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Research Article

Thermal Asymmetry of the Eyes and Ears in Holstein Dairy Cows: Association with Physiological Stress During the Postpartum Period

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Abstract

Background and Objective: Animal welfare has increased interest in developing simple, non-invasive methods for detecting stress and disease in farm animals. Infrared thermography (IRT) has emerged as a promising tool for monitoring physiological and pathological conditions. Although emotional stress has been associated with thermal asymmetry between the left and right sides of the face, the effect of physiological stress, particularly calving, on ocular and auricular thermal asymmetry remains unclear. This study investigated whether calving induces thermal asymmetry in the eyes and ears of Holstein dairy cows and evaluated the influence of subclinical hypocalcemia on these thermal responses. **Materials and Methods:** A total of 29 Holstein dairy cows were monitored using infrared thermography at four sampling points: Approximately one week before the expected calving date and once daily during the first three days postpartum. Cows with clinical disease or recent treatment were excluded. Based on serum Ionized Calcium (iCa^{2+}) concentrations, cows were classified as healthy ($n = 13$; $iCa^{2+} \geq 1.00$ mmol/L at all sampling points) or having subclinical hypocalcemia ($n = 16$; $iCa^{2+} < 1.00$ mmol/L at least once). Paired t-tests were used to compare left and right eye and ear temperatures, with statistical significance set at $p < 0.05$. **Results:** No significant thermal asymmetry was detected before calving. In healthy cows, significant left-right differences in eye temperature were observed on the first and second days postpartum, while ear temperature showed significant asymmetry on the second postpartum day ($p < 0.05$). Thermal symmetry returned by the third postpartum day. In contrast, cows with subclinical hypocalcemia exhibited no significant thermal asymmetry at any sampling point. **Conclusion:** Physiological stress associated with calving induces a transient thermal asymmetry in the eyes and ears of healthy Holstein dairy cows, which can be detected using infrared thermography. However, this response is not evident in cows with subclinical hypocalcemia, suggesting that metabolic disorders may alter thermographic patterns. These findings support the application of infrared thermography as a non-invasive tool for monitoring calving-related stress while highlighting the importance of considering calcium status when interpreting postpartum thermal assessments.

Key words: Thermal asymmetry, Holstein, infrared thermography, calving, stress, subclinical hypocalcemia

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Milk production is in a state of constant evolution, utilizing genetic improvement techniques and intensive management in order to maximize milk yield, the most palpable indicator of productivity and profit. This increase in milk yield often results in the decreased ability of the animals in dealing with the metabolic challenges required by the high milk production¹, by the consumption of energy dense feed² and the lack of space that comes with intensive productions, suppressing natural behaviors³⁻⁵. From the consumer's side, the access to information about the production systems has generated a concern about the welfare of the animals being used by the industry and a preference for products that come from farms that take measures in favor of the comfort and well-being of the cows has emerged in the recent years^{6,7}. Realistically, in order to generate adherence from the farmers to the practices that protect animal welfare, those practices should also result in an economical benefit, or at least not impair productivity in a significant way^{8,9}. Thus, developing methods that enable easy recognition of stressful factors to the cows without the need of specialized labor can prove to be a fruitful step towards this goal.

Infrared thermography has been studied for its potential in assessing the health status of animals and humans in a non-invasive way^{10,11}. In the veterinary field, it has already shown potential in detecting cows with infectious diseases such as mastitis in its early stages¹² and pathologies such as foot-mouth disease¹³⁻¹⁵, hoof diseases¹⁶ and subclinical hypocalcemia^{17,18}. Studies have also reported on the ability of thermography to identify alterations in the facial surface temperature of cows and calves caused by the emotional state of the subject, often resulting in a bilateral asymmetry, with one side of the face showing a higher surface temperature than the other^{19,20}. Therefore, thermal symmetry of the face could be a valuable indicator of animal welfare.

It is widely known that dairy cows go through a lot of sudden adaptations and stress during their peripartum²¹. Dairy cows are susceptible to many metabolic disorders during this period, such as hypocalcemia and ketosis, as well as infectious diseases such as metritis and mastitis^{21,22}. However, the calving process can be a stressful factor by itself. Cortisol levels have been reported to be increased in cows in the immediate peripartum, both originating from the fetus as a preparation for calving and from the stress of parturition²³. Therefore, the physiological stress from calving could be affecting the surface temperature of the face during this period, making it imperative to clarify if any physiological thermal asymmetries occur on the peripartum.

