm 0.05^{1,a*}$	$37.56 \pm 0.05^{1,a}$
5	$36.92 \pm 0.07^{1,b,c*}$	$37.57 \pm 0.07^{1,a}$	$36.60 \pm 0.08^{1,c*}$	$37.03 \pm 0.08^{1,b}$	$37.30 \pm 0.05^{1,a,b*}$	$37.50 \pm 0.05^{1,a}$	$37.40 \pm 0.05^{1,a*}$	$37.54 \pm 0.05^{1,a}$

Linear mixed model analysis with a Tuckey *post-hoc* test using the GraphPad program (VER. 9.4.0).

n, number of calves; Weight of calves according to category: Q_1, 37.8–41.2 kg; Q_2, 41.3–46.3 kg; Q_3, 46.4–56.3 kg; Q_4, 56.4–60.3 kg. Mean ± standard error.

[a,b,c,d] Different lowercase indicate a statistical difference between groups.

[1,2,3] Different numbers indicate statistically significant differences between days in the same weight group.

*Indicates a significant statistical difference in temperature due to the effect of time of day.

TABLE 7 Mean surface temperature of the different thermal windows ± standard error (SE) in water buffaloes ($n = 109$) with different birth weights (Q_1, 37.8–41.2 kg; Q_2, 41.3–46.3 kg; Q_3, 46.4–56.3 kg; Q_4, 56.4–60.3 kg.).

	Lacrimal gland	Lacrimal caruncle	Periocular region	Auditory canal	Nostrils	Pelvic limb
$Q_{1n=25}$	$36.82 \pm 0.05^{2,b}$	$36.98 \pm 0.04^{2,a}$	$36.08 \pm 0.03^{2,c}$	$35.08 \pm 0.08^{3,d}$	$34.21 \pm 0.05^{3,e}$	$32.74 \pm 0.10^{2,f}$
$Q_{2n=29}$	$36.34 \pm 0.04^{2,a}$	$36.40 \pm 0.03^{3,a}$	$35.53 \pm 0.03^{3,b}$	$34.51 \pm 0.08^{4,c}$	$33.66 \pm 0.05^{4,d}$	$32.22 \pm 0.09^{3,f}$
$Q_{3n=29}$	$37.33 \pm 0.02^{1,a}$	$37.13 \pm 0.02^{2,b}$	$36.46 \pm 0.03^{1,c}$	$36.26 \pm 0.04^{2,d}$	$35.07 \pm 0.06^{2,e}$	$34.50 \pm 0.12^{1,e}$
$Q_{4n=26}$	$37.44 \pm 0.02^{1,a}$	$37.33 \pm 0.02^{1,b}$	$36.60 \pm 0.02^{1,c}$	$36.49 \pm 0.03^{1,c}$	$35.40 \pm 0.05^{1,d}$	$34.89 \pm 0.12^{1,d}$

Linear mixed model analysis with a Tuckey *post-hoc* test using the GraphPad program (VER. 9.4.0).

n, number of calves; Weight of calves according to category: Q_1, 37.8–41.2 kg; Q_2, 41.3–46.3 kg; Q_3, 46.4–56.3 kg; Q_4, 56.4–60.3 kg. Mean ± standard error.

[a,b,c,d,e,f] Different lowercase indicate a statistical difference between groups.

[1,2,3] Different numbers indicate statistically significant differences between days in the same weight group.

of substrates and the production of heat (25). The concept of thermoneutrality considers the range where the basal metabolism maintains body temperature (26). In this way, the previous explanation would confirm the fact that an animal with a low birth weight would present low levels of substrates to compensate for heat loss and reach the thermal comfort zone.

The association between basal metabolism and the ability to thermoregulate could explain why a low-birth-weight animal has a lower body temperature. In this sense, Diesch et al. (27) showed that calves born in windy and humid regions with an ambient temperature of 10°C delayed the time for first standing. This could be due to the organism using the energetic reserves to activate thermoregulatory mechanisms, such as vasomotor changes, and shivering and non-shivering thermogenesis (1). These mechanisms depend on the animal's metabolic rate, which is lower per unit area in low-weight neonates and reduces their tolerance to cold temperatures. The basal metabolic rate (rate of energy expenditure per unit time) is higher in smaller animals because they have a higher surface/mass ratio which results in increased corporal heat loss (28–30) in other words, large surface area relative to mass shows a disadvantage in retaining heat (25, 31). Consequently, calves with low body weight may be more susceptible to hypothermia when heat loss exceeds heat production (32).

On the other hand, it is necessary to mention that the surface temperature was significantly lower during days 0, 1, and 2 compared to the rest of the days ($p < 0.05$). This event is probably due to age since the low critical temperature threshold is higher due to the development of thermoregulatory capacity (2). In other words, the growth of the animal leads to an increase in subcutaneous fat reserves, but also an increase in the thickness of the skin and the length of the hair that allow a greater tolerance to cold (27). Johanson and Berger (33) determined that birth weight can be considered a predictor of perinatal mortality, since animals with higher weights have a reduced incidence of mortality due to hypothermia, as has been reported for cattle in eastern Montana. In this study, animals that weighed over 17.42 kg experienced lower temperature decreases when exposed to cold stress (34). Likewise, for Nili-Ravi buffaloes, Hassan et al. (35) indicated that birth weight had a significant effect on mortality rates, as they found a relationship between low birth weight, poor thermoregulation and deficient dam-calf bonding. It has been suggested that newborns with low birth weight and those with insufficient milk supply from the mother are prone to perform compensatory behaviors, such as allosuckling, to prevent nutritional deficiency (36, 37). Similarly, Chnitier et al. (38) found that newborns lambs with low weights had a higher mortality rate (52.2%) than medium- and high-weight animals (24.4 and 1.8%, respectively). Those authors further determined that during winter, when lambs were exposed

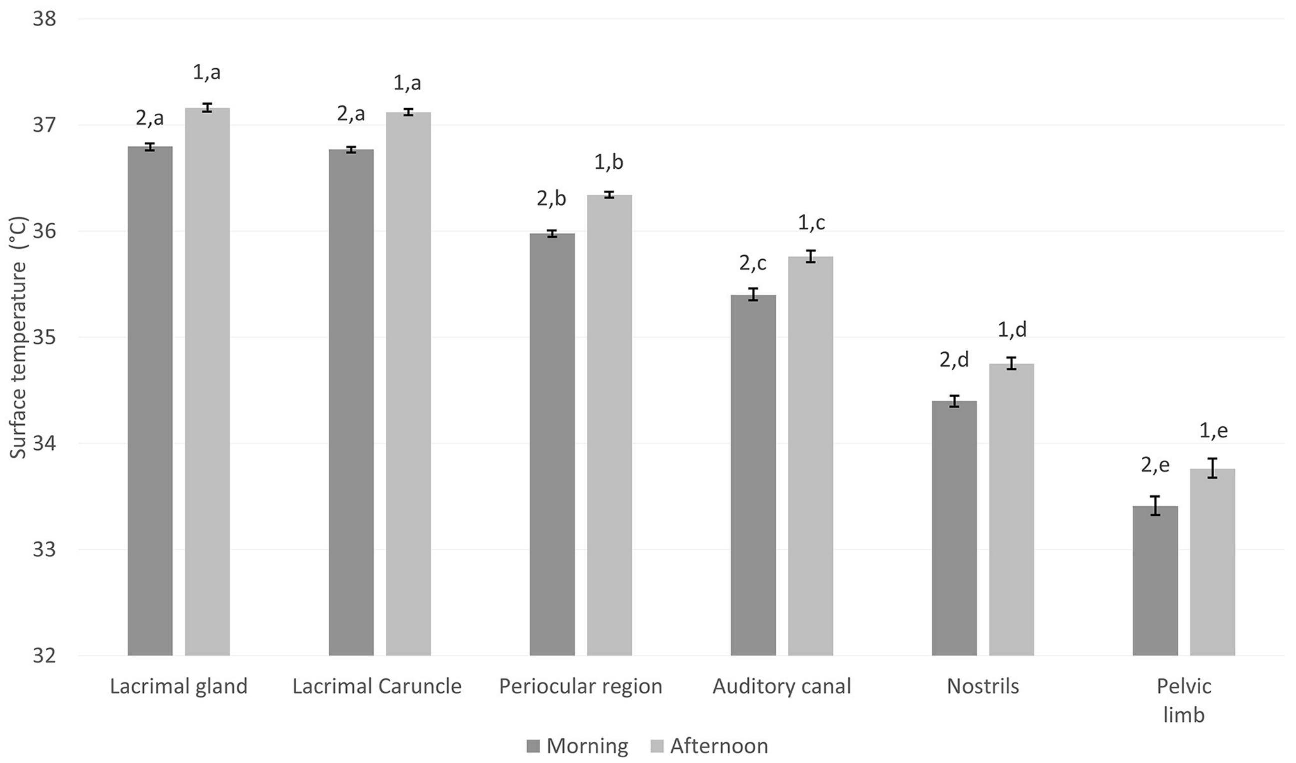

FIGURE 2
Mean surface temperature ± standard error (SE) of six thermal windows of water buffalo neonates ($n = 109$) at two different times of day (morning from 8:00 to 9:00 a.m., afternoon from 15:00 to 16:00 p.m.). Linear mixed model analysis with a Tuckey *post-hoc* test using the GraphPad program (VER. 9.4.0). n, number of calves; Weight of calves according to category: Q_1, 37.8–41.25 kg; Q_2, 41.3–46.3 kg; Q_3, 46.4–56.3 kg; Q_4, 56.4–60.3 kg. Mean ± standard error. A significance level of $p < 0.05$ was established. ^{a,b,c,d,e}Different literals indicate a statistical difference between the weights of the animals. ^{1,2}Different numbers indicate statistically significant differences between days in the same weight group.

to a decrease in environmental temperature, the mortality rate was 23.5%, 14% higher than that during the summer.

