



Infrared thermography as a potential non-invasive tool to study seasonal stress in late gestation Sahiwal cows and their neonate calves

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ABSTRACT

Stress triggers a multitude of physiological responses including alterations in surface temperature aimed at maintaining homeostasis of animal. The present study was undertaken to evaluate the comparative seasonal effect of thermal stress on body surface temperature during the critical period of late gestation and early life. For this, late gestational Sahiwal cows (n=48) were selected and categorized into four groups: natural heat stressed (NHS), cooling treated (CLT), spring and winter groups, and their neonate calves born in summer (IUHS-intra uterine heat stressed and IUCL-intra uterine cooled), spring and winter season. The thermal profile obtained from infrared thermography (IRT) showed a trend of surface temperature for each region examined over varied THI (temperature humidity index). Body surface temperature in NHS was significantly higher, followed by spring, CLT and least in winter group cows. Within the group, study revealed numerically high surface temperature of eye, front and udder on the day of parturition, while slightly lower temperature of flank portion. NHS, CLT and spring cows had non-significant variations in udder temperature. Insulation breakage from the legs at a colder temperature was observed. In neonates, significantly high surface temperature was observed in IUHS, followed by IUCL and spring calves, while significantly low surface temperature in winter calves. Thus, cooling treatment in the late gestation significantly lowered body surface temperature in both dams and calves. Further, it is also concluded that out of different anatomical regions, eye and feet temperature is the most reliable indicator for assessing seasonal stress with changing THI.

Keywords: Body surface temperature, Heat stress, Late gestation, Neonate

The variation in climatic variables such as air temperature, relative humidity, wind speed and solar radiation causes stress in lactating cattle (Pragna *et al.* 2017) and are recognized as the possible threats to the growth and productivity of all domestic animals species. Bodies with temperatures above absolute zero emit radiation, which can be detected even in minimal temperature variations with the use of digital thermography (Knizkova *et al.* 2007 and Roberto *et al.* 2014). IRT technology is a simple, effective, on-site and non-invasive remote sensing method used in measuring changes in body temperature due to relative changes in blood flow and heat transfer (Roberto *et al.* 2014 and Nääs *et al.* 2020). This technique detects surface heat with high accuracy, which is emitted as infrared radiation and generates a pictorial image. The emissivity and conductivity of an object's surface as well as its temperature all play a role in how much radiation is emitted from that surface which is measured by the camera (Poikalainen *et al.* 2012). Simply in a thermogram, the warmest region appears as white or red, whereas the

coldest region appears as blue or black. Such detail easily aids in the comprehension of thermoregulation caused by variations in surface temperature and the effects of environmental factors on animal welfare (Kotrba *et al.* 2007 and Bowers *et al.* 2009). Accordingly, this technology in bovines was found useful as a general indicator of stress (Schaefer *et al.* 2002 and Stewart *et al.* 2007), heat and methane production (Montanholi *et al.* 2008 and Barros *et al.* 2016), thermoregulatory capacity (Sevegnani *et al.* 2016) and can indicate thermal biometric changes in animal metabolism in response to environmental or physiological conditions (McManus *et al.* 2016).

Elevated ambient temperatures prompt increased blood flow from the core to the skin, facilitating heat dissipation as infrared energy through enhanced capillary blood flow (Knizkova *et al.* 2007). In contrast, winter and stressful conditions induce sympathetically-mediated vasoconstriction, reducing skin temperature (Blessing 2003). IRT detects temperature variations in animal body parts, offering insights into thermal regulation and stress. Unlike traditional rectal thermometers, recent advances in thermal imaging allow non-invasive estimation of calf core temperature, avoiding early life stress and anxiety-related effects (Cossa *et al.* 2021). Monitoring surface

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temperatures of different body parts under varying THI reveals inflection points, aiding accurate heat stress assessment and management refinement (Luzi *et al.* 2013, Weschenfelder *et al.* 2014 and Peng *et al.* 2019). Keeping these points in view, the aim of this study was to measure different body surface temperatures under different THI conditions during the critical period of late gestation in cows and early life in neonate calves.