Since it is possible to observe a causal link between stress and thermal asymmetry, it is also likely that an asymmetry could occur during a stressful transitional period such as the peripartum. As many thermographic studies on cows during this period utilize the surface temperature of the eyes and ears as samples, it is important to establish if any physiological alterations occur in those regions, which could be affecting results and reducing overall accuracy. It is especially important to assess if hypocalcemic cows, who are known to show cold ears as a symptom^{24,25}, show a thermal asymmetry between the left and right sides, which could be an important factor when trying to diagnose the disease using surface temperature. If thermal asymmetry was to be observed in dairy cows in their peripartum, the dairy industry be one step closer to a non-invasive, easy-to-use stress detection method. Moreover, studies on the detection of diseases and other abnormalities through the surface temperature of the ears and eyes would benefit from the knowledge of whether the right or left sides would have different thermal dynamics during the transition period after calving, requiring separate analysis and thresholds for each side.

The objective of this study is to clarify if a thermal asymmetry occurs between the left and right eyes and ears in the first 3 days after calving and to compare those results with cows that developed subclinical hypocalcemia.

MATERIALS AND METHODS

Study area and duration: This study was conducted at the Field Center of Animal Science and Agriculture, Obihiro University of Agriculture and Veterinary Medicine (Obihiro, Japan, North Latitude 42° 87', East Longitude 143° 17'). Obihiro is located in the southeastern area of Hokkaido prefecture and is part of the Tokachi region, one of the largest food production areas of Japan, often referred to as the "Food Valley". The climate of Obihiro has a marked seasonality with significant differences between seasons, with summers often surpassing 30°C in July and August and winters often falling below -10°C in January and February. Samplings for the study began in April, 2024 and continued until April, 2026.

Cows, housing and sampling schedule: The university farm of the Obihiro University of Agriculture and Veterinary Medicine had approximately 50 to 60 lactating cows composing its herd during the study period. They were kept in a free-barn confinement, with total mixed ration (TMR) being mixed and distributed by a robot and with a ventilation system turned on 24 hrs a day, despite the season. In total, 29 Holstein cows in the perinatal period, 4 primiparous and 25 multiparous, were used in this study, with the number of calvings averaging at 2.82 per cow.

In order to determine if the cows were close to parturition, the farm staff conducted daily measurements of rectal temperature of the cows housed at the close-up pen, which included cows within 3 weeks from their due date. When a significant drop in body temperature was observed by the staff, the cow was deemed close to giving birth^{26,27} and therefore moved to a maternity pen suited with cameras, making it possible for remote monitoring of the animals. Upon calving, the cows were kept in the maternity pen for about 24 hrs, after which they were moved to a hospital pen, together with other cows that were being treated for diseases. After being observed for at least 5 days in the hospital pen, the cows were then moved to the milking pen, together with the other lactating cows.

The close-up pen had windows and a gate that gave the animals free access to the outside of the barn. The maternity pen neither had gates connecting to the outside nor windows close by, but it was close to a gate used by the staff, which made it possible for the outside wind to flow inwards.

Samples were taken at 4 distinct moments: Once at around 1 week prior to the due date of the cows (close-up period) and once a day on the first 3 days in milk (DIM), with a sum of 4 samples being obtained from each cow. The time of sampling for the cows in the close-up period averaged at 7.3 ± 0.59 days (average $\pm$ SE) before parturition.

All protocols and procedures were approved by the Animal Care and Use Committee, Obihiro University of Agriculture and Veterinary Medicine (approval no. 24-51, 25-86).

Physical examination and environmental data: After restraining the animal inside the pen by tying it to a fence, a physical examination was performed by a veterinarian. Other animals inside the same pen were locked in feeding stanchions for the duration of the examination. Rectal temperature, heart rate, respiratory rate, rumen motility and presence of ping sound were examined in order to exclude cows with concomitant diseases. While performing the physical examination, a digital thermometer and hygrometer was used to measure ambient temperature and humidity, with data being annotated after 5 min of the device being turned on (CHE-TP1, Sanwa Supply, Okayama City, Okayama Prefecture, Japan).

By the end, the ambient temperature averaged at 15.2°C (SD = 6.68), humidity at 70.9% (SD = 15.8) and the temperature-humidity index (THI)³¹ at 58.6 (SD = 5.4). No samples where the THI surpassed the threshold for heat stress were used in the present study²⁸.

Thermography photos and analysis: The thermographic camera used was a FLIR ONE Pro-Android (Teledyne Technologies, Wilsonville, Oregon, United States), which was mounted on a Google Pixel 8 smartphone (Google LLC, Mountain View, California, USA). This camera has a sensitivity of 70 mK and was set to 95% emissivity during the shootings, as recommended by previous reports on thermography in cattle²⁹. The pictures were taken using the first party software designed for that camera (FLIR ONE for Android, Ver. 5.3.6, Teledyne Technologies, Wilsonville, Oregon, United States) and the settings were set to quality mode. The distance from the camera to the targets was measured using a laser distance measure (Makita LD030P, Makita Corporation, Anjo, Aichi, Japan).

