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Evaluation of ear skin temperature as a cow-side test to predict postpartum calcium status in dairy cows

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ABSTRACT

Subclinical hypocalcemia is considered a gateway disease that increases susceptibility to other metabolic and infectious diseases in transition dairy cows. In the absence of a cow-side test, however, it is difficult to identify hypocalcemic cows. The objective of this study was to evaluate ear skin temperature as a diagnostic predictor of serum calcium concentration. We conducted a cross-sectional study on 7 commercial dairy farms, involving 251 cows 0 to 48 h after calving. Skin temperature of the ears (STEar) was scored manually by palpating both ears. An infrared thermometer was used to measure ear temperature, skin temperature on the coxal tuber (STCox), and ambient temperature. Rectal temperature was measured using a digital thermometer. A blood sample was drawn to determine serum calcium concentration. Hypocalcemia was defined as serum calcium below 2.0 mmol/L, irrespective of clinical symptoms. Serum calcium concentration <2.0 mmol/L in connection with clinical symptoms was defined as clinical milk fever; serum calcium concentration <2.0 mmol/L without clinical symptoms was defined as subclinical hypocalcemia. Multivariate analysis using the GENLINMIXED procedure and receiver operating characteristic analysis were performed to evaluate whether serum calcium concentration could be predicted using ear temperature and other temperature estimates. The prevalence of hypocalcemia was 3.3, 27.3, 32.8, and 69.6% for cows in first, second, third, and fourth or greater lactation, respectively. None of the cows in first and second lactation had clinical milk fever. The prevalence of clinical milk fever was 6.0 and 20.3% for cows in their third and fourth or greater lactation, respectively. A decrease in ear temperature of 0.39°C [95% confidence interval (CI): 0.25–0.54] was associated with a decrease of 0.1 mmol/L in serum calcium concentration. Ambient temperature, however, was a major confounder for ear temperature. With an increase in ambient temperature of 1°C, STEar rose by 0.78°C (95% CI: 0.67–0.90). Hypothermia was more pronounced in clinical milk fever (median 21.8°C; interquartile range 14.7–27.0°C) compared with subclinical hypocalcemia (median 27.6°C, interquartile range 22.1–30.8°C). All temperature estimates had only accurate test characteristics based on their area under the curve for prediction of subclinical hypocalcemia (area under the curve for STEar, STCox, and rectal temperature were 0.641, 0.668, and 0.606, respectively) when cows with clinical milk fever were excluded. Although ear temperature has been associated with serum calcium concentration, ear temperature cannot be recommended for diagnosis of subclinical hypocalcemia.
Key words: subclinical hypocalcemia, milk fever, ear skin temperature

INTRODUCTION

Hypocalcemia is a potentially life-threatening metabolic disorder in dairy cows that predisposes them to various other metabolic and infectious disorders (Goff, 2008); it can be clinical or subclinical. Typically, the nadir in blood calcium concentration occurs between 12 and 24 h after calving, and only blood samples obtained at this time can reveal the extent of hypocalcemia experienced by a dairy herd (Kimura et al., 2006; Goff, 2008). Subclinical hypocalcemia is defined as a concentration of calcium in serum <2.0 mmol/L and affects approximately 50% of animals in second and greater lactation and up to 25% of animals in first lactation (Reinhardt et al., 2011).

Hypocalcemia around calving is associated with reduced milk yield, increased risk for clinical diseases (e.g., displaced abomasum, metritis) and increased risk of culling in early lactation (Chapinal et al., 2011, 2012a,b; Seifi et al., 2011; Roberts et al., 2012). Subclinical hypocalcemia is more costly than clinical milk fever because it affects a higher percentage of cows in the herd.

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Supplementation with oral calcium formulations around the time of calving in cows with subclinical hypocalcemia can reduce the risk of postpartum problems and increase milk yield (Oetzel, 2013). However, no comprehensively validated cow-side blood calcium test is available to identify cows with subclinical hypocalcemia, except for a hand-held photometric test that has been validated with only 20 samples and described in a preliminary report (Bootz et al., 2014). Known risk factors for hypocalcemia (e.g., parity, high milk yield in previous lactation, lameness) have been recommended as a way of identifying subpopulations of cows in which oral calcium supplementation would be beneficial (Oetzel and Miller, 2012), but this approach may lead to the unnecessary treatment of cows that have risk factors but not hypocalcemia (i.e., false-positive treatment decisions). More importantly, cows that have hypocalcemia but not these risk factors (false negatives) will be missed.

