

## Infrared thermography to assess thermoregulatory reactions of buffaloes supplemented with antioxidant and dense energy source in summer season

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### ABSTRACT

Twenty-four lactating buffaloes were chosen and subsequently divided into 4 groups i.e. group-I (control), group-II (supplemented astaxanthin at 0.25 mg kg<sup>-1</sup> BW/day), group-III (prill fat at 100 g day<sup>-1</sup>), and group-IV (combination). Surface body temperature at different anatomical regions of buffaloes was recorded using infrared thermography (IRT), rectal temperature using a digital thermometer, and cortisol hormone by ELISA kit at the fortnightly interval. Forehead region temperature showed a higher correlation (0.390) with THI compared to other anatomical regions. The change in surface body temperature was positively correlated with THI and cortisol levels. The increase in the IRT temperature at different anatomical sites of buffaloes was at a lower magnitude in treatment groups compared to the control group. Udder surface temperature was higher in peak lactation and high producing buffaloes. Forehead region temperature showed a close relationship with rectal temperature and cortisol levels of buffaloes. According to the research findings, astaxanthin and prill fat can be used in ameliorating heat stress. Infrared thermography (non-invasive method) of the forehead and udder can be used as indicators for measuring the heat stress and production levels of buffaloes, respectively.

**Key words:** Astaxanthin, buffalo, infrared thermography, prill fat, THI

Invasive approaches are commonly used for assessing physiological and metabolic changes in farm animals (Stewart *et al.*, 2008). However, to assess these changes, the animal has to be restrained, which causes handling stress to animals (Maziero *et al.*, 2012). These invasive techniques may cause anxiety-related effects as a result of the procedure itself, for that reason, it is advised to use digital thermography (thermography camera) in measuring surface body temperature and to indicate stress level in animals during summer (Soerensen and Pedersen, 2015).

Infrared thermography (IRT) is a non-invasive remote sensing method employed in measuring changes in thermal transfer, blood circulation and is used to point out thermal biometric changes in the animal due to elevated temperature and blood flux changes in response to change in ambient temperature (Naas *et al.*, 2014). Stewart *et al.* (2007) reported infrared eye temperature as the main indicator for the detection of stress in cattle. Some other studies had also focused on the application of IRT in the dairy industry for mastitis (Berry *et al.*, 2003),

to predict physiological parameters and stress levels in farm animals (Soerensen and Pedersen, 2015) and as a measure for detection of stress in young buffalo bulls (Chikkagoudara *et al.*, 2020).

Astaxanthin is a potent antioxidant, naturally occurring as a xanthophyll carotenoid compound which is known as "Supervitamin E" (Pan *et al.*, 2003). Microalgae (*Haematococcus pluvialis*, *Chlorella zofingiensis*, and *Chlorococcum* species), red yeast (*Phaffia rhodozyma*), fungi, seafood, and certain birds *viz.*, flamingos and quail naturally contain astaxanthin. The antioxidant strength of astaxanthin is 10 times stronger than carotenoids and 100 times higher than  $\alpha$ -tocopherol compound and helps in ameliorating the adverse effect of heat stress. Supplementation of astaxanthin will cause vasodilator action and body temperature elevation due to increased nitric oxide in supplemented groups (Kanazashi *et al.*, 2014). The inclusion of astaxanthin in the diet of Karan Fries calves (Kumar *et al.*, 2019) and lactating buffaloes (Somagond *et al.*, 2020) reduced the negative effects of thermal stress. Prill fat is a form of bypass fat that resists

lipolysis, biohydrogenation and by passes the rumen, and reaches the lower digestive tract for further digestion and absorption. This bypass fat increases caloric density in the diet without affecting the composition and digestion of dietary fibers (Schauff and Clark, 1989). Taking into account the foregoing information, the current study was carried out to determine the effects of astaxanthin, prill fat, and their combination on indicators of heat stress and body surface temperature in lactating buffaloes through infrared thermography (IRT) during the summer season.