As our findings show, energy resources differ depending on the weight of newborns, so those with higher weight have a greater chance of using the energy that is available to them to compensate for temperature changes at birth (39). For example, newborn piglets weighing <1 kg have lower energy resources and greater sensitivity to cold stress during the 1st h of life (40). Similarly, data from newborn Holstein-Friesian calves immersed in water at a temperature of 15–17°C to induce cold stress showed 3-fold increases in catecholamine and blood glucose concentrations, suggesting that hypothermia activates the autonomic nervous system to increase blood glucose levels that contribute to shivering and non-shivering thermogenesis (41, 42).

Another factor linked to birth weight that can be altered is the newborn's vitality and ability to reach the mother's udder (43). Ingesting colostrum helps maintain metabolic resources that aid thermoregulation (7). When this behavior is limited, the ability of young animals to acquire more nutrients is reduced (1). A study by Stanko et al. (44) showed that adding exogenous sources of energy in the form of glucose or colostrum maintained thermostability during the 1st day after birth in newborn Brahman calves exposed to a cold environment (5–25°C). A study by Schmidek et al. (45) in cattle identified factors predisposing to the delayed first intake of colostrum and their implications for the calf mortality rate. Bueno et al. (46) also emphasize the importance of first-colostrum feeding latency, and its effect on calf cortisol levels, with adverse effects on immunoglobulin absorption.

In the present study, regardless of birth weight, the calves presented higher than surface temperatures on days 3, 4, and 5, possibly influenced by the quality of nutrition provided during the 1st days of life. Conte et al. (47) reported that the ingestion of good-quality colostrum can mitigate the negative impact of thermal stress because its lipidic and niacin contents participate in the metabolic and vasodilator responses that aid thermoregulation. The energy provided by colostrum is another element that contributes to obtaining resources and maintaining the thermal balance. According to Silva and Bittar (2), a domestic bovine calf requires 4.6–4.7 Mcal/kg to maintain thermoneutrality. In this regard, studies that have focused on colostrum composition have reported fat, protein, and total solids percentages of 11.31, 8.73, and 25.31%, respectively. These three elements also provide neonates to energy (48). These factors could explain the significantly lower temperatures on days 0, 1, and 2, suggesting a compensatory effect of colostrum ingestion that, in a study of newborn Holstein calves, brought about an increase of ∼1°C in prescapular temperature (49). The differences found in the days after birth support the hypothesis that colostrum consumption is essential for newborn ruminants to achieve thermoregulatory success (1).

TABLE 8 Significant correlations between the time of day and the surface temperature of the different thermal windows.

Thermal window	Variable	Correlation coefficient (*r*)	*P*-value
Periocular	Morning	0.9990	<0.0001
	Afternoon	0.9988	<0.0001
Lacrimal gland	Morning	0.9983	<0.0001
	Afternoon	0.9979	<0.0001
Lacrimal caruncle	Morning	0.9976	<0.0001
	Afternoon	0.9976	<0.0001
Auditory canal	Morning	0.9989	<0.0001
	Afternoon	0.9984	<0.0001
Nostrils	Morning	0.9988	<0.0001
	Afternoon	0.9989	<0.0001
Pelvic limb	Morning	0.9989	<0.0001
	Afternoon	0.9974	<0.0001

Spearman's rank correlation coefficients and their statistical significance between the sampling (morning/afternoon) and the surface temperature of the different thermal windows on day 0, significance level $p < 0$.

Other experimental data have shown that feeding neonates high-fat diets resulted in an increase in body temperature of 0.5°C under exposure to cold climates, an environmental factor also associated with enhanced muscle development in newborns (50, 51).

Therefore, the results presented affirm that the birth weight of buffalo calves is related to the amount of energy available at birth and can affect their thermoregulatory capacity. In addition, said thermoregulatory capacity is developed according to the age of the animals, which can lead to morphophysiological changes.

4.2. Temperature variation according to the thermal window

According to Mota-Rojas et al. (52), ruminants have some anatomical regions with a high disposition of blood capillaries, arteriovenous anastomoses, and glabrous skin. These areas are known as thermal windows. These regions facilitate heat exchange between the animal and its environment; therefore, exposure to cold temperatures generates a peripheral vasoconstriction response that decreases blood flow (41).

In the present study, temperatures of the lacrimal gland and lacrimal caruncle did not differ between groups Q_1 and Q_2. Moreover, the temperatures recorded in these two thermal windows were higher than those in the other evaluated regions. This suggests that the ocular region could be a useful window for non-invasive assessment of core temperature (23, 53). The difference between these two regions may be attributable to the activation of the sympathetic nervous system (SNS) and the consequent secretion of catecholamines that participate in peripheral vasoconstriction (54–56). This vasomotor response preserves the core temperature in metabolically active organs, such as the brain, heart, and abdomen (28, 57). The effects of this response were described by Shu et al. (58). This body of evidence suggests that the high temperatures exhibited in these two windows are due to their proximity to the brain (59).

Difference between the responses of the lacrimal caruncle and lacrimal gland may also reflect the fact that the latter is irrigated by the infraorbital artery, a structure innervated by the sympathetic branch of the facial nerve (52, 58, 60). Given these anatomical traits, these regions could be helpful for indirectly assessing autonomic nervous system activity as a stress response (61, 62).

The ocular or periocular window can provide a better approximation of core temperature (52, 59). Hoffman et al. (63), for example, established that the IRT readings (mean = 37°C) taken from the eyes of 22 cows and nine calves demonstrated that this is a suitable area for monitoring body temperature, unlike the ear and shoulder. Recently, studies have been done in buffaloes, in which it has been determined that the orbital thermal window could be a validated zone in this specie to record temperature (14, 52, 59).

In contrast to these regions, the auditory canal and pelvic limb registered low temperatures. The recorded values indicate heat loss through the skin due to dermal vasoconstriction and closing of arteriovenous anastomoses (28). IRT readings from the ears and limbs of newborn piglets should be useful to recognize the early stages of hypothermia, although it is important to mention that the concave shape of the auditory canal could alter radiation in that zone (23). For this reason, evaluating the thermal response of the limbs, where blood flow may be reduced due to the vasomotor changes that occur under exposure to cold, could be an alternative (1), even though this possibility requires further study.

The nostrils showed significantly lower temperatures than those recorded in the ocular, lacrimal caruncle, and auricular regions. These results are attributed to the tachypnea response as a means of achieving thermoneutrality (64). In this regard, the increase in oxygen intake compensates for one of the main consequences of hypothermia; namely, hypoxemia (57, 65). Even under conditions of regular respiratory rate, the convective processes associated with the inhaled and exhaled airflows, provide evaporation of fluid present in the nostrils that cause the corresponding cooling. The response in this window reflects increased airflow, which could be helpful in monitoring the breathing patterns of animals (66). On this topic, Kim and Hidaka (67) used IRT and computer vision systems to state breathing patterns in cattle. The authors obtained an accuracy of 76%. However, when evaluating temperatures in this region, elements such as the moist surface must be considered (68), as they can alter measurements due to heat dissipation by evaporation (69).

The IRT technique is useful because it responds to the vascular structure of each thermal window, thus allowing for more reliable approximations to detect changes in the body temperature of newborn buffaloes associated with hypothermic states.

4.3. Effect of time of day on thermoregulation in newborn buffaloes

The thermal responses of the six windows evaluated were affected by the time of the day (morning *vs.* afternoon). In addition to this, it was possible to observe that the temperatures of all the thermal windows presented positive correlation with the time of day ($p < 0.05$). The temperature readings of the buffalo calves were significantly higher in the afternoon. A possible explanation for this is the effect of environmental factors. Villanueva-García et al. (23), Mota-Rojas et al. (69), and Kozat (15) mentioned that elements such as wind and rain can cause hypothermia in newborns, whereas solar radiation has the opposite effect (70).