MATERIALS AND METHODS

The present study was carried out at the Livestock Research Centre (LRC) and Climate Resilient Livestock Research Centre (under NICRA), National Dairy Research Institute (NDRI), Karnal, Haryana. The animal experiments performed were acceptable to the ethical standards, performed as per the guidelines of Institute Animal Ethics Committee (IAEC). The investigation was conducted on 48 Sahiwal cows and their neonate calves maintained in good health, clinically normal state, and free from any physiological, anatomical and infectious disorder. Experimental animals were kept under the same feeding and management guidelines as those followed at NDRI (ICAR 2013).

The Sahiwal cows in late gestation were grouped based on season of parturition, viz. summer, spring and winter season. Summer season cows were further divided into two groups: Group-I (natural heat stressed-NHS), in which cows were kept under the summer environment, i.e. directly under the sun for 8 h, while Group-II (cooling treated-CLT), in which cows were kept under controlled temperature (24-25°C) in climatic chamber for 8 h, i.e. 9:00-5:00 PM. Similarly, calves born from these cows were grouped into *in-utero* heat-stressed (IUHS) calves from NHS cows, *in-utero* cooled calves (IUCL) from the CLT cows, calves born in spring season and calves born in winter season.

The study was performed in summer (May to August), winter (November to January) and spring months (February to April). The microclimatic variables such as

dry bulb temperature (T_{db}), wet bulb temperature (T_{wb}) were recorded in morning at 7.30 AM and afternoon at 2.30 PM in experimental sheds by analog hygrometer (Zeal, UK) during the whole experimental period. Based on the climatic data, temperature humidity index (THI) was calculated as:

$$THI = 0.72 \times (T_{db} + T_{wb}) + 40.6 \text{ (McDowell, 1972)}$$

The thermography profile of different body points was determined by the infrared thermal camera (ThermaCamTMS2000; FLIR Systems, Wilsonville, USA) with high resolution (384×288). This type of portable camera transforms natural radiation emitted from the target (in this case, the dairy cow's skin surface) at a wavelength of 8-12 mm into a thermally processible electric signal. The equipment was calibrated for an emissivity of 0.96 indicated for biological tissues, even temperature differences as small as 0.1°C can be detected by the camera. Before using the infrared thermographic camera, it was calibrated at the ambient conditions (Montanholi *et al.* 2008). The individual animals were restrained in a particular shaded area before thermography. The multiple IRT images of different body location such as eye, front, flank, and udder were taken at a distance 1.0 m from each of the body locations and best quality image in terms of focus and precise location were selected for analysis. Thermal images that were in focus, were analysed, using the Darvi TI analysis software. Using a drawing pad, all temperatures on the different points were measured. Images were saved on a portable flash card inside the camera as JPEG files.

Statistical analysis of the data was performed using software version (22) of the SPSS system. A two-way analysis of variance (ANOVA) was carried out to test the significant difference at 5% level between groups. The pairwise comparison of mean was carried out using Tukey's multiple comparison tests.

RESULTS AND DISCUSSION

The changes in average maximum and minimum

Table 1. Mean environmental variables during different season of study

Month	Max Temp (°C)	Min Temp (°C)	Tdb (°C)	Twb (°C)	RH %	THI
<i>Spring season</i>						
Mid Feb-March	27.62	12.09	27.15	22.52	65.87	76.37
Mid Mar-April	34.08	15.12	33.29	21.46	34.5	80.02
Mid April-30 April	36.27	18.29	35.63	21.91	27.07	82.02
<i>Summer season</i>						
Mid May-June	36.07	23.56	35.25	25.74	46.1	84.51
Mid June-July	36.49	26.36	34.45	26.92	55.13	84.78
Mid July-Aug	32.52	26.61	31.09	28.19	80.35	83.29
<i>Winter season</i>						
Mid Nov-Dec	24.49	9.42	23.45	16.51	46.97	69.37
Mid Dec-Jan	17.89	7.37	17.03	14.67	77.43	63.42
Mid Jan-Feb	16.57	7.31	16.34	13.25	71.61	61.91

Max Temp, Maximum temperature; Min Temp, Minimum temperature, Tdb, Dry bulb temperature; Twb, Wet bulb temperature; RH, Relative humidity and THI, Temperature humidity index.