Before the beginning of every thermography shooting session, the cows were tied to a fence inside the pen, away from direct sunlight and natural wind²⁹. The ventilation was then turned off and the gates closest to the cows were closed. The cows waited standing up for at least 15 min, in order for their surface temperature to stabilize and acclimate to the ambient temperature^{30,31}. Images of both eyes and the back side of both ears were taken from a distance of 30 to 50 cm, with the targets as parallel as possible to the camera (Fig. 1). A minimum of 5 images were taken of each region and the one with the best focus was chosen for analysis.

The images were then transferred to a personal computer and analyzed using the software FLIR Thermal Studio Pro for Windows (Ver. 2.0.53, Teledyne Technologies, Wilsonville, Oregon, United States). For the ears, an area was manually drawn following the borders of the ear and closed at the thinnest part of their base. Ear tags or holes were excluded from the area when drawing (Fig. 1). The temperature data of every pixel was then converted into a Comma Separated Values file (.csv), which was used to create a histogram of the temperature of the ears. Since the temperature profile of each ear did not show a normal distribution, the median surface temperature of each ear (EarST) was selected for statistical analyses.

However, it was impossible to determine the limits of the eyes in the thermographic pictures, making it difficult to design an objective and reproducible drawing method that extracted the temperature profile of the whole area of the eyes. Therefore, the maximum surface temperature of each eye (EyeST) was chosen for further analysis, which was obtained by drawing a square area containing the whole eye in it. Even though the square area contained parts of the face close to the eyes, the maximum temperature spot was located inside the eye in every picture (Fig. 1).

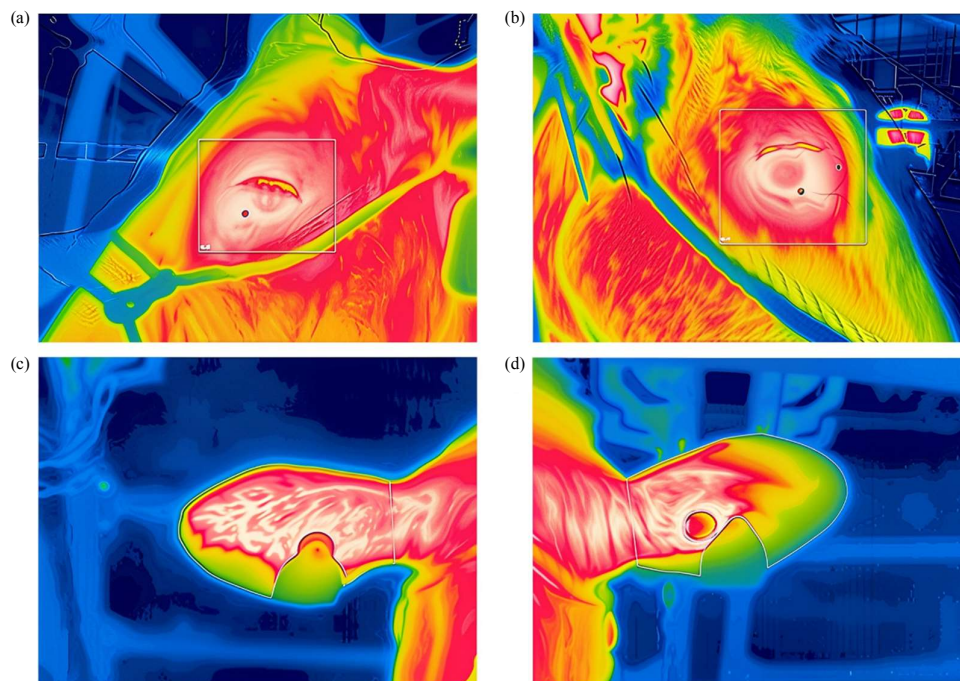


Fig. 1(a-d): Shooting and drawing methods for the (a-b) Thermography of the eyes and (c-d) Thermography of the ears

After confirming the distance with a laser distance measure (30-50 cm), the image was taken using the first-party application FLIR ONE (Ver. 5.3.6), with the thermographic camera mounted on a smartphone positioned as parallel as possible to the eyes and to the back of the ears. Using the software FLIR Thermal Studio Pro (Ver. 2.0.53), the polygon tool was selected and an area was drawn around the edges of the ear, finishing at the point where the base of the ear was the thinnest. Ear tags were excluded from the analyzed area (c-d). As for the eyes (a-b), a square area was drawn containing the entirety of the eye in it, with the pixel with the highest temperature inside this area, represented by the red mark, being annotated

Table 1: Serum iCa^{2+} concentrations, THI¹, both EyeSTs² and both EarSTs³ for each sampling time in the healthy and SCH₄ groups (average $\pm$ SE)