In the six thermal windows, during the afternoon, heat loss by evaporation is attenuated, so the amount of blood in the dermal tissue increases due to the activation of the SNA (71, 72). A study of Murrah water buffaloes reported that exposure to 45°C during the summer increased blood flow by 5–17 units (in the dorsal, abdominal, and auricular areas), as well as the animals' respiratory frequency (73). Marai and Heeeb (74) mention that the characteristics for the water buffalo to remain in an optimal productive state are an air temperature of 13–18°C, relative humidity of 55–60%, and an air speed of 5–8 km/h. An environmental temperature lower than 15°C is a crucial factor that could challenge thermoregulatory mechanisms in newborn, while wind speed and solar radiation could affect the IRT reading. This could be a limitation within the present study and an element to consider for future studies.

The ability of water buffaloes to maintain thermoneutrality during periods of intense heat also depends on characteristics such as the presence of a thick epidermis, low hair density, and abundant melanin (14, 52). Interestingly, these features may help in a certain way to increase resilience to cold because the difficulty that these animals experience in dissipating heat could be beneficial by preventing greater heat loss through evaporation (73).

According to our findings, the time of the day influences the thermostability of newborn water buffaloes (75); therefore, this must be considered an important factor for the perinatal management of the offspring of this species.

5. Conclusion

According to the results obtained, the birth weight of water buffalo calves is a factor that can alter their thermoregulation, as calves with higher weight at birth achieve thermostability more quickly than those with lower weights. However, individual characteristics are not the only factor that should be considered, as the half-period of day can also alter the surface temperatures of animals, since they tend to be lower in the morning and higher in the afternoon. Considering these findings, IRT can be used as an additional tool to monitor normothermic states in newborn water buffaloes, where timely diagnosis of critical states can promote better productive management of buffalo calves in the face of possible exposed to cold. The findings in the present article show that lacrimal caruncle and periocular surface can be considered as more stable thermal windows to monitor the thermal state of the newborns, followed by nostrils.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animals monitored in this study were not touched or stressed, as infrared thermography is a non-invasive technique; therefore, the protocol did not require approval from an Ethics Committee. During the study, handling of the animals was carried out under the guidelines of the Official Mexican Standard, NOM062-ZOO-1999, which stipulates the technical specifications for the production, care, and ethical use of animals.

Author contributions

All authors contributed to the conceptualization, writing, reading, and approval of the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Assessment of thermal changes in water buffalo mobilized from the paddock and transported by short journeys

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Evaluating the welfare of buffaloes during transport is key to obtaining and commercializing high-quality meat products; however, effective assessments require recognizing several stressors that activate physiological mechanisms that can have repercussions on the health and productive performance of species. The aim of this study was to evaluate the surface temperatures of different body and head regions in this species during events prior, and posterior, to transport for short periods; that is, from paddock to loading. The second goal was to determine the level of correlation between thermal windows. This study used infrared thermography (IRT) to evaluate the surface temperature of 624 water buffaloes (Buffalypso breed) during 12 short trips (average duration=2 h±20 min) by focusing on 11 regions of the body (*Regio corporis*), in the head regions (*Regiones capitis*) the face regions (*Regiones faciei*), Orbital region (*Regio orbitalis*) with special attention to structures such as the lacrimal caruncle, periocular area and lower eyelid (*Regio palpebralis inferior*); nasal region (*Regio nasalis*) with special attention to nostril thermal window; and regions of the skull (*Regiones cranii*) such as auricular region (*Regio auricularis*) with special attention to auditory canal and frontal–parietal region (*Regio frontalis-parietalis*) and trunk region (*Truncus regionis*) such as thoracic and abdominal regions, regions of the vertebral column (*Columna vertebralis*) with the thoracic vertebral region (*Regio vertebralis thoracis*) and lumbar region (*Regio lumbalis*); and regions of the pelvis limb (*Regiones membri pelvini*). Recordings were made during seven phases: paddock (P1), herding (P2), corral (P3), chute handling (P4), shipping (P5), pre- (P6), and post-transport (P7). A total of 48,048 readings were obtained from 11 thermal windows. The results showed that the surface temperatures of the windows increased by as much as 5°C during P2, P3, P5, P6, and P7 compared to P1 and P4 ($p<0.0001$). Differences of at least 1°C were also observed between thermal windows in the craniofacial, lateral corporal, and peripheral zones ($p<0.0001$). Finally, a strong positive correlation ($r=0.9$, $p<0.0001$) was found between the thermal windows.

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These findings lead to the conclusion that the surface temperature of the craniofacial and corporal regions of buffaloes transported for short periods varied in relation to the phase of mobilization (from paddock to post-transport), likely as a response to stressful factors, since herding and loading increased the thermal values in each window. The second conclusion is that there are strong positive correlations between central and peripheral thermal windows.

KEYWORDS

***Bubalus bubalis*, infrared thermography, animal welfare, transport, herding method**

1. Introduction

Animal transport is an integral element in the process of breeding and fattening buffaloes (1–3) to obtain and commercialize meat products (4–7). However, transport is considered one of the most stressful procedures for farm animals because it exposes them to environmental factors, drivers' aptitude, road conditions, and trip duration, all of which can affect their welfare by provoking injuries or negative mental states such as fear and anxiety, due to the triggering of physiological and behavioral mechanisms that can modify muscular metabolism and muscle-to-meat conversion, and affect the amount, quality, and innocuity of the final products (8–11). Obviously, this means economic losses for the producers.

In light of the negative effects of mobilization-induced stress, diverse behavioral, pathological, and physiological indicators are being used to evaluate the levels of animal welfare during this phase, where observable physiological changes include dehydration, hyporexia, tissue damage, smoke inhalation, and physical, thermal, and psychological stress (12–15), which can inhibit the immune system under long exposure to adverse environments (16). Endocrine and metabolic alterations, increased plasma cortisol and serum protein concentrations, and glycemia levels have been documented after 16 h of transport, while loss of live weight (from 7.9 to 10.5%) (17, 18) has been associated with energy deficits caused by muscular and hepatic glycogenolysis (19). Another physiological indicator suggested for monitoring is body temperature, as increases can aid in recognizing stressful processes and may be related to the overall health condition of animals (20). Regarding behavioral changes in buffaloes, reports have emphasized that transport conditions increase the frequency of aggressive and dominant behaviors (21, 22), urination (28%), defecation, and urination and defecation (72%), as well as attempts to jump from vehicles (12%) (7) which results in falls and injuries that affect both the animals and the quality of the final product.

Water buffaloes have shown a greater predisposition to transport-induced stress due to certain morphophysiological characteristics that make them susceptible to thermal stress, a very common consequence of transport. Buffaloes have fewer sweat glands and a lower proportion in relation to body weight than other livestock species (394/cm^2), low hair density (100–200/cm^2), and hide with more pigmentation that impedes adequate thermal exchange (23, 24). Evaluating body temperature in buffaloes is a parameter that permits the determination of the degree of thermal stress. The "gold standard" for monitoring this factor was obtained via invasive methods, like measuring rectal and vaginal temperatures (25). However, these techniques can generate stress in animals due to the handling requirements. For this reason, non-invasive technologies such as IRT have been proposed as tools to evaluate surface temperatures in these animals (26, 27).

IRT enables evaluation of the amount of heat that a body radiates, a phenomenon related to changes in peripheral blood flow through activation of the autonomic nervous system (ANS) in the face of diverse stressors (28). Because transport entails a certain degree of stress that has not been widely studied in water buffaloes, the aim of this study was to evaluate the surface temperatures of different body and craniofacial regions in this species during events prior, and posterior, to transport for short periods; that is, from paddock to loading. The second goal was to determine the level of correlation between central (abdominal and thoracic region) and peripheral (limb and nasal) thermal windows.

2. Materials and methods

2.1. Study location

The study was conducted in the state of Veracruz in south-southeast Mexico, from June 2021 to August 2022. The production unit is in a zone with a tropical humid climate, with a mean temperature of 31 ± 2°C, relative humidity of 86%, an elevation of 20 m.a.s.l., and annual rainfall of 1,500–2,000 mm (29).

2.2. The animals and the distribution of the phases

For this study, 624 male Buffalypso buffaloes destined for fattening were selected. The mean weight of the animals was 230 ± 21.78 kg. They were transported on 12 short trips covering 110 km with a mean duration of 2 h ± 20 min and an average velocity of 55 km/h.

The buffaloes were divided for the 12 trips according to the capacity of the first floor of the truck as follows: 53, 51, 49, 56, 53, 50, 53, 52, 54, 51, 52, and 50. Before each trip, they were housed in the same paddock and herded gently (no use of physical utensils or shouting by handlers).