Table 2. Mean ($\pm$ SE) surface temperature ($^{\circ}$ C) in different groups of late gestation Sahiwal cows at various time intervals

Season	Day	Summer		Spring	Winter
		NHS	CLT		
Eye	-14	36.80 $\pm$ 0.29	35.72 $\pm$ 0.33	36.03 $\pm$ 0.66	32.17 $\pm$ 1.00
	-7	37.57 $\pm$ 0.22	35.85 $\pm$ 0.63	36.63 $\pm$ 0.44	31.55 $\pm$ 1.26
	0	37.57 $\pm$ 0.52	35.93 $\pm$ 0.43	37.02 $\pm$ 0.37	32.83 $\pm$ 0.82
	Overall	37.31 $\pm$ 0.22	35.83 $\pm$ 0.26	36.56 $\pm$ 0.29	32.18 $\pm$ 0.58
Front	-14	33.95 $\pm$ 0.46	32.87 $\pm$ 0.58	33.65 $\pm$ 0.36	28.43 $\pm$ 0.56
	-7	35.8 $\pm$ 0.45	32.7 $\pm$ 0.72	34.47 $\pm$ 0.35	27.50 $\pm$ 1.11
	0	34.83 $\pm$ 0.72	32.95 $\pm$ 0.23	35.00 $\pm$ 0.42	28.52 $\pm$ 0.73
	Overall	34.86 $\pm$ 0.35	32.84 $\pm$ 0.30	34.37 $\pm$ 0.25	28.15 $\pm$ 0.47
Flank	-14	36.17 $\pm$ 0.33	35.48 $\pm$ 0.46	35.17 $\pm$ 0.51	29.7 $\pm$ 0.69
	-7	37 $\pm$ 0.25	35.62 $\pm$ 0.47	35.65 $\pm$ 0.39	29.23 $\pm$ 0.19
	0	35.98 $\pm$ 0.78	34.12 $\pm$ 0.87	36.55 $\pm$ 0.37	29.45 $\pm$ 0.23
	Overall	36.38 $\pm$ 0.3	35.07 $\pm$ 0.38	35.79 $\pm$ 0.27	29.46 $\pm$ 0.24
Udder	-14	35.7 $\pm$ 0.25	36.02 $\pm$ 0.6	36.32 $\pm$ 0.43	33.67 $\pm$ 0.16
	-7	37.23 $\pm$ 0.19	36.1 $\pm$ 0.48	36.52 $\pm$ 0.45	33.63 $\pm$ 0.52
	0	37.48 $\pm$ 0.11	36.53 $\pm$ 0.39	36.9 $\pm$ 0.48	32.67 $\pm$ 0.97
	Overall	36.81 $\pm$ 0.22	36.22 $\pm$ 0.28	36.58 $\pm$ 0.25	33.32 $\pm$ 0.36
Feet	-14	32.22 $\pm$ 0.69	30.12 $\pm$ 0.74	31.87 $\pm$ 0.59	25.08 $\pm$ 0.71
	-7	32.63 $\pm$ 0.67	28.55 $\pm$ 1.04	31.35 $\pm$ 0.66	26.75 $\pm$ 0.61
	0	31.95 $\pm$ 0.63	30.8 $\pm$ 0.69	31.37 $\pm$ 0.61	27.33 $\pm$ 0.49
	Overall	32.27 $\pm$ 0.37	29.82 $\pm$ 0.51	31.53 $\pm$ 0.34	26.39 $\pm$ 0.40

Values with different superscript in rows (a, b, c, d) varied ($p < 0.05$). NHS, natural heat stressed; CLT, cooling treated.

temperature, dry and wet bulb temperature, relative humidity and THI during summer, spring and winter season are delineated in the Table 1. During summer season the maximum temperature observed was $>36^{\circ}\text{C}$ in NHS group while it remains restricted to 25°C in climatic chamber. There was a steady increase in maximum and minimum temperature, dry and wet bulb temperature, relative humidity and THI from winter (lowest) to summer season (highest). The data depict that the Sahiwal cows of NHS

and spring groups faced most stressful condition.