Groups and timings ¹	iCa^{2+} (mmol/L)	THI	Left EyeST (°C)	Right EyeST (°C)	Left EarST (°C)	Right EarST (°C)
Healthy (n = 13)						
Close-up	1.235 $\pm$ 0.016	57.4 $\pm$ 1.1	34.95 $\pm$ 0.85	36.01 $\pm$ 0.54	30.37 $\pm$ 0.87	30.29 $\pm$ 1.06
1 DIM	1.089 $\pm$ 0.017	59.1 $\pm$ 1.5	33.66 $\pm$ 0.55	36.38 $\pm$ 0.54	27.53 $\pm$ 2.17	29.71 $\pm$ 2.43
2 DIM	1.098 $\pm$ 0.011	58.5 $\pm$ 1.6	33.77 $\pm$ 0.48	36.81 $\pm$ 0.44	27.49 $\pm$ 1.36	31.00 $\pm$ 1.46
3 DIM	1.162 $\pm$ 0.015	58.2 $\pm$ 1.6	34.48 $\pm$ 0.68	35.43 $\pm$ 0.71	28.75 $\pm$ 1.62	29.46 $\pm$ 1.63
SCH (n = 16)						
Close-up	1.238 $\pm$ 0.011	60.2 $\pm$ 1.4	34.15 $\pm$ 0.87	34.59 $\pm$ 0.85	28.72 $\pm$ 1.87	30.07 $\pm$ 1.78
1 DIM	0.935 $\pm$ 0.025	58.0 $\pm$ 1.5	33.93 $\pm$ 0.85	34.87 $\pm$ 0.83	21.03 $\pm$ 3.14	21.24 $\pm$ 2.99
2 DIM	0.894 $\pm$ 0.025	58.1 $\pm$ 1.5	34.69 $\pm$ 0.64	33.86 $\pm$ 0.93	27.46 $\pm$ 1.86	29.07 $\pm$ 1.75
3 DIM	1.072 $\pm$ 0.020	58.7 $\pm$ 1.4	34.14 $\pm$ 0.75	34.56 $\pm$ 0.63	29.26 $\pm$ 0.80	30.15 $\pm$ 0.77

¹THI: Temperature-humidity index Mader *et al.*²⁸, ²EyeST: Eye surface temperature, ³EarST: Ear surface temperature and ⁴SCH: Group of cows with subclinical hypocalcemia (at least one instance of serum iCa^{2+} concentration below 1 mmol/L)

Blood sampling: Blood was collected by a veterinarian through the tail vein using vacuum tubes containing blood clot accelerant and serum separating gel (SIM-K0706S-Blue, Sekisui Chemical Co., Ltd., Chuo, Tokyo, Japan) and was sent to a commercial laboratory (Obihiro Center of Clinical Analysis, Obihiro, Hokkaido, Japan) for the estimation of serum iCa^{2+} concentrations. Individuals with serum iCa^{2+} concentrations below 1.00 mmol/L with no apparent clinical symptoms were diagnosed with subclinical hypocalcemia^{32,33}. None of the 29 cows used in this study showed any symptoms of milk fever.

Grouping method and statistical analysis: In order to be included in this study, the sampled cows were required to not show any clinical signs of disease in any of the 4 sampling instances. They also could not have received any kind of treatment between 5 days before and 5 days after each sampling date, which was confirmed through their medical history. Two groups were then created based on their serum iCa^{2+} status at the time of sampling: The healthy group, containing cows with a serum iCa^{2+} concentration ≥ 1.00 mmol/L in all four sampling moments and the subclinical hypocalcemia group (SCH), which contained

asymptomatic cows that showed at least one instance of serum iCa^{2+} below 1.00 mmol/L. By the end, the healthy group contained 13 cows and the SCH group had 16. This setup fits the standards published by Idris *et al.*³⁴ which recommended that thermography studies investigating meaningful changes in the temperature of eyes should be composed of at least two sessions in groups containing 14~16 cows and at least 3 sessions on groups with 10~12 cows³⁴.

To assess if air temperature and humidity could be confounding factors for EarST in this study, Repeated Measures ANOVA comparing the THI of the 4 timings (close-up, 1 DIM, 2 DIM and 3 DIM) were performed for both the healthy and SCH groups. Then, to identify the presence of bilateral thermal asymmetry in both healthy and SCH cows, Paired Samples t-tests were performed between the right and left EyeST and EarST in each of the four instances of sampling. This analysis was selected due to its ability of circumventing the effect of environmental temperature by grouping and analyzing the data in pairs the left side and the right side of the same cow. In order to assess which side suffered a change in surface temperature and caused an asymmetry, Repeated Measures ANOVA were performed in both healthy and SCH groups for each target region alone left ear, right ear, left eye or right eye from the close-up stage until 3 DIM. To account for the multiple tests, Bonferroni corrections were applied to the results of all analyzes by multiplying their p-values by 4 (the number of comparisons done for each target region).

