RESEARCH ARTICLE

Infrared Thermography as a Non-Invasive Supportive Diagnostic Tool for Subclinical Hypocalcaemia in Frieswal Dairy Cows during the Transition Period

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Abstract

Subclinical hypocalcaemia (SCH) remains a pervasive and underdiagnosed metabolic disorder in high-yielding dairy cows, contributing to compromised postpartum health and productivity. This study investigates the utility of infrared thermography (IRT) as a non-invasive physiological indicator for the early detection of SCH in Frieswal cows during the transition period under subtropical climatic conditions in India. A total of 22 pregnant Frieswal cows were monitored from 7 (±1) days prepartum to 7 days postpartum. Animals were classified as normocalcaemic (≥8.0 mg/dL) or SCH (6.0-8.0 mg/dL) based on serum calcium concentrations. The highest incidence of SCH occurred on the day of calving (day 0, 68%) and the third postpartum day (64%), with 27% of cows persistently hypocalcaemic across both timepoints. Thermal imaging was conducted using a calibrated high-resolution infrared camera targeting ear surfaces, and rectal temperature (RT) was recorded. On day 0, SCH cows had significantly lower serum calcium (6.80 ± 0.10 mg/dL) and average ear surface temperature (100.60 ± 0.40 °F) compared to normocalcaemic cows. Strong negative correlations (r = –0.91 to –0.96) were observed between serum calcium and IRT parameters, RT, and temperature-humidity index (THI) on day 0. By day 3, these relationships reversed, yielding strong positive correlations (r = 0.97 to 0.99). IRT parameters remained consistently correlated with RT and THI (r = 0.92–0.96). These findings underscore the diagnostic value of IRT as a real-time, non-invasive tool for early SCH identification. This approach offers a practical solution for integrating precision livestock farming technologies into metabolic health monitoring protocols, particularly in tropical dairy production systems where heat stress amplifies physiological vulnerabilities.

Key words: Infrared thermography, Frieswal cows, Postpartum, Subclinical hypocalcaemia.

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Introduction

India remains the global leader in both milk production and cattle population, with an output of 247.87 million tonnes during 2024-25 and 193.46 million cattle as of the 2019 census (BAHS, 2024). Despite this impressive macro-scale productivity, individual animal performance lags significantly behind global standards, particularly when compared to the United States, where average daily milk yield reaches 36 kg (USDA, 2024), in contrast to India's 9.05 kg for exotic/crossbred milch cattle and 3.86 kg for indigenous breeds (BAHS, 2024). This disparity underscores persistent productivity bottlenecks in Indian dairying, particularly in terms of reproductive efficiency and periparturient metabolic health.

High-producing cows such as Frieswal, a Holstein × Sahiwal cross, are especially susceptible to transition period disorders, including subclinical hypocalcaemia (SCH), due to the sudden spike in calcium demand associated with colostrogenesis and lactation onset. SCH, which affects up to 54% of multiparous dairy cows (Reinhardt *et al.,* 2011), is a silent precursor to multiple postpartum complications such as retained placenta, metritis, mastitis, and reduced fertility (Džermeikaitė *et al.,* 2023). In India, these issues are further exacerbated by environmental stressors, inefficient estrus detection, and inconsistent transition cow management practices (Mohtashamipour *et al.,* 2020).

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Traditional diagnostic methods such as serum calcium assays, rectal temperature checks, and physical palpation are limited by invasiveness, labour intensity, and variability (Lukas *et al.,* 2009). Recent advances in Precision Dairy Farming (PDF), which deploy real-time data from wearables, milk sensors, and environmental monitors, offer viable, scalable alternatives (Neethirajan, 2020). Among these, infrared thermography (IRT) has emerged as a promising, non-invasive tool for detecting metabolic and febrile disease. IRT enables continuous measurement of peripheral tissue temperatures, which may correlate with physiological stressors like SCH (Wang *et al.,* 2023; Brezov *et al.,* 2023).