## MATERIALS AND METHOD

### *Animal and weather data*

The current research was carried out at the Kaithal district of Haryana state of India during the summer months (July to October 2018). Twenty-four lactating buffaloes that are in good health (I to III parity and 1.5 - 2.5 months of lactation) were selected and divided based on body weight and milk yield, into four groups i.e., group-I (control), group-II, group-III, and group-IV. All buffaloes were fed with a ration consisting of concentrate mixture and roughage (wheat and rice straw, maize, sorghum, etc.) (NRC, 2001) and buffaloes received free access to water. The control group of buffaloes did not receive any treatment and served as control. While animals of group II, III, and IV were additionally supplemented with astaxanthin at 0.25 mg kg<sup>-1</sup> body wt/day, 100 g of prill fat /animal/day, and the combination (Astaxanthin and Prill fat), respectively. Astaxanthin (Herbal antioxidant) was purchased from Herbo Nutra, New Delhi, and Prill fat (Bergafat T-300) from Nutriprof Company for the supplementation to the experimental animals during the summer season. Approval of this experimental study was taken from Institutional Animal Ethics Committee (IAEC), Government of India (Approval No. 42-IAEC-18-7).

Summer stress during the study period was measured by using climatic data. Microclimatic data viz., dry bulb temperature (Tdb) and wet bulb temperature (Twb) in degree Celsius (°C) were recorded with dry and wet bulb hygrometer (Zeal, UK) every day during the experimental period. The temperature-humidity index (THI) was calculated from the dry bulb and wet bulb temperatures using the equation given by NRC (1971). The THI during the experiment period was ranged from 76.73 to 83.10.

$$\text{THI} = 0.72 (\text{Tdb} + \text{Twb}) + 40.6$$

Where, Tdb = dry bulb temperature (°C) and Twb = wet bulb temperature (°C)

### *Infrared thermography*

An IR camera was used to conduct infrared thermography (ThermaCam™SC2000; FLIR Systems, Wilsonville, USA). 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, temperature differences as small as 0.1 °C can be detected by the camera. At various points on the body i.e., forehead, neck, dorsal, ventral, and udder region infrared images were taken at fortnightly intervals (Fig 1). Multiple photos were taken from the same location to choose the best image in terms of focus and precise location. Images were saved on a portable flash card inside the camera as JPEG files. ThermaCam™ Researcher 2001 software was used to interpret the IRT.

Recording of rectal temperature and blood samples were collected at the fortnightly interval. Cortisol level in plasma was analysed by using 'Bovine Cortisol ELISA Kit' (Cat No.E0110Bo) distributed by Bioassay Technology, 1713 Junjiang International Building, 218 Ningguo Rd. Yangpu Dist. SH. China.