2.3. Housing prior to loading and vehicle type

To carry out the experimental protocol, the transport process was divided into seven phases (Figure 1):

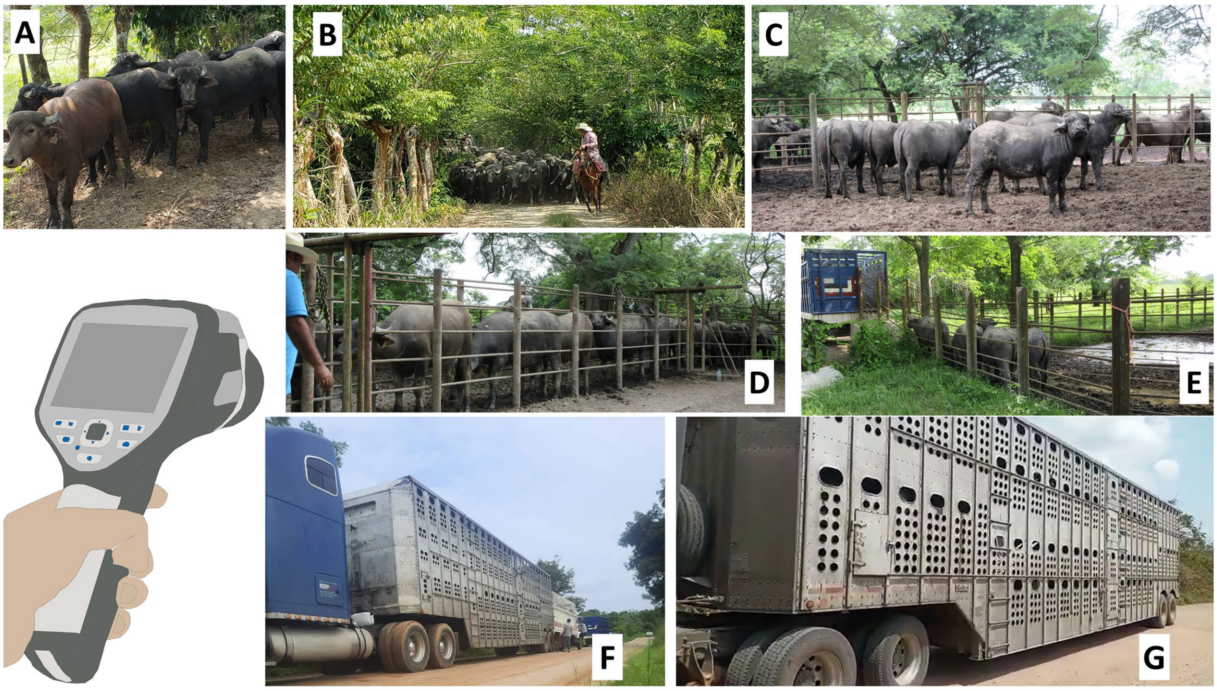

FIGURE 1
Areas where the evaluation phases were carried out. **(A)** The paddock of the production unit; basal sampling (P1). **(B)** After herding by men on horseback (P2). **(C)** After spending the night in the cattle corral system, prior to entering the handling chute (P3). **(D)** Handling chute (P4). **(E)** Entering the vehicle via a loading ramp (P5). **(F,G)** The vehicle prior to transport (P6) and upon arrival at the reception site (P7).

Phase 1 (P1). Paddock, basal monitoring: The animals were housed in paddocks with natural shade until they reached a weight of 208–251 kg. Alimentation consisted of native grasses (*Paspalum fasciculatum*) (percentage of dry matter = 14.7% with 56.7% neutral detergent fiber, 38.8% acid detergent fiber, 6.2% raw protein (30), and 50 g/animal of mineral complements).

Phase 2 (P2). Herding by handlers on horseback: Once the steers reached the desired weight, they were herded gently by men on the horseback without physical utensils or electric prods. For all trips, herding from the paddock to the corral system (P3) required an average of 25–35 min. This was always performed at 18:00. The animals remained in the cattle corral system for 12 h (including the night before transport).

Phase 3 (P3). Cattle corral system: The pen had a surface area of 630 m^2 and a dirt floor. It was framed by 1.6 m high tubular structures with an entrance gate and an exit gate that led to the handling chute. The buffaloes remained there for 8 h without solid food but with *ad libitum* access to water.

Phase 4 (P4): Handling chute: The chute consisted of a hallway (1 m wide × 1.6 m high × 3.5 m long), framed by tubular structures, and connected to an individual weight scale. This hallway led to a loading ramp. The average duration was 50 min.

Phase 5 (P5). Loading: During an interval of approximately 30–50 min, the buffaloes were led up with a concrete loading ramp made of anti-slip grooving. Slope was 20°.

Phase 6 (P6). Pre-transport: The time spent in the vehicle before transport, counted from the moment when all the animals had been loaded into the truck.

Phase 7 (P7). Post-transport: The time when the animals were still inside the vehicle, immediately upon arrival at the reception site, after transport over dirt and paved roads.

The topography of the roads was classified as unpaved or paved. The former had the specifications of Class E roads, according to the classification of Mexico's Department of State (*Secretaría de Gobernación*) (31), with a maximum slope of 13%.

The mean maximum velocity was 25 km/h. Dirt roads were used to exit the production unit (10 km) and enter the reception site (12.5 km). The total trip length on the unpaved roads was 22.5 km with a maximum overelevation of 10%, vertical curves of 4 m/%, and road width of 6 m. The other 87.5 km were covered on a paved road with a maximum transversal slope of 7%, a maximum speed of 55 km/h, vertical curves of 5 m/%, and a road width of 8 m.

The vehicle was a Wilson Trailers Silver Star (dimensions: 15.24 m long × 2.59 m wide × 4.6 m high) with a load capacity of 22,500 kg. The truck was designed especially for animal transport with two floors, sliding side doors, a rear guillotine door, a non-slip floor, a reinforced plastic roof, and fiberglass. The walls were made of galvanized steel and aluminum, with openings for ventilation during the trips.

2.4. Monitoring by infrared thermography

Thermographic monitoring was performed using a FLIR® Thermal TM E60 infrared camera (FLIR Systems, United States) with an IR resolution of 320 × 240 pixels, thermal sensitivity <0.045°C, and precision ±2°C or 2%. All the radiometric images were taken at a distance of 1–1.5 m from the buffaloes, focusing on one of the following regions: facial regions (*Regiones faciei*) and skull region (*Regiones cranii*), trunk region (*Truncus regionis*) left or right, or regions of the vertebral column (*Columna vertebralis*), and regions of the pelvis limb (*Regiones membri pelvini*). Eleven body thermal windows in the head (*Regiones capitis*) and the trunk (*Truncus* *regionis*) were selected and delimited using FLIR tools software. The facial regions (*Regiones faciei*) included the orbital region (*Regio orbitalis*) with special attention to structures such as the lacrimal caruncle, periocular area and lower eyelid (*Regio palpebralis inferior*); nasal region (*Regio nasalis*) with special attention to nostril thermal window and regions of the skull (*Regiones cranii*) such as auricular region (*Regio auricularis*) with special attention to auditory canal and frontal–parietal region (*Regio frontalis-parietalis*) (Figure 2).

The trunk region (*Truncus regionis*) thoracic region and abdominal region, thermal window of the trunk, and regions of the pelvis limb (*Regiones membri pelvini*) with two anatomical regions indicated the femoral region (*Region femoris*) and the tarsus region (*Regio tarsi*), thermal windows of the regions of the pelvis limb (*Regiones membri pelvini*) are shown in Figure 3.

Figure 4 shows the regions of the vertebral column (*Columna vertebralis*) with the thoracic vertebral region (*Regio vertebralis thoracis*) and lumbar region (*Regio lumbalis*).

2.5. Recording and reading of the IRT

In accordance with the seven experimental phases, IRT readings were recorded as follows: P1, prior to the buffaloes being herded by men on horseback. Recordings were made while they were at rest for 1 h under shade, between 17:00 and 18:00 h; P2, inside the corral on the day prior to transport; P3, at 08:00 h, prior to guiding the animals into the handling chute to be weighed and loaded; P4, while the buffaloes were in the handling chute waiting to be weighed; P5, during loading as the animals walked up the ramp and entered the truck; P6, for 40 min after all the buffaloes were loaded into the truck but with the motor shut off, and P7, for 40 min immediately after concluding the short trip, but with the truck's motor still turned on.

All digital and radiometric images were stored in JPG format for later analysis using FLIR software (Tools Systems, United States) to obtain the maximum, minimum, and average temperature readings, which were then entered into a database.

The ambient temperature (AT) and relative humidity (RH) were monitored at different times, in accordance with the experimental phase. P1: AT and RH ranged from 23 to 25°C and 81–90%, respectively (17:00–18:00 h). P2: AT and RH values were 21–25°C and 81–88%, respectively (18:00–18:30 h). P3: AT and RH fluctuated around 20–23°C and 81–86%, respectively (08:00 h). P4: AT and RH were 22–25°C and 81–88%, respectively (09:00 h). P5: values were 22–27°C and 81–88% at 10:00 h. P6 and P7: data for AT and RH ranged from 22–28°C and 81–89% at 11:00 h and 23–30°C and 83–94% at 14:00–15:00 h.