Despite global momentum in sensor-based livestock management, few studies have explored the diagnostic utility of IRT for SCH in tropical crossbred breeds like Frieswal, especially under heat stress conditions prevalent in India. Addressing this gap, the present study hypothesizes that ear surface temperature measured by infrared thermography correlates significantly with blood calcium levels and can thus serve as a non-invasive diagnostic marker for subclinical hypocalcaemia in Frieswal cows. The objectives of the current study were threefold: (i) to assess the incidence of SCH across the periparturient window; (ii) to determine the relationship between ear thermographic data, serum calcium levels, and rectal temperature; and (iii) to evaluate the feasibility of IRT as a predictive tool for early detection and intervention in SCH under Indian farming conditions.

Materials and Methods

Study Area

The study was conducted at the Livestock Research Centre, ICAR–National Dairy Research Institute (NDRI), Karnal, Haryana, India, using Frieswal cows from April to September 2024. Karnal experiences a subtropical climate with extreme seasonal temperatures. Summer temperatures may reach up to 48°C, while winter temperatures can drop to 6°C, with diurnal fluctuations of 15-20°C. The annual rainfall ranges between 760 and 960 mm, predominantly during July and August. Relative humidity varies from 41% to 85%. Meteorological data, including dry and wet bulb temperatures, were recorded daily at the milking parlour using instruments provided by the Indian Meteorological Unit at the Krishi Vigyan Kendra, Karnal. The temperature-humidity index (THI) was calculated using the National Research Council (NRC, 1971) formula: THI = 0.72 (T_{db}+ T_{wb}) + 40.6, where, T_{db} represents the dry bulb temperature in ^{o}C and T_{wb} denotes the wet bulb temperature in ^{o}C. THI-based heat stress was classified as follows (Armstrong, 1994): THI <72 = none, 72-78 = mild, 79-88 = moderate, 89-98 = severe, and >98 = danger.

Animal Selection

A total of 22 advanced pregnant Frieswal cows, with parity ranging from 1 to 6, were selected for the study. All animals were housed in a loose housing system with open paddocks. The average 305-day lactation yield of these cows was 3988.42 ± 278.21 kg, with an average daily milk yield of 13.07 kg.

Management Practices

Animals were group-managed in a dedicated shed for advanced pregnant cows. Housing adhered to BIS standards, with proper space allocation for resting, feeding, and watering. Natural tree shade and structural ventilation were employed to maintain thermal comfort throughout the year. Green fodder was provided *ad libitum* three times daily (10:00 AM, 1:00 PM, and 4:00 PM), and a maintenance-level concentrate (1.5 kg/animal) was fed at 8:00 AM. Milking cows received additional concentrate at 1.0 kg per 2.5 kg of milk yield above 5.0 kg, distributed across the three daily milking sessions (6:00 AM, 12:00 PM, and 6:00 PM). Dry cows received 2.0 kg of concentrate in addition to the maintenance ration.

Rectal Temperature Measurement

Rectal temperature (RT) was measured 7 (±1) days before the expected calving date, the day of calving (day 0) to the 7 days postpartum using a commercial flexible digital thermometer. Measurements were conducted between 8:00 and 9:00 AM with cows restrained in a Travis.

Infrared Ear Temperature Measurement and Image Analysis

Infrared thermography was performed using a high-resolution handheld IR camera (TESTO, Germany; resolution: 320 × 240 pixels; operating range: −30°C to +650°C). Calibration was conducted using a cap at room temperature, and ambient conditions were accounted for variations. Animals were allowed a 20-30 min acclimatization period prior to imaging. Thermal images were captured every morning (8:00-9:00 AM) on 7 (±1) day before the expected calving date, days 0 (calving), 1, 2, 3, 5, and 7 postpartum, focusing on both the inner and outer surfaces of the left and right ears. A fixed distance of 1 m and an angle of 5°-10° were maintained for image capture. Analysis was conducted using Testo IR analysis software.

Blood Sampling and Serum Calcium Analysis

Ethical clearance for blood sampling was obtained from the Institutional Animal Ethics Committee (IAEC), ICAR-NDRI, Karnal, Haryana, India. Blood samples were collected via jugular venipuncture 7 (±1) days prepartum, and on days 0, 1, 2, 3, 5, and 7 postpartum, using 9 mL serum collection tubes. Samples were allowed to clot for 30 min at room temperature and centrifuged at 1400 x g for 10 min. Serum was stored at −40°C in sterile 1.5 mL microcentrifuge tubes until further analysis. Serum calcium concentrations were determined using a fully automated clinical chemistry analyzer (EM-200, Erba Mannheim, Dubai, UAE).