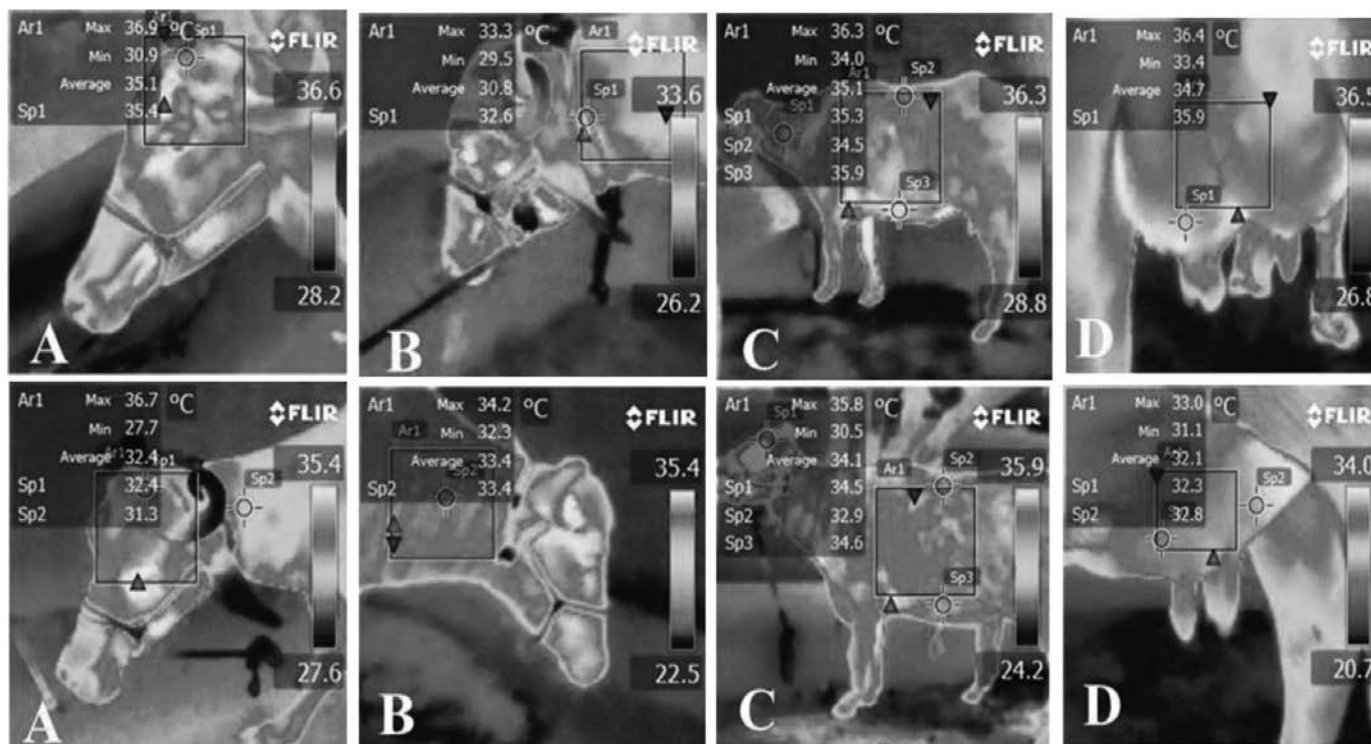
### *Statistical analysis*

Statistical analysis of the data was performed using software version (22) of the SPSS system and Prism 5. A two-way analysis of variance (ANOVA) was carried out to test the significant difference at 1 and 5% level between treatments, intervals, and their interaction. The pairwise comparison of mean was carried out using Tukey's multiple comparison tests. The correlation coefficient was evaluated at the level of probability (P<0.01 and P<0.05).

## RESULTS AND DISCUSSION

### *IRT of forehead, neck, dorsal and ventral region*

Overall mean values of surface temperature of different anatomical locations viz., fore head, neck, dorsal, ventral and udder region of different groups of buffaloes have been presented in Table 1. The values of forehead temperature for group I, II, III, and IV of buffaloes were 35.78±0.26, 35.17±0.35, 35.54±0.28, and 34.98±0.38 °C respectively (Table 1). Significant (P<0.05) change in overall average values of forehead temperature of the control and treatment group (IV) was noticed. However,



**Fig. 1 :** Infrared thermography of forehead (A), Neck (B), Dorsal and ventral (C), and Udder (D) region of buffaloes in different groups.

**Table 1:** The overall mean values of IRT temperature ( $^{\circ}\text{C}$ ) at different anatomical regions of different groups of buffaloes

IRT	Control	Supplementation			Overall Mean value
		Astaxanthin	Prill fat	Combination	
Fore head	35.78 <sup>Y</sup> ±0.26	35.17 <sup>XY</sup> ±0.35	35.54 <sup>XY</sup> ±0.28	34.98 <sup>X</sup> ±0.38	35.37±0.18
Neck	33.64 <sup>X</sup> ±0.43	33.14 <sup>X</sup> ±0.36	33.45 <sup>X</sup> ±0.23	32.69 <sup>X</sup> ±0.41	33.23±0.21
Dorsal	34.39 <sup>Y</sup> ±0.41	33.01 <sup>X</sup> ±0.38	34.47 <sup>Y</sup> ±0.43	33.62 <sup>XY</sup> ±0.40	33.87±0.35
Ventral	35.23 <sup>Y</sup> ±0.37	34.48 <sup>XY</sup> ±0.32	34.93 <sup>XY</sup> ±0.38	34.21 <sup>X</sup> ±0.33	34.71±0.23
Udder	35.93 <sup>X</sup> ±0.32	36.19 <sup>X</sup> ±0.31	36.77 <sup>XY</sup> ±0.31	37.2 <sup>Y</sup> ±0.36	36.52±0.29

The values with different superscripts X, Y, and Z within a row differed significantly ( $P < 0.05$ ).