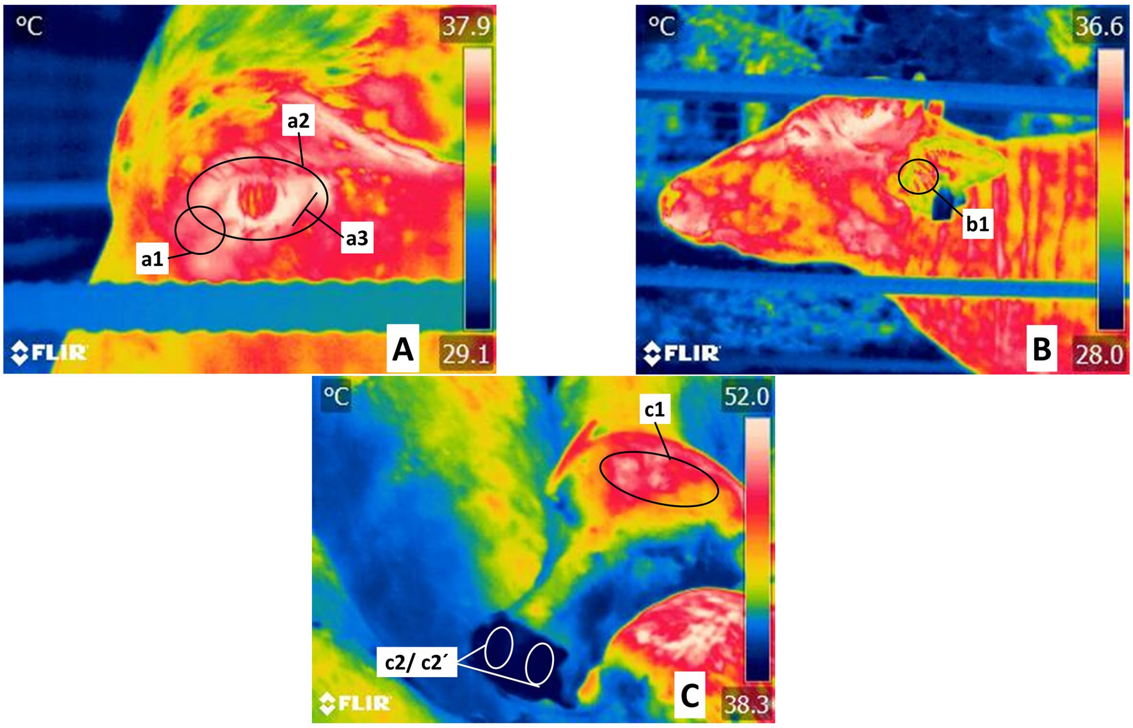

FIGURE 2
Delimitation of thermal windows in the head regions (*Regiones capitis*). **(A)** Ocular thermal windows in Orbital region (*Regio orbitalis*). Recordings in this zone centered on the lacrimal caruncle, delimited as circle a1, and considered the medial commissure of the eyelids up to half a centimeter toward the cranium. This area reflects the radiation of blood flow from the infraorbital artery innervated by the sympathetic branches of the facial nerve. The periocular thermal window, marked by circle a2, spans the periphery of the upper and lower eyelids, irrigated by both the supraorbital and infraorbital arteries. The lower eyelid thermal window, defined by a line approximately 3 cm long (a3), was used to evaluate radiation from the conjunctival mucosa and was irrigated by the infraorbital artery. **(B)** Regions of the skull (*Regiones cranii*), thermal window of the auricular pavilion in the auricular region (*Regio auricularis*). This area is framed by circle b1 in the region of the auditory canal, the antihelix ridge, and the lateral peduncle of the helix, irrigated by the media and the lateral and central auricular arteries. **(C)** Thermal window of the frontal-parietal region (*Regio frontalis-parietalis*). Outlined by an oval (c1) that encompasses the frontal-parietal region (*Regio frontalis*), cornual region (*Regio cornualis*) limits, and parietal region (*Regio parietalis*), a zone irrigated by cornual and supraorbital arteries. *Regio naris* thermal window in the frontal region (*Regio frontalis*): ovals c2 and c2´. It was used to evaluate the radiation from the maxillary artery.

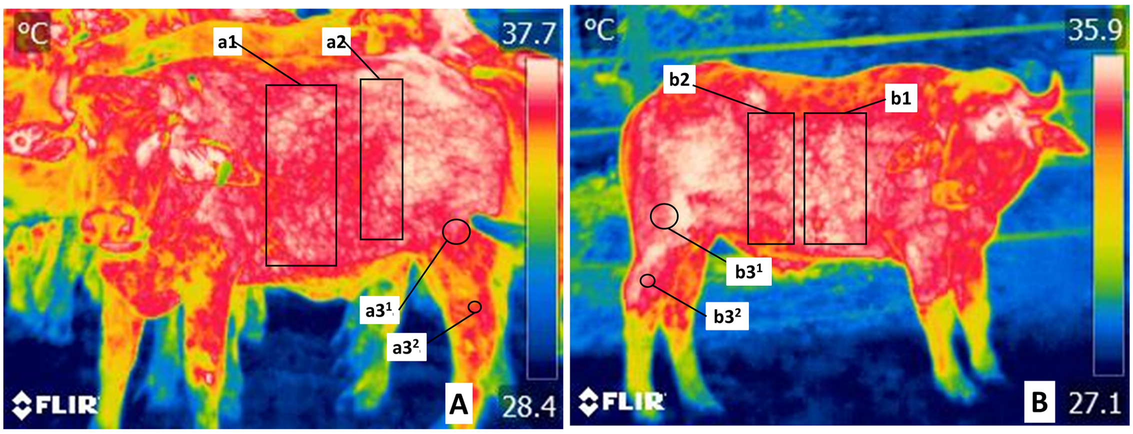

FIGURE 3
Delimitation of the trunk thermal windows in the trunk region (*Truncus regionis*). **(A)** The thermal window of the thoracic region in costal region (*Regio costalis*) is shown by a rectangle (a1, b1) traced from the costal arch from 1° to 12°. The cranial abdominal region (*Regio abdominis cranialis*) delimited by a rectangle (a2, b2), includes the oblique abdominal and rectus abdominis muscles irrigated by the surface epigastric artery (*A. epigastrica cranialis superficiales*). **(B)** The thermal window of the regions of the pelvis limb (*Regiones membri pelvini*) were marked by two circles at the *Regio femoris* (a3^1, b3^1), and at the *Regio tarsi* (a3^2, b3^2). It includes the femoral region the femoral muscle and the tarsus region lateral projection of the region of the common calcaneal tendon (*Regio tendinis calcanei communis*), regions irrigated by the femoral artery, and branches of the saphenous artery.

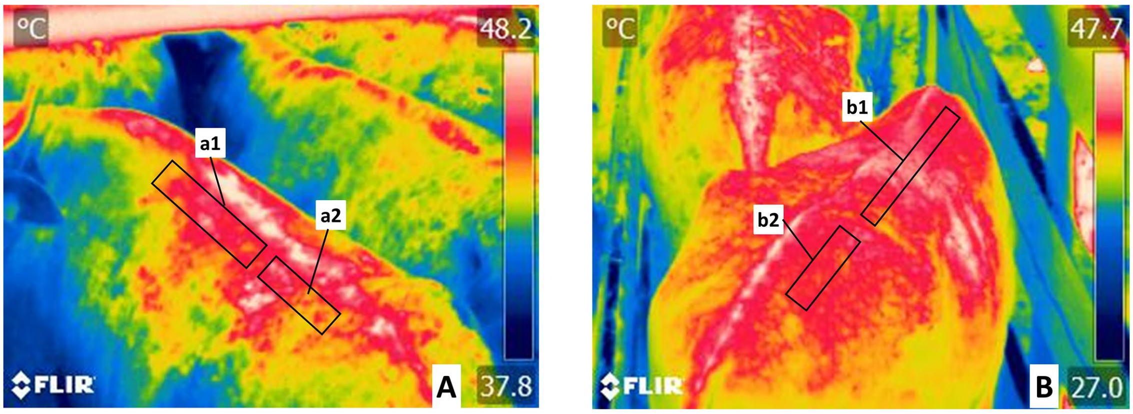

FIGURE 4
Delimitation of the regions of the vertebral column (*Columna vertebralis*) thermal windows. **(A)** The thermal window of the thoracic vertebral region (*Regio vertebralis thoracis*) is marked with a rectangle. It includes the left (a1) and right (b1) projections from the thoracic vertebrae and the thoracic vertebrae and the with of the transverse processes from thoracic vertebrae 1ª–12ª. This area is irrigated by spinal branch (*Ramus spinalis*) from the dorsal branches of the abdominal aorta artery, in the thoracic vertebral region. **(B)** The thermal window of the lumbar vertebral region (*Regio vertebralis lumbalis*). This rectangle encompasses the left (a2) and right (b2) projections from the lumbar vertebrae above the transverse processes of lumbar vertebrae 1ª–7ª. This permits the evaluation of radiation from the spinal branch (*Ramus spinalis*) from the dorsal branches of the abdominal aorta artery, in the lumbar vertebral region.