Surface temperature of late gestation Sahiwal cows: The thermographic images revealed significantly ($P < 0.05$) higher surface temperature in NHS while significantly ($P < 0.05$) lower surface temperature in winter as compared to other groups which have been presented in Table 2 and Fig. 1. Besides, the data revealed that within the group, surface temperature of eye, front and udder was numerically high on the day of parturition, while temperature of

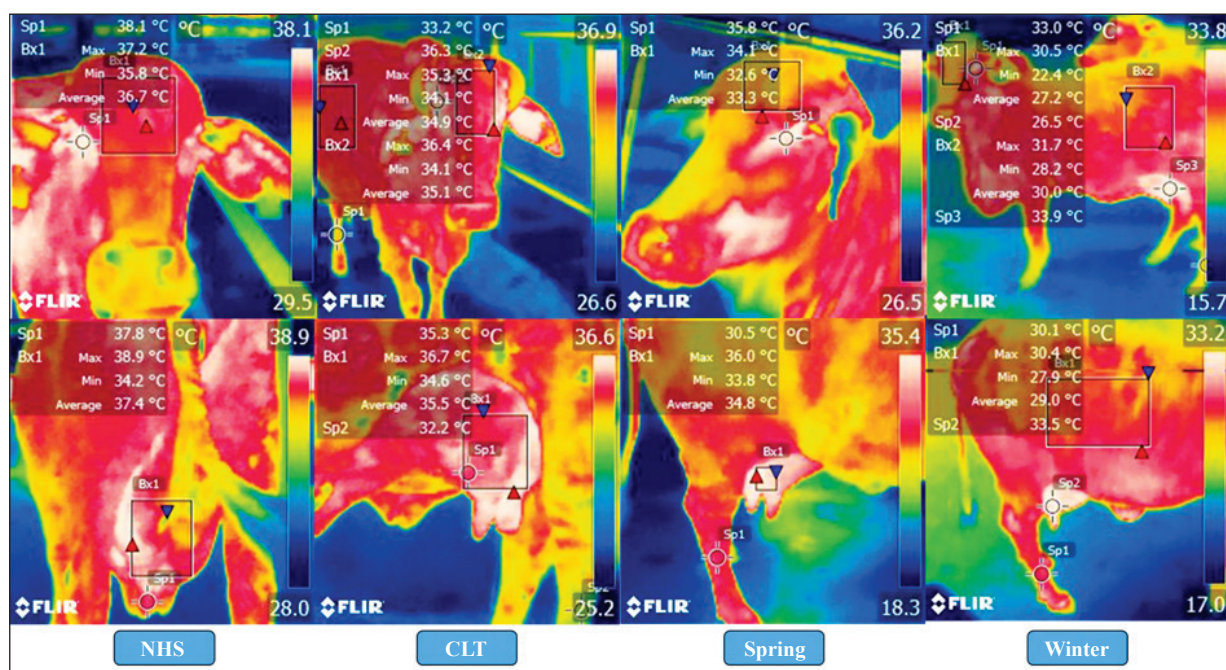


Fig. 1. Infrared thermography of different region of late gestation Sahiwal cows in different groups (NHS, CLT, Spring and winter).

Table 3. Mean ($\pm$ SE) surface temperature ($^{\circ}$ C) at different time intervals in neonate calves during different seasons