**Table 2:** Mean values of THI, rectal temperature ( $^{\circ}\text{C}$ ), cortisol levels, and forehead temperature ( $^{\circ}\text{C}$ ) of buffaloes in the control group

Fortnight interval	THI	Rectal temperature ( $^{\circ}\text{C}$ )	Cortisol (ng/ml)	Forehead temperature $^{\circ}\text{C}$ )
I	82.94±0.71	39.25±0.14	12.72±0.61	37.86±0.33
II	83.10±0.56	39.16±0.10	12.56±0.80	36.78±0.60
III	81.81±0.67	39.40±0.24	12.28±0.50	35.72±0.25
IV	79.95±0.72	39.11±0.28	11.85±0.59	35.12±0.62
V	79.30±0.73	38.33±0.19	11.29±0.86	35.58±0.14
VI	76.73±0.31	38.93±0.29	11.03±0.78	33.65±0.15
Mean ± S.E		39.10 ±0.09	12.07±0.25	35.78±0.26

mean values for forehead temperature in treatment groups did not show any significant change. Further, a significant decrease was noticed in forehead temperature

at IV<sup>th</sup> intervals when the THI level was decreased from 81.23±0.27 to 78.24±0.34. Analysis of variance showed significant variation between intervals (Table 3). Forehead

**Table 3:** Analysis of variance (ANOVA) of Infrared thermography temperature in buffaloes

Source of Variation	df	Mean Sum of Square				
		Forehead	Neck	Dorsal	Ventral	Udder
Groups	3	4.755	6.185	17.321*	7.455	11.731*
Intervals	5	44.628**	26.428**	45.685**	46.775**	23.041**
Groups x Intervals	15	2.142	3.529	3.488	1.879	1.052
Error	120	2.415	4.269	4.679	3.166	3.561

\*\* (p&lt;0.01); \*(p&lt;0.05)

**Table 4:** Correlation coefficient of the temperature-humidity index (THI) with infrared thermography of different anatomical regions of buffaloes

Parameters	THI	Forehead	Neck	Dorsal	Ventral	Udder	Rectal temperature
THI	1.00						
Forehead	0.390**	1.00					
Neck	0.231**	0.331**	1.00				
Dorsal	0.375**	0.369**	0.265**	1.00			
Ventral	0.280**	0.383**	0.293**	0.300**	1		
Udder	0.347**	0.285**	0.280**	0.180*	0.187*	1	
Rectal temp.	0.271**	0.08	0.13	0.08	0.174*	0.314**	1

\*\* (p&lt;0.01); \*(p&lt;0.05)

temperature showed higher positive correlation values (0.390) with THI compared to values as other anatomical sites (Table 4). Mean values of IRT of forehead varied at different intervals in a similar pattern as the change in THI and cortisol level at different intervals in the control group (Table 2).

The overall mean values for neck temperature of control (I) and treatments (II, III, and IV) were  $33.64 \pm 0.43$ ,  $33.14 \pm 0.36$ ,  $33.45 \pm 0.23$ , and  $32.69 \pm 0.41^\circ\text{C}$ , respectively (Table 1). No significant ( $P < 0.05$ ) difference in overall mean values of neck temperature of control and treatment groups. However, mean values of neck temperature in astaxanthin supplemented groups were numerically lower than the control group. Further, a significant ( $P < 0.05$ ) change in neck temperature was noticed at different intervals in the astaxanthin and combination group of buffaloes during the summer season.

Mean values of dorsal and ventral skin temperature varied at different intervals. The overall mean value of dorsal skin temperature in group II ( $33.01 \pm 0.38^\circ\text{C}$ ) was significantly ( $P < 0.05$ ) lower than group I ( $34.39 \pm 0.41^\circ\text{C}$ ) and III ( $34.47 \pm 0.43^\circ\text{C}$ ). Dorsal skin temperature in group IV ( $33.62 \pm 0.40^\circ\text{C}$ ) was numerically lower than group I ( $34.39 \pm 0.41^\circ\text{C}$ ) and III ( $34.47 \pm 0.43^\circ\text{C}$ ). Mean values for ventral skin surface temperature were higher than

dorsal skin surface temperature. The overall mean values of ventral skin temperature in group IV ( $34.21 \pm 0.33^\circ\text{C}$ ) were significantly lower than group I ( $35.23 \pm 0.37^\circ\text{C}$ ). Ventral skin temperature in group I ( $35.23 \pm 0.37^\circ\text{C}$ ) was numerically higher than group II ( $34.48 \pm 0.32^\circ\text{C}$ ) and III ( $34.93 \pm 0.38^\circ\text{C}$ ). No significant ( $P < 0.05$ ) change in ventral skin temperature of supplemented groups was observed (Table 1).