2.6. Statistical analysis

The GraphPad Prism statistical package (ver. 9.4.1) was used for all analyses. First, descriptive statistics were obtained for each thermal window (periocular, lacrimal caruncle, lower eyelid, auditory canal, nostrils, parieto-frontal area, and the thoracic, abdominal, appendicular, lumbar, and dorsal areas) and event (P1–P7). Results are expressed as mean ± standard error (SE). The Shapiro-Wilk test was used for normality analysis.

The events and thermal windows were considered as independent variables, and the surface temperature was considered as the dependent variable. Analysis of variance (ANOVA) was performed to evaluate the effect of these variables in a linear mixed model using the following statistical model:

$$Y_{ijak} = \mu + \tau i + \tau j + \tau i \tau j + \beta k + e_{ijk}$$

where:
Y = response variable (surface temperature).
τi = effect of the thermal window.
τj = effect of the event.
$\tau i \tau j$ = interaction effect.
β = random effect (animal).

μ = population mean.

e = error.

Differences between means were analyzed using a *post-hoc* Tukey test. The level of significance was set at $p < 0.05$. Correlation analysis was conducted using the Pearson's correlation coeficient.

2.7. Ethics statement

The experimental protocol was approved by the Scientific Commission of the Master in Science (CAMCA) "Maestría en Ciencias Agropecuarias" of the Faculty of Veterinary Medicine and Animal Husbandry, Universidad Autónoma Metropolitana, Mexico City, Mexico. The animals used in this study were handled gently, without the use of physical utensils that could cause stress, in accordance with the guidelines of the Official Mexican Standards NOM-062-ZOO-1999, which establish technical specifications for the production, care, and ethical use of animals during experimentation; and NOM-051-ZOO which establish technical specifications for humanitarian handling in the mobilization of animals published by the Department of Agriculture, Rural Development, Fisheries and Alimentation. It is also important to consider that the animals were not touched or stressed, since infrared thermography is a non-invasive technique.

3. Results

During the seven phases analyzed, we obtained 48,048 surface temperature readings from 11 thermal windows of 624 water buffaloes transported on 12 short trips. Table 1 shows the mean temperatures of the thermal windows in the head regions (*Regiones capitis*) and their corresponding standard errors.

Observations showed the thermal windows considered in the orbital region (*Regio orbitalis*) such as the lacrimal caruncle, periocular region, and lower eyelid thermal windows had significant average increases of 4.3°C, 4.8°C, and 3.7°C, respectively, during P2, P3, and P4, compared to P1 ($p < 0.0001$). Readings for the Regions of the skull (*Cranii regions*), the auditory canal thermal window showed increases of 1.3°C, 0.8°C, and 1.3°C in those windows compared to P5 ($p < 0.0001$), while the comparison of P3 to P2 and P4 revealed a significant reduction of 1.3° ($p < 0.0001$). Observations during P6 and P7 showed a gradual increase in surface temperature compared with P5 ($p < 0.0001$).

Readings for the auditory canal window showed an increase of 7.7°C from P1 to P2 ($p < 0.0001$), but the auricular temperature decreased by 2.6°C from P2 to P3 ($p < 0.0001$). Later, it increased by 1°C and 0.8°C during P4 and P5, respectively, compared to P2 ($p < 0.0001$). Recordings for P6 and P7 showed significant increases of 2.7°C and 2.5°C compared to P4 and P5 ($p < 0.0001$).

For the thermal windows the nasal region (*Regio nasalis*), the nostrils and frontal-parietal region, the study found that temperatures in P1 were 4.7°C and 11.6°C lower, respectively, than those in P2 ($p < 0.0001$), but those temperatures decreased by 1.3°C and 6.4°C, respectively, in P3 ($p < 0.0001$). In P4 and P5, the temperatures increased by 0.5°C and 0.8°C, respectively ($p < 0.0001$). This increase was maintained in P6 (2°C) and P7 (2.6°C), compared to P5 ($p < 0.0001$). Readings from the thermal window of frontal- parietal region showed increases of 8.4°C in P4, 4.5°C in P5 ($p < 0.0001$), and 8.6°C in P7 compared to P5 and P6 ($p < 0.0001$).

Considering the different phases of this study and the thermal responses in the windows described, during P1, temperatures in the orbital region (*Regio orbitalis*) such as the lacrimal caruncle, periocular, and lower eyelid were significantly 2°C higher than in the auditory canal, nostrils, and thermal window of frontal-parietal region ($p < 0.0001$). During P2, the parieto-frontal window presented the highest temperature among all windows ($p < 0.0001$). The orbital region (*Regio orbitalis*) such as the lacrimal caruncle, periocular, and auditory canal had temperatures 0.7°C higher than those of the lower eyelid ($p < 0.0001$), but the latter was 1.9°C higher than the nostrils window ($p < 0.0001$).

Observations from P3 showed that the temperature of the periocular window was 0.3°C higher than that of the lacrimal caruncle ($p < 0.0001$), and 1°C higher than that of the lower eyelid ($p < 0.0001$). Similarly, the temperature of the lower eyelid was 1.2°C higher than that in the nostril window ($p < 0.0001$). For P4, P5, P6, and P7, the thermal window of frontal–parietal region showed the highest temperatures in all regions ($p < 0.0001$), followed in the orbital region (*Regio orbitalis*) by the lacrimal caruncle, periocular region, and lower eyelid region ($p < 0.0001$).

The temperatures of the thermal windows of the lateral region of the trunk are presented in Table 2. In P1, the thermal window of the thoracic region presented a lower temperature than the other phases but was 8.6°C lower in P2 ($p < 0.0001$). Readings for phases P3, P4, and P5 showed reductions of 5.4°C, 3°C, and 3.3°C, respectively, compared to P2 ($p < 0.0001$), but increases of 3.4°C in P6 and 4.9°C in P7 were determined ($p < 0.0001$). The thermal window of the abdominal region recorded a temperature increase of 6.8°C in P2 compared to P1 ($p < 0.0001$). With respect to P3, P4, and P5, we observed decreases of 4°C, 1.7°C, and 2.8°C, respectively, compared to P2 ($p < 0.0001$), whereas P6 and P7 registered increases of 4.6°C and 5.3°C, respectively ($p < 0.0001$). Temperature readings for the thermal window of the pelvic limb in P1 were the lowest of all events ($p < 0.0001$), but the temperatures recorded in P2 and P6 were significantly higher than those in P3, P4, and P5 by 2.7, 0.2, and 2.3°C, respectively ($p < 0.0001$).

For P1 and P6, we found that the thermal window had values 0.5 and 0.4°C above those of the thermal window of the thoracic region ($p < 0.0001$). Readings for the latter, compared to the limbs regions, showed increases of 0.9 and 3.7°C, respectively ($p < 0.0001$). During P2 and P7, the study found that the thermal window of the thoracic region was 1.3 and 0.4°C higher than the thermal window of the abdominal region ($p < 0.0001$) while the latter was 1.6 and 3.7°C higher than the thermal window of the pelvic limb regions ($p < 0.0001$).

Table 3 presents the thermal values recorded in the thermal windows of the regions of the vertebral column. The reading for the thermal window of the thoracic vertebral region (*Regio vertebralis thoracis*) in P1 was lower than in all other events ($p < 0.0001$). The temperature of this window was 6°C and 6.6°C higher in P2 and P4 than in P3 ($p < 0.0001$) but decreased by 2.1°C and 1.9°C in P5 and P6, respectively ($p < 0.0001$). Later, the surface temperature of this thermal window increased by 4.3°C compared to P6 ($p < 0.0001$). Observations of the thermal window of the lumbar region (*Regio lumbalis*) showed that P6 had the highest temperature of all thermal windows ($p < 0.0001$), although in P1, it obtained the lowest value ($p < 0.0001$). In P2 and P4, this zone registered temperatures of 6.4°C and 6.7°C, respectively, higher than in P3 ($p < 0.0001$). Finally, the surface

TABLE 1 Mean±standard error (SE) temperatures for the thermal windows of the head regions (*Regiones capitis*) of 624 water buffaloes in the 7 phases of transport for short periods.