The p-values < 0.05 after Bonferroni correction were considered significant. Statistical analysis was performed using JASP for Windows (JASP Team (2024). A JASP (Version 0.96) [Computer software]). A summary of the raw data can be seen at Table 1.

RESULTS

No significant differences were observed in Repeated Measures ANOVA that compared the THI across all the 4 sampling timings between the healthy and SCH groups ($p > 0.05$). The paired-samples t-tests performed in the healthy group indicated that a left-right difference could be observed at both 1 and 2 DIM for EyeST and at 2 DIM for EarST (Table 2). At 1 DIM, the left EyeST averaged at $33.66^{\circ}\text{C} \pm 0.55$ (average $\pm$ SE) and the right side at $36.38^{\circ}\text{C} \pm 0.54$. At 2 DIM, they averaged at $33.77^{\circ}\text{C} \pm 0.48$ and at $36.81^{\circ}\text{C} \pm 0.44$, respectively. The Cohen's d observed after comparing both sides were -0.889 ± 0.51 (Cohen's d $\pm$ SE) for 1 DIM and 1.725 ± 0.46 for 2 DIM. As for EarST at 2 DIM, the left side averaged at $27.49^{\circ}\text{C} \pm 1.36$ and the right at $31.00^{\circ}\text{C} \pm 1.46$, with a Cohen's d of -0.942 ± 0.24 . Thus, it is possible to affirm that a physiological thermal asymmetry occurs in the ears and

eyes of healthy cows during the first 48 hrs after calving. Graphs showing the alterations in EarST and EyeST were created to facilitate the visualization of how each side changes in each sampling period (Fig. 2a-b). On the other hand, the Repeated Measures ANOVA for the healthy group comparing the surface temperatures of the same side across the 4 sampling moments did not show any significant differences for neither EyeST nor EarST ($p > 0.05$).

The SCH group showed no instance of asymmetry in any of the sampling times after performing the Paired Samples t-tests (Table 2, Fig. 3a-b). The Repeated Measures ANOVA showed a significant drop in EarST at 1 DIM for the SCH group both for the left and for the right sides ($p < 0.05$), but they were still not significantly different from each other.

DISCUSSION

Instances of thermal asymmetry were observed in healthy cows at 1 and 2 DIM for the eyes and at 2 DIM for the ears, according to the results from the paired-samples t-tests ($p < 0.05$). All of the instances of asymmetry had a Cohen's d with a magnitude greater than 0.8, indicating that side exerts a large effect on the surface temperature of the eyes and ears at those moments. Since EyeST and EarST did not show any statistically significant differences between the same side across all 4 sampling times, it is not possible to affirm that there was a significant drop or increase in surface temperature between sampling moments, making it hard to pinpoint with precision which side suffered changes that resulted in a thermal asymmetry. However, it is possible to see in the time-lapse graphs (Fig. 2) and in the summarized data (Table 1) that the bilateral asymmetries in both EyeST and EarST were possibly caused by a decrease of the left side at 1 and 2 DIM when compared to the close-up stage, while the right side remained relatively stable.

Air temperature and humidity have been reported to have a positive correlation with the temperature of the ears^{17,18,29}, which can be confounding factors when trying to diagnose diseases such as hypocalcemia with the ear's temperature. The temperature of the eyes, however, have been reported to be more correlated with the core temperature of the subject, not suffering from the influence of ambient temperature¹⁵. Since the samples of each cow were taken in relatively short intervals, the THI did not vary much from the beginning to the end of the sampling period of each animal. Thus, it was possible to conclude that the effect of THI was around the same between all the groups, making it reasonable to perform direct comparisons between the EyeST and EarST of different timings.