Season	Day	Summer		Spring	Winter
		IUHS	IUCL		
Eye	1	37.37 $\pm$ 0.34	33.72 $\pm$ 0.84	36.88 $\pm$ 0.73	34.85 $\pm$ 0.34
	4	37.42 $\pm$ 0.87	36.15 $\pm$ 0.71	36.5 $\pm$ 0.56	34.08 $\pm$ 0.66
	15	36.88 $\pm$ 0.52	35.62 $\pm$ 0.68	35.03 $\pm$ 0.58	33.18 $\pm$ 0.66
	Overall	37.22 ^a $\pm$ 0.34	35.16 ^b $\pm$ 0.48	36.14 ^c $\pm$ 0.39	34.04 ^d $\pm$ 0.35
Front	1	34.2 $\pm$ 0.96	32.35 $\pm$ 0.86	33.22 $\pm$ 0.72	29.12 $\pm$ 0.89
	4	35.28 $\pm$ 1.33	33.18 $\pm$ 0.94	32.22 $\pm$ 0.23	30.42 $\pm$ 0.57
	15	33.03 $\pm$ 0.73	33.42 $\pm$ 1.05	33.1 $\pm$ 0.66	29.53 $\pm$ 0.46
	Overall	34.17 ^a $\pm$ 0.6	32.98 ^b $\pm$ 0.53	32.84 ^b $\pm$ 0.33	29.69 ^a $\pm$ 0.39
Flank	1	33.28 $\pm$ 1.31	30.82 $\pm$ 0.95	33.77 $\pm$ 0.83	28.25 $\pm$ 0.42
	4	35.92 $\pm$ 0.63	34.75 $\pm$ 0.5	34.2 $\pm$ 0.83	30.17 $\pm$ 0.52
	15	37.3 $\pm$ 0.54	36.98 $\pm$ 0.42	33.97 $\pm$ 0.49	30.65 $\pm$ 0.68
	Overall	35.5 ^a $\pm$ 0.63	34.18 ^b $\pm$ 0.72	33.98 ^b $\pm$ 0.4	29.69 ^a $\pm$ 0.39
Feet	1	33.48 $\pm$ 1.08	30.38 $\pm$ 0.34	30.8 $\pm$ 0.62	28.45 $\pm$ 0.36
	4	33.37 $\pm$ 0.86	31.82 $\pm$ 0.60	30.57 $\pm$ 0.37	26.97 $\pm$ 0.56
	15	32.22 $\pm$ 0.46	32.00 $\pm$ 0.52	31.13 $\pm$ 1.12	27.20 $\pm$ 0.54
	Overall	33.02 ^a $\pm$ 0.48	31.40 ^b $\pm$ 0.32	30.83 ^b $\pm$ 0.42	27.54 ^a $\pm$ 0.31

Values with different superscript in rows (^{a, b, c}) and columns (^{X, Y}) varied ($p < 0.05$). IUHS, Intra uterine heat stressed, IUCL, Intra uterine cooled.

the flank portion was slightly lower on that day. When the temperature threshold limit was chosen using this software, the eyes were the hottest place with the highest temperature. The elevation in ambient temperature leads to a greater flow of blood from the core to the skin, facilitating the dissipation of more heat through both sensible and insensible mechanisms (Marai *et al.* 2007). Silanikove (2000) also reported that HS increases cardiac output and cutaneous blood flow, due to blood redistribution from deep splanchnic to more peripheral body regions. The software's insulation isotherm identified insulation breakage from the legs at a colder temperature. Vasoconstriction, which is sympathetically mediated and occurs under stressful circumstances, causes blood to be redirected from cutaneous capillary beds and lowers skin temperature (Blessing 2003). This demonstrated an insulation inadequacy when the insulation level is below a thermal index that depicts energy loss and extremities vasoconstriction (Renuka *et al.* 2018). The findings of this study are consistent with those of many other researchers, viz. Salles *et al.* (2018)

and Somagond *et al.* (2021) recorded a stronger connection between forehead temperatures and THI. Mean values of IRT of different body parts in NHS, CLT and winter groups varied at different intervals in a similar pattern as the change in THI. Further mean values for eye, forehead, flank and udder temperature in spring season did not show any significant difference with NHS group indicating THI value above 76 elicits thermal stress and may be associated with similar thermoregulatory mechanism. Higher IRT temperature variability in feet portion is suggestive of possible effects of higher ambient temperature as well as floor temperature in NHS and spring groups. In consonance with the present findings Montanholi *et al.* (2008) also reported higher udder temperatures compared to other regions (except the eye), particularly around parturition irrespective of the THI. Moreover NHS, CLT and spring cows had non-significant variations in udder temperature which may be due to higher metabolic activity, increased blood supply, and greater generation of heat during milk production in CLT and spring counterparts.