		Phase						
Thermal window		P1. Paddock	P2. Herding	P3. Corral	P4. Handling chute	P5. Loading	P6. Pre-transport	P7. Post-transport
Facial region (*Regions faciei*)	**Orbital region** *(Regio orbitalis)* Lacrimal caruncle	$33.9 \pm 0.03^{e,1}$	$38.9 \pm 0.06^{c,2}$	$37.4 \pm 0.02^{e,2}$	$38.7 \pm 0.03^{c,3}$	$38.3 \pm 0.03^{d,3}$	$40.2 \pm 0.04^{b,2}$	$41.1 \pm 0.09^{a,4}$
	Periocular region	$33.7 \pm 0.03^{f,2}$	$39.0 \pm 0.04^{c,2}$	$37.7 \pm 0.02^{e,1}$	$39.0 \pm 0.03^{c,2}$	$38.5 \pm 0.03^{d,2}$	$40.0 \pm 0.03^{b,2,3}$	$41.5 \pm 0.09^{a,2}$
	Lower eyelid	$33.9 \pm 0.04^{f,1,2}$	$38.3 \pm 0.02^{c,3}$	$36.4 \pm 0.06^{e,3}$	$38.1 \pm 0.04^{c,4}$	$37.7 \pm 0.02^{d,4}$	$40.3 \pm 0.08^{b,2}$	$41.3 \pm 0.09^{a,3}$
	Nasal region (*Regio nasalis*) Nostrils	$31.7 \pm 0.04^{g,3}$	$36.4 \pm 0.04^{c,4}$	$35.1 \pm 0.03^{f,5}$	$35.6 \pm 0.02^{e,6}$	$35.9 \pm 0.03^{d,6}$	$37.3 \pm 0.03^{b,4}$	$38.5 \pm 0.03^{a,6}$
Regions of the skull (*Cranii regions*)	Auricular pavilion	$31.2 \pm 0.05^{f,3}$	$38.9 \pm 0.03^{b,2}$	$36.3 \pm 0.05^{e,4}$	$37.3 \pm 0.03^{c,5}$	$37.1 \pm 0.02^{d,5}$	$39.8 \pm 0.03^{a,3}$	$39.7 \pm 0.04^{a,5}$
	Frontal-parietal region	$31.5 \pm 0.06^{g,3}$	$43.1 \pm 0.08^{c,1}$	$36.7 \pm 0.03^{f,3}$	$45.1 \pm 0.07^{b,4}$	$41.2 \pm 0.06^{e,1}$	$41.1 \pm 0.09^{e,1}$	$49.7 \pm 0.15^{a,1}$

Different literals a, b, c, d, e, f, g indicate statistically significant differences among the temperatures of the animals according to the phase.
Different numbers 1, 2, 3, 4, 5, 6 indicate statistically significant differences between the temperatures of the thermal windows.

TABLE 2 Mean±standard error (SE) of the temperatures in the thermal windows of the lateral region of the trunk of 624 water buffaloes transported during seven phases of short trips.

		Phase						
Thermal window		P1. Paddock	P2. Herding	P3. Corral	P4. Handling chute	P5. Loading	P6. Pre-transport	P7. Post-transport
Lateral region of the trunk	Thoracic region	$33.0 \pm 0.03^{e,2}$	$41.6 \pm 0.06^{b,1}$	$36.2 \pm 0.02^{d,1}$	$38.6 \pm 0.03^{c,1}$	$38.3 \pm 0.07^{c,1}$	$41.7 \pm 0.06^{b,2}$	$43.2 \pm 0.08^{a,1}$
	Abdominal region	$33.5 \pm 0.02^{g,1}$	$40.3 \pm 0.05^{c,2}$	$36.3 \pm 0.03^{f,1}$	$38.6 \pm 0.03^{d,1}$	$37.5 \pm 0.05^{e,2}$	$42.1 \pm 0.07^{b,1}$	$42.8 \pm 0.08^{a,2}$
Limbs region	Pelvic limb	$32.1 \pm 0.04^{f,3}$	$38.7 \pm 0.02^{b,3}$	$35.9 \pm 0.03^{e,2}$	$38.4 \pm 0.03^{c,1}$	$36.3 \pm 0.02^{d,3}$	$38.6 \pm 0.04^{b,3}$	$39.1 \pm 0.04^{a,3}$

Different literals a, b, c, d, e, f, g indicate statistically significant differences among the temperatures of the animals according to the phase.
Different numbers 1, 2, 3 indicate statistically significant differences between the windows.

TABLE 3 Mean±standard error (SE) temperatures for the thermal windows of the trunk region (*Truncus regionis*) left or right of 624 water buffaloes during 7 phases of short periods of transport.

	Phase						
Thermal window	P1. Paddock	P2. Herding	P3. Corral	P4. Handling chute	P5. Loading	P6. Pre-transport	P7. Post-transport
Thoracic vertebral region	$32.1 \pm 0.05^{f,2}$	$41.6 \pm 0.09^{b,1}$	$35.6 \pm 0.04^{e,1}$	$42.2 \pm 0.06^{b,1}$	$40.1 \pm 0.10^{d,1}$	$40.8 \pm 0.08^{c,2}$	$45.1 \pm 0.11^{a,2}$
Lumbar vertebral region	$33.4 \pm 0.04^{g,1}$	$42.0 \pm 0.08^{c,1}$	$35.6 \pm 0.04^{f,1}$	$42.3 \pm 0.03^{b,1}$	$40.4 \pm 0.13^{e,1}$	$41.4 \pm 0.07^{d,1}$	$44.6 \pm 0.07^{a,1}$

Different literals a, b, c, d, e, f, g indicate statistically significant differences between the temperatures of the animals according to the phase.
Different numbers 1, 2 indicate statistically significant differences between the thermal windows.

temperature decreased in P5 and P6 by 1.9°C and 1°C, respectively, compared to P4 ($p < 0.0001$).

Regarding statistically significant differences between the thermal windows of the thoracic vertebral region and lumbar region, the findings showed that in P1 and P6, the temperatures of the thermal window of the lumbar region were 1.3 and 0.6°C higher, respectively, than in the thermal window of the thoracic vertebral region ($p < 0.0001$). At P7, the temperature of the thermal window of the thoracic vertebral region was 0.5°C higher than that in the thermal window of the lumbar region ($p < 0.0001$).

Table 4 shows the level of correlation observed between the 11 thermal windows. Overall, the correlations among the different thermal windows were positive, strong, and statistically significant ($p < 0.0001$).

4. Discussion

The results obtained for the buffaloes transported for short periods showed that the surface temperatures of thermal windows of the head and trunk increased significantly in P2, P4, P6, and P7, that is, during the events that required handling ($p < 0.0001$). These responses may be associated with the loss of thermal stability in the buffaloes, a species whose optimal ambient for achieving thermoneutrality is in the range of 13–18°C (32, 33).

Although adequate temperatures have been reported for water buffaloes, there is limited literature on the thermal responses of these species during transport. For conventional bovines, a study on the transport by land and sea of 481 animals reported a significant association between increases in such biomarkers as glucose, creatine kinase, and lactate levels with IRT temperature readings (34). A study of 120 pigs transported for 40 min reported temperature increases in the orbital region and behind the ear, which correlated positively with increased salivary cortisol concentrations ($r = 0.49$ and $r = 0.50$, respectively) (35). Studies of this type in other livestock species subjected to transport suggest that surface temperatures can be useful indicators of physiological changes triggered in response to transport-induced stress (36, 37), which may be reflected as central and peripheral nervous system reply with activation of vasomotor modulation hyperthermia (38).

In our study, the largest surface temperature increases in the buffaloes were recorded in the post-transport phase ($p < 0.0001$). This can be explained by the activation of Autonomic nervous system (ANS) and its sympathetic branch, which generates catecholamine production as a short-term response (24). Catecholamines act on metabolically active organs such as brown adipose tissue to initiate thermogenesis, thus increasing heat irradiation (39, 40). Activation of the hypothalamic-pituitary-adrenal axis (HPA) causes the secretion of glucocorticoids (e.g., cortisol) that promote gluconeogenesis and lipolysis (41, 42). In this regard, studies on ruminants have reported increases in plasma cortisol by up to 10-fold after 30 and 45 min, 1 h, 2 h, and up to 4 h post-transport (43–47). Mitchell et al. (48), cited in Ali-Gholi and Daryoush (49), stated that sympathetic, adrenal, and medullar reactions are related to physiological responses during transport, while hypothalamus, hypophysis, and suprarenal reactions are responsible for cortisol secretion under adverse environmental conditions. Therefore, this physiological response is responsible for heat production during the

TABLE 4 Correlations between the thermal windows of 624 water buffaloes during all seven phases of short periods of transport.