Statistical Analysis

All statistical analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). The distribution of IR temperature data from each ear surface on all sampled days was assessed using Quantile-Quantile (Q-Q) plots to determine the suitability of parametric tests. Descriptive statistics were computed separately for cows classified as healthy and those with subclinical hypocalcaemia (SCH). Pearson's correlation coefficient was used to evaluate linear associations between serum calcium levels and IR parameters across the days of observation. Correlation strength was interpreted based on Landis and Koch (1977)'s quintile classification.

Results and Discussion

The Frieswal cows in this study were categorized as normocalcaemic or subclinically hypocalcaemic (SCH) based on serum calcium concentrations. Normocalcaemic cows exhibited calcium levels ≥8.0 mg/dL, while SCH animals had serum calcium levels ranging from 6.0 to 8.0 mg/dL, following the criteria outlined by Reinhardt *et al.* (2011). On the 7th day prepartum, 9% of animals exhibited SCH, while on the day of calving, this figure rose markedly to 68%. The incidence on the 7 (±1) days before expected calving date, day 0 (calving) and 1st, 2nd, 3rd, 5th, and 7th days postpartum was 9%, 68%, 18%, 18%, 64%, 14%, and 18%, respectively. Notably, the highest incidence occurred on the day of calving (68%) and the 3rd day postpartum (64%). A subset of 27% of animals was identified as persistently SCH, as they exhibited low calcium levels on both the day of calving and the 3rd day postpartum.

Prediction of SCH based on Ear Surface Temperature by use of IRT

Infrared thermographic imaging was employed to assess ear surface temperatures as a potential predictive marker for SCH. On the day of calving (day 0), 22 cows were evaluated, of which 15 were SCH and 7 were normocalcaemic. On the third day of calving, our study identified 14 animals suffering from SCH, and 8 were normocalcaemic. Parameters assessed included serum calcium, average ear inner surface temperature (AEIST), average ear outer surface temperature (AEOST), average ear surface temperature (AEST), rectal temperature (RT), and THI. An infrared thermographic image of the ear surface is shown Figure 1.

On the 0th day, the SCH group had a mean serum calcium level of 6.80 ± 0.10 mg/dL, compared to 8.30 ± 0.10 mg/dL in the normocalcaemic group. Correspondingly, mean AEST was slightly higher in the SCH group (100.60 ± 0.40 °F) than in the normocalcaemic group (100.10 ± 1.00 °F). Mean rectal temperatures were 102.90 ± 0.30 °F and 102.50 ± 0.40 °F in the SCH and normocalcaemic groups, respectively.

On the third day postpartum, the SCH group displayed a reduced serum calcium level (6.81 ± 0.15 mg/dL) compared to normocalcaemic cows (9.50 ± 0.23 mg/dL), similar to the 0th day. AEST was also slightly lower in SCH cows (100.37 ± 0.56 °F vs. 100.51 ± 0.75 °F).