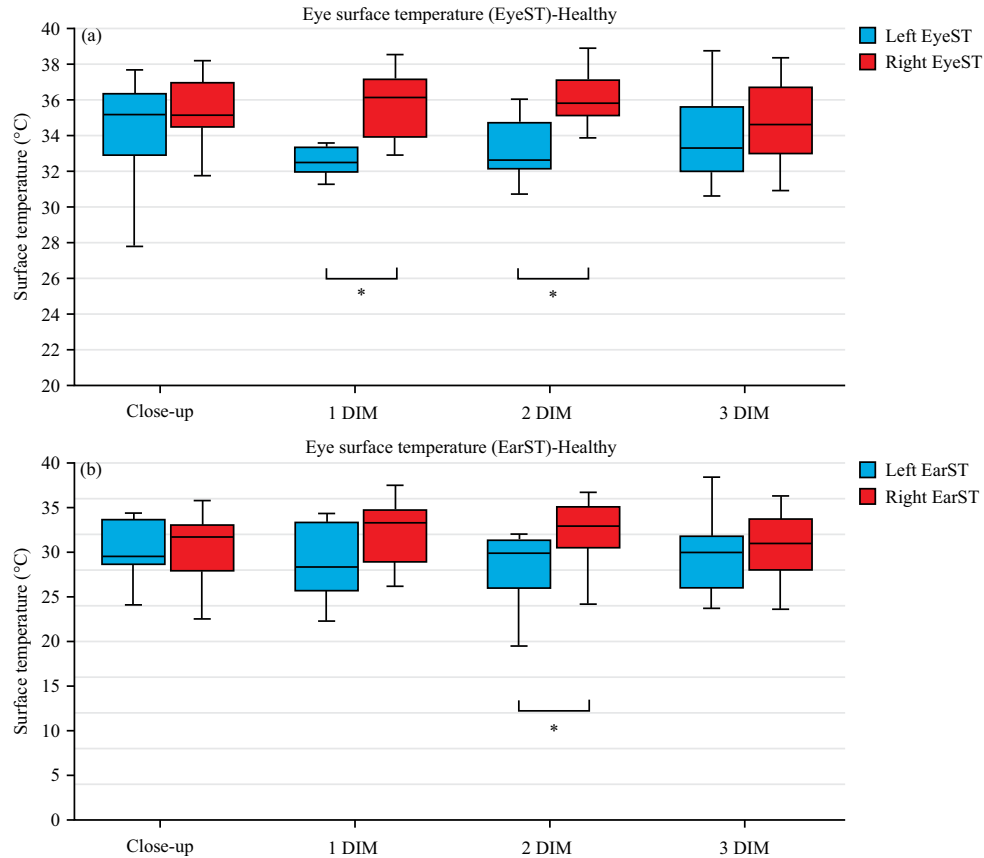


Fig. 2(a-b): Box plot graphs showing the changes in the left and right (a) Eye's surface temperature (EyeST) and (b) Ear's surface temperature (EarST) of healthy dairy cows, from their close-up period to 3 days in milk (DIM)

After performing Paired Samples t-tests, significant differences (*) between the left and right side were observed at 1 and 2 DIM for EyeST and at 2 DIM for EarST ($p < 0.05$)

Table 2: Paired samples t-tests comparing the left and right EyeST and EarST of the healthy and SCH cows on four separate moments (Close-up, 1, 2 and 3 DIM)

					95% CI for Cohen's d	
Groups and timings ¹	p-value	p-value (bonf)	Cohen's d	SE Cohen's d	Lower	Upper
Healthy-EyeST (n = 13)						
Close-up	0.296	1.184	-0.303	0.383	-0.854	0.259
1 DIM	0.008	0.032*	-0.889	0.506	-1.523	-0.228
2 DIM	0.001	0.004*	-1.725	0.461	-2.583	-0.839
3 DIM	0.378	1.512	-0.254	0.420	-0.802	0.304
Healthy-EarST (n = 13)						
Close-up	0.878	3.512	0.044	0.136	-0.501	0.587
1 DIM	0.078	0.312	-0.535	0.142	-1.109	0.058
2 DIM	0.005	0.020*	-0.942	0.242	-1.588	-0.27
3 DIM	0.349	1.396	-0.270	0.127	-0.819	0.289
SCH-EyeST (n = 16)						
Close-up	0.683	2.732	-0.104	0.305	-0.594	0.389
1 DIM	0.402	1.608	-0.216	0.329	-0.708	0.284
2 DIM	0.452	1.808	0.193	0.337	-0.305	0.684
3 DIM	0.712	2.848	-0.094	0.41	-0.584	0.399
SCH - EarST (n = 16)						
Close-up	0.040	0.160	-0.563	0.087	-1.084	-0.025
1 DIM	0.816	3.264	-0.059	0.073	-0.549	0.432
2 DIM	0.040	0.160	-0.563	0.105	-1.084	-0.026
3 DIM	0.270	1.080	-0.287	0.251	-0.782	0.218

¹EyeST: Eye surface temperature, ²EarST: Ear surface temperature, ³SCH: Subclinical hypocalcemia (at least one instance of serum iCa^{2+} concentration below 1 mmol/L),

⁴DIM: Days in milk and *p-values < 0.05 after Bonferroni correction

As for the pattern of asymmetry observed by this study in healthy cows, the right side seemed to be consistently warmer than the left, which in turn showed a visual decrease in 1 and 2 DIM (Fig. 2). Contrary to what other studies reported on cows and calves submitted to emotional distress showing an increase in the surface temperature of the eyes^{19,20}, it does not seem like the thermal asymmetry observed in the present study occurred from the left side getting warmer due to activation of the right hemisphere of the brain.