It is well known that decreased temperature of the ear (Guterbock, 2004; Radostits et al., 2007; Peek and Divers, 2008) and skin (Larsen et al., 2001) are clinical symptoms indicative of hypocalcemia in periparturient dairy cows. Therefore, it is common practice for veterinarians and farmers to use ear temperature determined by manual palpation as an estimate for the presence or absence of milk fever (i.e., calcium status) of a periparturient cow. To our knowledge, however, temperature of the ear skin has never been validated as a potential predictor of calcium status.

Infrared thermography allows for the noninvasive measurement of surface skin temperature and does not lead to radiation exposure (Eddy et al., 2001; Schaefer et al., 2004). These characteristics have led to an emerging interest in infrared thermography in both human and veterinarian medicine. The technique has been used previously in cows to assess the skin temperature of the udder and hooves as a way of identifying subclinical mastitis (Colak et al., 2008) and laminitis (Nikkhah et al., 2005), respectively.

Measuring the skin temperature of the ears would be a quick and noninvasive way to assess a cow's calcium status. The objective of our study was to evaluate the diagnostic performance of ear skin temperature in identifying cows with hypocalcemia.

MATERIALS AND METHODS

The experimental procedures reported herein were conducted with the approval of the Institutional Animal Care and Use Committee of the Freie Universität Berlin. Cows were managed according to the guidelines set by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medical Products (Hellmann and Radeloff, 2000)

Cows, Housing, and Feeding

We conducted a cross-sectional clinical study from January 2015 to August 2015. A convenience sample of 7 commercial dairy farms was recruited within a 165-km radius of a veterinary practice in Brandenburg, Germany. The average herd size was 717 (range 270–1,425). The average milk production (305-d ECM, 4.0% fat, 3.4% protein) was 8766 kg (range 7,470–9,634 kg). All cows were Holsteins except for herd 3, in which Jerseys were the dominant breed. Lactating cows were milked twice daily, and for all cows TMR was delivered once daily and pushed up multiple times per day. The TMR from close-up and fresh cows was formulated to meet or exceed minimum nutritional requirements for high-producing dairy cows (NRC, 2001). None of the herds used anionic salts as a prevention strategy for milk fever. Only herd 1 implemented a blanket treatment for cows in third or greater parity, with a fat-coated bolus directly after parturition that consisted of 43 g of calcium (Bovikalc, Boehringer Ingelheim Pharma GmbH and Co. KG, Ingelheim am Rhein, Germany).

Milking cows in all herds were housed in freestalls. In herds 1, 2, and 4, dry cows were housed in freestall barns with slatted floors and beds equipped with straw (herd 4) or wood shavings (herds 1 and 2). Dry cows in herds 3, 5, 6, and 7 were housed in deep straw bedding.

Overall, 251 animals were enrolled. Inclusion criteria were calving within the last 48 h on a given day (i.e., during a routine herd-health visit).

Experimental Procedures

Cows were restrained in headlocks during all experimental procedures. All cows enrolled in this study were examined 0 to 48 h after calving by one veterinarian according to a written standard operating procedure. Briefly, skin temperature of the ears (**STEar**) was scored manually by palpation of both ears on a 3-point scale (1 = 5–15°C; 2 = 16–25°C; 3 = 26–35°C). For measuring STEar (Figure 1), we used an infrared thermometer (Fluke 568 IR Thermometer, Fluke Deutschland GmbH, Glottertal, Germany) on the front and rear side of each ear. The device featured a range of −40 to 900°C, with a resolution of 0.1°C, a spectral range of 8 to 14 μm and a sensitivity of ±1% or ±1°C. We used an emissivity of 0.98 as previously described for the skin of calves (Hoffmann et al., 2013), other mammals (Kastberger and Stachl, 2003), and humans (Wolfe and Zissis, 1985).