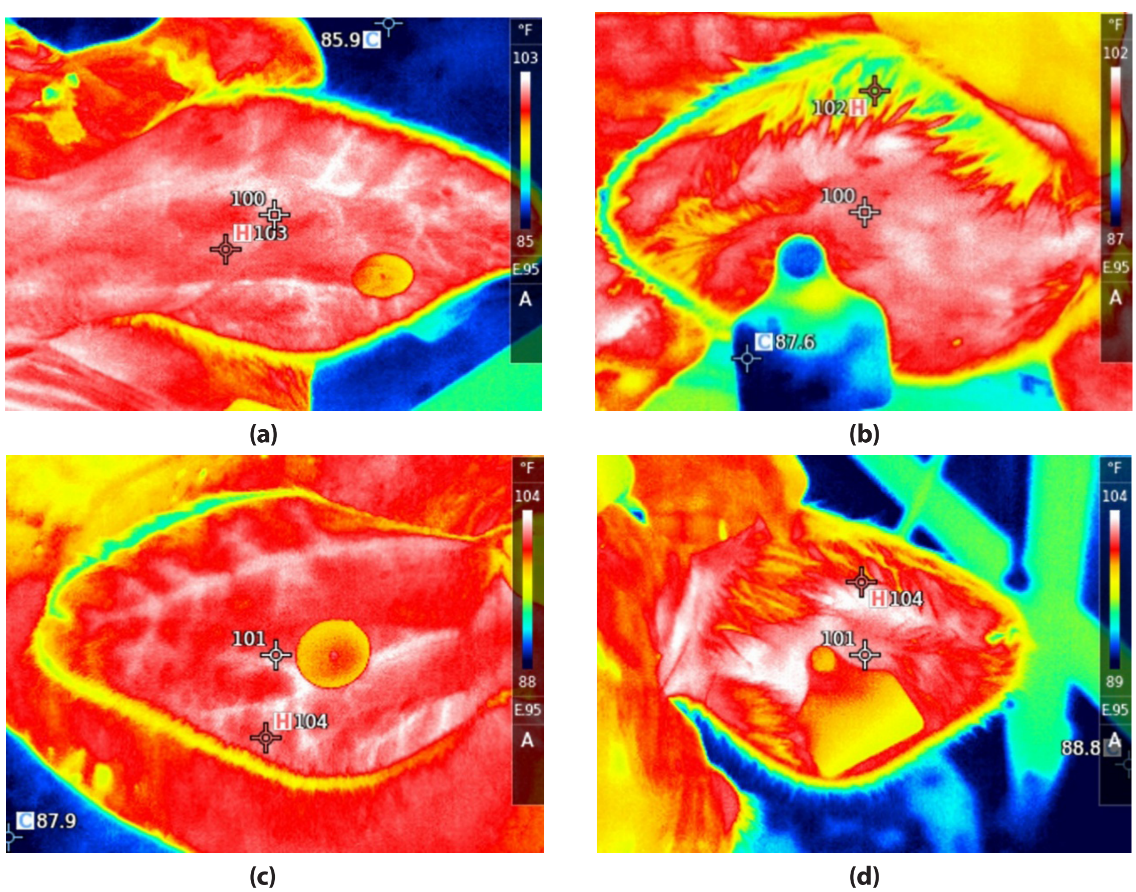

Fig. 1: (a) Right ear inner surface temperature (b) Left ear outer surface temperature (c) Left ear inner surface temperature (d) Right ear outer surface temperature

The comparison of the mean Ca++, thermographic parameters, rectal temperature, and THI did not show any difference between the 0th day and 3rd day of the SCH group and the normocalcemic group (Table 1).

Correlation Analysis

Correlation analysis of blood calcium levels, infrared (IR) thermographic parameters, and THI on the 0th and 3rd days postpartum is presented in Table 2. On the day of parturition (0th day), blood calcium exhibited a very strong negative correlation ($r = -0.91$ to -0.96, $p < 0.01$) with IR thermographic parameters, rectal temperature, and THI. In contrast, on the 3rd day postpartum, blood calcium levels demonstrated a very strong positive correlation ($r = 0.97$ to 0.99, $p < 0.01$) with the same parameters. Additionally, on the 0th day, IR thermographic parameters were strongly correlated with rectal temperature ($r \approx 0.92$, $p < 0.01$) and THI ($r = 0.94$ to 0.96, $p < 0.01$). A comparable trend was observed on the 3rd day postpartum. These findings indicate that blood calcium, IR thermographic parameters, and THI are strongly interrelated during the immediate postpartum period.

Subclinical hypocalcaemia (SCH) remains a major metabolic challenge in high-producing dairy breeds such as Frieswal cows, particularly during the periparturient period. The Frieswal breed, a cross of Holstein and Sahiwal, is widely distributed across diverse agro-climatic zones in India, and its high milk yield predisposes it to a range of production-related disorders, including hypocalcaemia. The transition from late gestation to early lactation involves a sharp surge in calcium demand due to the initiation of colostrogenesis and lactogenesis, elevating energy needs by up to 2.5-fold (Reynolds *et al.,* 2003).