Several studies have hypothesized that asymmetries in brain activity causes asymmetry in facial surface temperature. However, some studies have shown increases in body surface temperature on the right side^{35,36}, while others have shown changes on the left side¹⁹. Thus, the contralateral warming of the eyes and ears due to the activation of one of the brain's hemispheres does not seem to apply uniformly to all mammals and/or sources of stress. Two different studies, one on marmosets that were captured³⁵ and another on cats that were put into transporter containers³⁶, reported that the emotional stress from being restrained caused a rise in the tympanic membrane temperature of the right ear, with no differences being observed in the left side. On the other hand, calves that went through a disbudding procedure, as well as calves that spectated it, have been reported to develop a thermal asymmetry in the face that tended to show higher values in the left side, in both eyes and ears¹⁹. It was hypothesized in this study that the rise in temperature in the left side of the face is derived from the innervation predominantly originating from the contralateral hemisphere of the brain, even though it also receives some innervation from the ipsilateral one.

The aforementioned studies on marmosets and cats^{35,36} hypothesized that the activation of the right hemisphere of the brain as a response to emotional stress is what caused the rise in the right ear's temperature, with no mention of the contra laterality of the innervation. The activation of the right hemisphere as a response to stress has already been established in humans during panic attacks and anticipation to painful stimuli³⁷. In rats subjected to chronic stress by being restrained for 6 hrs a day, the right hemisphere of the brain responded by shortening the length of dendrites, creating a morphological asymmetry between both hemispheres³⁸. Unfortunately, while there is sufficient proof that the right hemisphere of the brain is responsible for processing stress, the mechanism through which the temperature of the eyes and ears change as a result of it is still unclear.

If the thermal asymmetry observed in the present study does indeed occur from a unilateral activation of the brain, it is possible that the physiological stress from calving activates

different cerebral pathways when compared to emotional stress, the most studied form of stress to the moment. It is also possible that different animal species show distinct patterns of asymmetry when under stress, thus explaining the different patterns observed in the cows from this study and in the marmosets and cats from the aforementioned reports. Nevertheless, further studies on the specific pathways of those mechanisms are necessary in order to make any conclusive observations.

As far as we know, the apparent decrease in the ear's surface temperature as a response to stress observed in the present study has only been reported in one other study, which utilized infrared thermography to detect fear-related responses in heifers by startling them with plastic bags and tubes³⁹. That study only measured the left eye's surface temperature, though, making it impossible to stipulate if any kind of asymmetry occurred or not. A decrease in nasal temperature was also reported in rhesus monkeys that were approached by people carrying capture nets⁴⁰. It was hypothesized that the left eye of those heifers and the nose of those monkeys got colder due to the activation of the sympathetic nervous system, which has been reported to cause cutaneous vasoconstriction⁴¹. This would lead to a general decrease in the surface temperature of the body, especially in the extremities.

To the moment, those decreases in surface temperature have only been reported as a response to acute fear stimuli and none reported on the presence of an asymmetry between the left and right sides. The origin for the asymmetry observed in the present study at the first two days after calving could be the result of both sympathetically-mediated cutaneous vasoconstriction and ipsilateral brain hemisphere activation. While vasoconstriction derived from sympathetic activation would generate a decrease in surface temperature in the whole body, the activity of the brain's right hemisphere could be causing an increase in blood flow in the right side of the face, thus warming it up. However, this reasoning is still only conjecture and requires more specific experimentation in order to be solidified.

Detecting stress at a specific point through thermal asymmetry could prove to be a challenging endeavor. Calves have been previously reported to show a unilateral alteration in eye temperature just by being approached by a human being²⁰ and other animals showed fast changes in temperature from acute fear stimuli^{35,36,39,40}. Even in this present study, no cows showed an identical surface temperature for both ears or eyes, at any given moment. Therefore, the ideal thermography shooting conditions would require automated systems, with no human interacting with