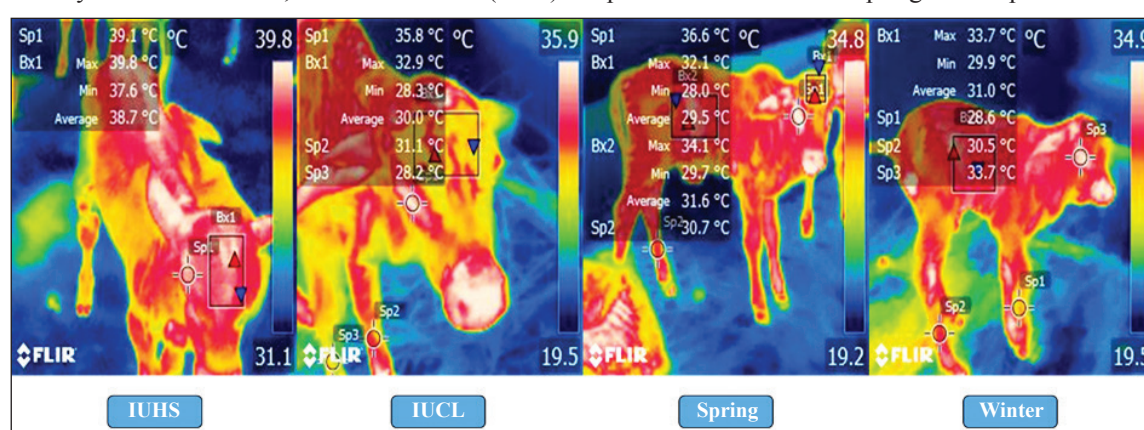


Fig. 2. Infrared thermography of different region of neonate Sahiwal calves in different groups IUHS, IUCL, Spring and winter).

Surface temperature of neonate calves: The thermographic images revealed significantly ($P < 0.05$) high surface temperature in IUHS calves, followed by IUCL and spring calves while significantly ($P < 0.05$) low surface temperature in winter calves which are presented in Table 3 and Fig. 2. Besides, the data revealed that within the group, surface temperature of flank increased with advancement of age. It was reported that more efficient animals have a lower body surface temperature, have smaller maintenance energy requirements and were associated with less methane and heat production (Montanholi *et al.* 2007). Thus in agreement with the above findings, present study revealed that cooling treatment in the late gestation significantly lowered body surface temperature in both dams and calves. Relatively higher surface temperature in IUHS and spring calves may be due to carryover effect of stress encountered during late gestation. This is also evidenced by the fact that inflammation causes an increase in capillary blood flow, which transmits heat to the skin and become visible to an infrared camera (Schaefer *et al.* 2004). Inconsistent variations observed with advancement of age may be due to the fact that environmental and microclimatic factors are also known to affect the accuracy of infrared cameras. The other variables like ground temperature, physical and physiological influences, such as variations in hair colour and skin vascularisation (Cardoso *et al.* 2015) as well as rumen development with advancement of age may have caused wider temperature gradient between the groups. In calves, IRT temperature of eye varied significantly between the groups, which is suggestive of cumulative effect of both prenatal and postnatal experiences. Since eye has rich capillary beds innervated by the sympathetic system and response to change in blood flow (Pavlidis *et al.* 2002, Lowe *et al.* 2020), the increased sensitivity of the eye can be reflective of prenatal adaptation to thermal stress. In consonance with the present findings, Zotti *et al.* (2011) evaluated surface temperature of feet as most appropriate measure to predict the thermal comfort condition particularly in young animals as there was a direct influence of environment and ground temperature in the heat absorption.

Thus, it can be concluded that temperatures of eye, forehead, flank and feet as recorded by infrared thermographs were excellent predictors of comfort levels, and are highly associated with THI. IRT as a non-invasive techniques was suitable in indicating stress level under changing THI conditions and can predict calves that will be more enduring, less vulnerable, and more productive. Thermal stress occurs when a combination of environmental factors surpasses a threshold, causing the core body as well as surface temperature of a species to exceed, which may be exacerbated during late gestation period due to disruptions in metabolism and hormonal balance. In addition to measuring stress and pain responses, in future under changing climatic conditions, the automation of physiological measures in livestock like IRT could also be

beneficial for other applications including genetic selection and breeding.

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