The findings of our study reaffirm the widespread prevalence and physiological implications of SCH. Approximately 68% of Frieswal cows exhibited SCH on the day of calving, with 64% remaining affected by the third day postpartum. These rates are consistent with prior reports citing SCH incidences ranging from 41% to 54% in multiparous cows (Reinhardt *et al.,* 2011; Venjakob *et al.,* 2017). The condition often remains undetected due to the absence of overt clinical signs but contributes significantly to postpartum disorders, including metritis, retained placenta, mastitis, and delayed reproductive performance (Martinez *et al.,* 2012; Caixeta *et al.,* 2017).

Infrared thermography (IRT), as applied in our study, presents a promising non-invasive modality for early detection of SCH. The moderate negative correlations between calcium concentration and ear surface temperature recorded on the day of calving correlates with Venjakob *et al.* (2016), who demonstrated the utility of IRT in predicting postpartum calcium status via ear skin temperatures. As peripheral perfusion declines during hypocalcaemia, extremity temperatures drop, which can be captured reliably through thermal imaging. This complements traditional assessments, such as manual palpation of the ears, which though widespread, remain subjective.

Interestingly, by the third day postpartum, our data reveal a reversal in the trend of calcium levels correlated positively and significantly with average ear surface temperatures. This suggests that as cows recover from acute calcium depletion,

Table 1: Comparison of mean calcium and temperature parameters on the 0th and 3rd day of postpartum in the SCH and normal groups

Group	SCH		Normal	
Days postpartum	0	3	0	3
Ca++	6.8 ± 0.10	6.85 ± 0.15	8.3 ± 0.10	9.50 ± 0.20
AEIST	100.3 ± 0.40	99.80 ± 0.60	100.1 ± 1.00	100.10 ± 0.80
AEOST	101.0 ± 0.30	100.90 ± 0.50	100.0 ± 0.90	100.80 ± 0.70
AEST	100.6 ± 0.40	100.30 ± 0.50	100.1 ± 1.00	100.50 ± 0.70
RT	102.9 ± 0.30	102.90 ± 0.30	102.5 ± 0.40	103.30 ± 0.50
THI	84.0 ± 0.60	84.30 ± 0.50	84.4 ± 0.40	84.70 ± 0.20

Table 2: Correlation of calcium, THI, and IR parameters on the 0th and 3rd day of postpartum

	Ca (mg/dL)	Avg. ear Inner surface	Avg. ear outer surface	Avg. ear surface tem.	Avg. RT	THI
Ca (mg/dL)	1	-0.942**	-0.967**	-0.961**	-0.914**	-0.926**
Avg. ear Inner surface	0.992**	1	0.974**	0.994**	0.922**	0.945**
Avg. ear outer surface	0.990**	0.996**	1	0.987**	0.928**	0.950**
Avg. ear surface tem.	0.989**	0.992**	0.990**	1	0.929**	0.960**
Rectal temperature	0.990**	0.977**	0.973**	0.974**	1	0.847**
THI	0.973**	0.975**	0.980**	0.976**	0.968**	1
Correlation on 0th day of postpartum						
Correlation on 3rd day of postpartum						

**Significant at p<0.01 level (2-tailed).

thermographic profiles also normalize. A parallel conclusion was drawn by Emam *et al*. *(*2024), who highlighted the dynamic utility of thermal indicators in the transition phase for early disease detection.

Mechanistically, SCH disrupts neuromuscular function and immune competency, explaining its association with diverse postpartum disorders. Cortisol secretion during SCH impairs neutrophil function, increasing susceptibility to infections (Wang *et al.,* 1991). Moreover, SCH alters ovarian dynamics, delaying estrus and conception rates (Jonsson and Daniel 1997; Caixeta *et al.,* 2017). These systemic effects render SCH a gateway condition, underpinning multiple periparturient diseases. Dietary imbalances also play a critical role in SCH etiology. High dietary potassium, excessive phosphorus intake (>0.5% DMI), or insufficient anions during the dry period have been shown to elevate SCH risk (Seifi *et al.,* 2004; Goff, 2008; Grünberg, 2014).

Conclusions

Thus, our findings reinforce the high prevalence of subclinical hypocalcaemia in high-yielding Frieswal cows and demonstrate the potential of infrared thermography as a cost-effective, field-adaptable diagnostic tool. Future studies should emphasize integrating IRT with behavioural sensors and AI-driven platforms for real-time prediction, as supported by recent modelling frameworks (Van Leerdam *et al.,* 2024).

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