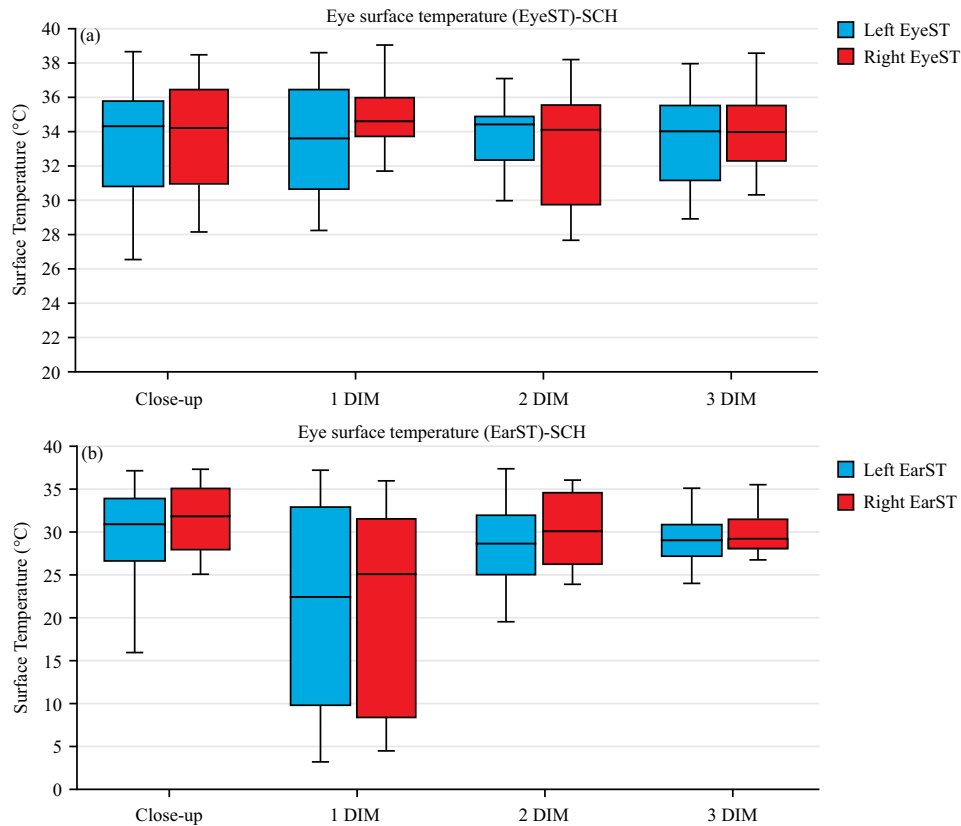


Fig.3(a-b): Box plot graphs showing the changes in the left and right (a) Eye's surface temperature (EyeST) and (b) Ear's surface temperature (EarST) of dairy cows with subclinical hypocalcemia (SCH), from their close-up period to 3 days in milk (DIM)

No significant differences were observed at any time point between the left and right sides after performing paired samples t-tests ($p < 0.05$)

the animals, during extended periods of time. Also, before being able to stipulate a threshold for detecting thermal asymmetry associated with stress, it is necessary to clarify the physiological range of those differences when the cow is at a relaxed state.

As for the SCH group, no instance of thermal asymmetry was observed in either eyes nor ears, despite the significant drop in EarST at 1 DIM (Table 2, Fig. 3). While still unclear, it has been hypothesized that the mechanism through which the ears get cold in hypocalcemic cows is related to the lower cardiac output caused by weaker contractions of the heart⁴². This would result in less blood flow to the extremities of the body, affecting the homeostasis of its surface temperature, which could explain the lack of an asymmetry in the ears and eyes observed in this study.

One study that aimed to diagnose cows with subclinical hypocalcemia using the surface temperature of the ear utilized both ears in their analysis, but it was ultimately not recommended as a diagnostic method due to low overall accuracy¹⁸. In our previous study, we also analyzed the

potential of utilizing the ear surface temperature for identifying cows with SCH, but only the right ear's temperature was utilized¹⁷. We hypothesized that this difference in accuracy could be due to the method through which the temperature was obtained and by the fact that only the back side of the ear was utilized in our study. However, it is possible that the difference in results could also be due to our previous study only using the right ear, which seems to not change in temperature during the peripartum in healthy cows, while the left does (Fig. 2).

The results from the present study suggest that a thermal asymmetry between the ears and eyes does occur in dairy cows in the first 2 days postpartum. Studies involving infrared thermography, or any other kind of thermometry, of the eyes and ears of cows in the peripartum could benefit from taking into account the physiological asymmetry that occurs in this period. Moreover, studies utilizing the temperature of the eyes or the ears to diagnose diseases in the peripartum, such as hypocalcemia, could achieve more accurate results by analyzing both sides separately.

CONCLUSION

The present study was able to demonstrate that healthy Holstein dairy cows go through a physiological thermal asymmetry between the right and left eyes and ears in the first 2 days after calving, most likely caused by the stress from parturition. Studies utilizing the surface temperature of those places to diagnose diseases could benefit from taking this phenomenon into account, raising the accuracy of their diagnostic models. However, the mechanism through which this thermal asymmetry occurs still requires clarification. Moreover, the detection of asymmetry in single individuals still requires further analysis in order to establish a threshold for the difference in temperature between both sides.

SIGNIFICANCE STATEMENT

This study demonstrates that physiological stress associated with calving induces measurable thermal asymmetry in the eyes and ears of healthy Holstein dairy cows, as detected by infrared thermography. The findings highlight thermal asymmetry as a potential non-invasive biomarker for monitoring calving-related stress and improving animal welfare. Furthermore, the absence of thermal asymmetry in cows with subclinical hypocalcemia indicates that metabolic disorders can influence thermal responses, emphasizing the need to consider health status when interpreting infrared thermographic assessments in dairy cattle.

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