	Lacrimal caruncle	Periocular region	Lower eyelid	Auricular pavilion	Nostrils	Frontal-parietal region	Thoracic region	Abdominal region	Limbs region	Lumbar region	Thoracic vertebral region
Lacrimal caruncle	1*	0.96*	0.97*	0.94*	0.95*	0.95*	0.94*	0.94*	0.95*	0.95*	0.93*
Periocular region		1*	0.97*	0.99*	0.99*	0.98*	0.97*	0.96*	0.99*	0.97*	0.95*
Lower eyelid			1*	0.96*	0.97*	0.98*	0.97*	0.97*	0.96*	0.98*	0.97*
Auricular pavilion				1*	0.99*	0.96*	0.96*	0.94*	1*	0.96*	0.95*
Nostrils					1*	0.98*	0.98*	0.97*	0.99*	0.98*	0.97*
Frontal-parietal region						1*	1*	1*	0.96*	0.99*	0.99*
Thoracic region							1*	1*	0.96*	0.99	0.99*
Abdominal region								1*	0.95*	0.99*	0.98*
Limbs region									1*	0.97*	0.96*
Lumbar region										1*	0.99*
Thoracic vertebral region											1*

*$p < 0.0001$; $^{NS}p > 0.05$.

perception of stressful factors under conditions of transport, handling, and environmental challenges.

This explanation may aid in understanding the report by Sakakibara et al. (50), who found a weak negative correlation between blood cortisol concentrations and surface temperatures ($r = -0.209$) in five bovines transported for 8 h. Robertson et al. (51) observed that activation of the ANS leads to catecholamine secretion (52) that can generate physiological effects like tachycardia, tachypnea, and hyperthermia (53, 54). This could be related to the level of stress experienced by the animals, as Hagenmaier et al. observed (55) in 80 calves transported under two levels of handling. Animals under intensive handling, with the use of electric prods and minimal trotting during loading, had higher lactate, epinephrine, norepinephrine, cortisol, and glucose levels but lower levels of blood pH and bicarbonate, with excess bases, compared to the animals that received minimal handling during transport. This means that the external factors which increase the stress response of animals during transport (e.g., higher surface temperatures) include social interaction with handlers and the training that stockpeople receive to mobilize animals of different species, two factors that are fundamental for preventing intense stress responses, even during short trips like those in the present study (2 h ± 20 min). Some authors have reported that even when the trip time is maintained below 12 h, some species are unable to become habituated immediately to the intrinsic and individual factors that cause stress (56, 57).

Another important condition observed during animal transport that needs to be emphasized is neophobia, that is, fear of new situations. This may be another factor involved in stress responses prior to transporting water buffaloes (58, 59). This fear involves activation of the basolateral region of the amygdala, which coordinates responses by the cerebral cortex and hypothalamus, the physiological center that modulates heart and respiratory rates, and body temperature during events that cause fear or anxiety (60, 61).

With respect to the temperature differences detected among the different thermal windows, the minimal increase of 1°C among the facial, skull and nasal regions in comparison with lateral corporal trunk limbs region, and lateral region of the trunk ($p < 0.0001$) can be explained by the extensive distribution of capillaries and arteriovenous anastomoses that allow heat exchange with the environment (62). When mechanisms that preserve thermoneutrality are activated, heat loss by evaporation (as water vapor), convection, or conduction is responsible for the changes in the amount of heat irradiated through the skin (38, 63). Specifically, the thermal windows of head and periocular, and frontal-parietal regions showed a difference of 4°C ($p < 0.0001$), possibly due to distribution of the blood vessels in those areas. For example, the head regions is were irrigated by the facial artery and its branches in the infraorbital artery, which carries blood to the lower eyelid and lacrimal caruncle (24, 64), whereas the frontal-parietal region is supplied by branches (*A. transversa faciei*, *A. auricularis rostralis*, *A. palpebralis inferior lateralis*, *A. palpebralis superior lateralis*) of the superficial temporal artery (*A. temporalis superficialis*) (64). This distribution of blood vessels could confer a thermoregulatory advantage to water buffaloes by serving as a pathway for heat loss. Taylor (65) found that circulation around the horns allowed Toggenburg goats to serve as a pathway for thermoregulation. This could explain the increase in the temperature of the frontal-parietal region recorded in our study.

The thermal windows in the trunk regions, such as the abdominal or thoracic regions, had temperatures 2°C higher than those in the limbs region ($p < 0.0001$). This is related to the presence of vital metabolically active organs, such as the heart and liver, which contribute to raising body temperature as animals try to compensate through peripheral vasomotor changes in the structures of the limbs (66). Temperatures in these regions are produced by blood flow from the metatarsal dorsal and saphenous arteries (64). Changes in the limbs temperatures in dogs and horses have been associated with levels of muscle activity that increase blood flow at the local level (67, 68); however, the specific muscular response was not evaluated in our study.

The thermal windows of the nostrils were the one that presented the lowest temperatures of all nasal region (*Regio nasalis*) ($p < 0.0001$). Although this area has a high density of surface blood vessels from the maxillary artery and vein (24), elimination of water vapor during the respiratory cycle and tachypnea that occurs during stress are two factors that may influence nostril temperatures, leading to a greater loss of water vapor and heat (28). Finally, it is important to mention that a strong, positive, and significant correlation was observed among the thermal windows ($r = 0.9$, $p < 0.0001$). This means that as the temperature of one body region increases, those of other regions also increase because of the greater radiation of surface heat in the different thermal windows (69, 70).

In a similar manner, Napolitano et al. (71) the surface temperature of 109 buffalo calves was evaluated with the aim of recognizing variations in various thermal windows (both regions of the body and head) with respect to their birth weight, finding that the head region registers higher values (except the thermal window of the nostril) and with less variation with respect to pelvic limbs in all groups studied.

The results of the present study confirm the usefulness of the IRT technique for evaluating large ruminants, for example, to identify changes in the surface temperatures of buffaloes subjected to short periods of transport. However, it is important to emphasize that one of the limitations of this study is that we did not evaluate other response variables, such as physiological (e.g., body temperature) and endocrine parameters (e.g., cortisol concentrations), to relate the temperature increases detected to other factors. Likewise, another limitation of the present study could be the lack of monitoring the cleanliness of the animals to prevent that soiling, fecal matter, sweat, or urine could alter the thermal readings. The inclusion of physiological indicators during the pre- and post-transport phases could provide a clearer picture of the stress experienced by water buffaloes during transport. We consider this an important field for future explorations that will assess the degree of relationship between the surface temperatures of thermal windows and physiological indicators that are modified by the effects of transport.

5. Conclusion

According to the surface temperature values of water buffaloes transported for a short period, transport and the practices applied during this activity affect the temperature of various body and head regions. The responses of each region depend largely on the phase in which they are evaluated (from the paddock to post-transport). Herding and loading were identified as the moments when the buffaloes had the most marked stress responses, which increased the recorded thermal values. The strong positive correlations between the central and peripheral windows can help understand the importance of good handling of buffalo species during transport, with the goal of minimizing the potential stress they may experience and physiological responses that can impact their welfare.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the animal study because the animals used in this study were handled gently, without the use of physical utensils that could cause stress, in accordance with the guidelines of the Official Mexican Standards NOM-062-ZOO-1999, which establish technical specifications for the production, care, and ethical use of animals during experimentation; and NOM-051-ZOO which establish technical specifications for humanitarian handling in the mobilization of animals published by the Department of Agriculture, Rural Development, Fisheries and Alimentation. It is also important to consider that written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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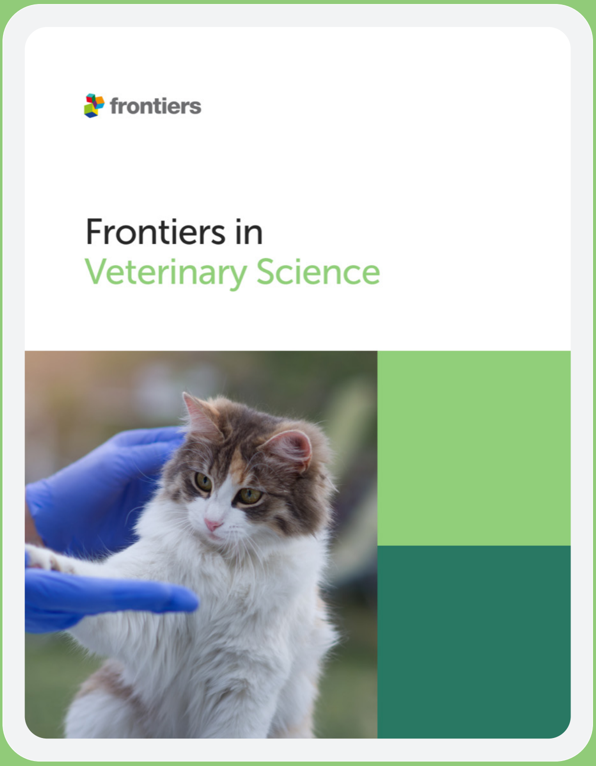

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