In the present study overall mean thermal imaging surface temperature of forehead, neck, dorsal, ventral and udder were  $35.37 \pm 0.18$ ,  $33.23 \pm 0.21$ ,  $33.87 \pm 0.35$ ,  $34.71 \pm 0.23$ , and  $36.65 \pm 0.29^\circ\text{C}$  respectively. Peng *et al.* (2019) reported during high THI conditions, higher value of average temperatures of the eye, fore, and rear udder i.e.,  $36.30$ ,  $36.08$ , and  $35.81^\circ\text{C}$  respectively compared to forehead value ( $32.80^\circ\text{C}$ ). A significant positive correlation (0.390) was observed in the present study between THI and IRT of different body regions (Table 4). Changes in IRT temperature at different anatomical sites indicating possible effects of change in ambient temperature would affect thermoregulatory responses of animals. The use of IRT of the eye in bulls as a non-invasive physiological indicator of thermal discomfort has been documented (Chikkagoudara *et al.*, 2020). Forehead temperature showed a significant positive correlation with



THI (0.390), plasma cortisol levels, and rectal temperature in the control group of buffaloes (Table 2). IRT temperature of the forehead is closest to the core body temperature of buffaloes compared to the temperature of other anatomical sites. The findings of this study are consistent with those of many other researchers *viz.* Martello *et al.* (2016) reported the most promising regions for estimating body temperature in cattle were the head and forehead. Kessel *et al.* (2010) also reported that proximity to the brain, the temperature of the forehead region (i.e., brain) has a reliable measure of core body temperature. Salles *et al.* (2018) recorded a stronger connection between forehead temperatures and THI. The rectal temperature has shown the highest correlation with udder temperature (0.314) in the present study.

### **IRT of udder**

The mean values of udder skin temperature in group IV were significantly ( $P < 0.05$ ) greater compared to the control ( $35.93 \pm 0.32$  °C) and group II ( $36.19 \pm 0.31$  °C) (Table 1). No significant ( $P < 0.05$ ) change in average values of group III ( $36.77 \pm 0.31$  °C) and group IV ( $37.2 \pm 0.36$  °C) was observed. Mean udder skin temperature was higher in group III and IV i.e., 0.84 and 1.27°C respectively over group I. Montanholi *et al.* (2008) also reported higher udder temperatures compared to other regions (except the eye) due to lower hair density and thinner skin in the udder region. The temperature of the body surface mainly depends on peripheral blood flow to that region (Sjaastad *et al.*, 2010). Overall udder temperature (36.65°C) in the present study was 1.5 to 2°C higher than other body regions studied (forehead (35.37°C), neck (33.23°C), dorsal (33.87°C) and ventral (34.71°C). Higher udder temperature in prill fat supplemented groups (III and IV) may be because of higher milk production in these animals than the control (group I) and astaxanthin supplemented group (group II). The result of the study corroborates with Jessica (2017) also reported a higher mean temperature of the udder during the period of peak lactation might be due to higher metabolic activity, increased blood supply, and greater generation of heat during milk production.

### **CONCLUSION**

IRT is the convenient non-invasive and accurate tool to distinguish minute variations at different anatomical regions and an efficient technique to evaluate stress levels under field/farm conditions. Surface temperature measurements at various anatomical regions can be used as an indicator to evaluate thermal discomfort in lactating buffaloes. Lower IRT temperature in astaxanthin

supplemented groups indicated their ameliorative effect on heat stress buffaloes. Whereas prill fat worked as a bypass fat and helped in improving the milk production of buffaloes. Further, higher udder temperature might be due to higher metabolic activity for the synthesis of milk in prill fat supplemented groups compared to other groups. According to the study's findings, astaxanthin can be used in ameliorating the adverse effects of heat stress and prill fat as a dense energy source during summer. Further, it is also concluded that out of different anatomical regions forehead temperature is the most reliable indicator for assessing stress levels of buffaloes.

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