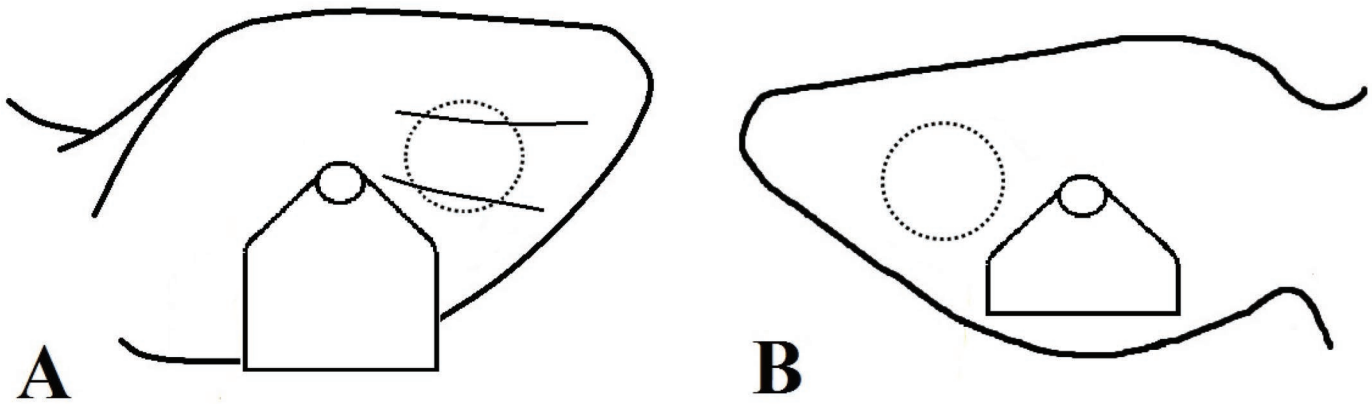

Figure 1. Schematic presentation of the measuring points for the infrared thermometer on the front (A) and rear side (B) of the ear.

Temperature was detected with a distance of approximately 1 m and a measured area of 2 cm in diameter. Skin temperature on the coxal tuber (**STCox**) was measured the same way as described for STEar. Rectal temperature (**RT**) was measured using a commercial thermometer (Veterinär-Thermometer SC 12, SCALA Electronic GmbH, Stahnsdorf, Germany).

Temperature of the feeding alley was recorded using the infrared thermometer to obtain an estimate of the ambient temperature.

Blood Sampling and Laboratory Analyses

Blood samples were taken immediately after evaluation of the skin temperature from the coccygeal vessels using Vacutainer systems (Vacuette 8 mL Z Serum Beads Clot Activator, Greiner Bio-One GmbH, Kremsmünster, Austria). Samples were kept at room temperature and allowed to clot. Within 5 h of blood collection, samples were centrifuged to harvest serum, which was frozen at −20°C. Analysis of blood samples was carried out by a commercial laboratory (Synlab Services GmbH, Augsburg, Germany). Total serum calcium concentration was analyzed using photometry (AU680, Beckman Coulter, Krefeld, Germany). The inter- and intraassay coefficients of variation were 1.6 and 0.7%, respectively.

Statistical Analyses

This study was carried out as an observational experiment. Animals were enrolled by convenience when a veterinarian visited a farm on a given day and an animal met the inclusion criteria of being within 48 h after parturition.

Individual cow data were transferred to Excel (Office 2010, Microsoft Deutschland Ltd., Munich, Germany). Statistical analyses were performed using SPSS for Windows (version 22.0, SPSS Inc., Ehningen, Germany). The individual cow was the experimental unit in all analyses.

We used univariate analyses to determine the association between calcium status and STEar, STCox, or rectal temperature. Calcium status was defined according to serum calcium concentration and the clinical appearance of the animal. Normocalcemia was defined as a serum calcium concentration greater than or equal to 2.0 mmol/L. Cows not affected clinically with a serum calcium concentration below 2.0 mmol/L were characterized as having subclinical hypocalcemia. Recumbent cows with a serum calcium concentration below 2.0 mmol/L were defined as having clinical milk fever.

Univariate analyses included STEar, STCox, or rectal temperature as dependent variables and calcium status as an independent variable. For evaluation of the association between serum calcium concentration and STEar, STCox, or rectal temperature, we used the GENLINMIXED procedure in SPSS. Herd was considered a random effect. According to the model-building strategies described by Dohoo et al. (2009), each parameter considered for the mixed model should be separately analyzed in a univariate model, including the parameter as a fixed factor (i.e., categorical parameter) or covariate (i.e., continuous parameter). Only parameters resulting in univariate models with $P \leq 0.20$ should be included in the final mixed model. The initial model contained the following explanatory variables as fixed effects: parity (1, 2, 3, or 4 or more), breed (Holstein or Jersey), time after parturition (continuous, 0 to 48 h), oral calcium supplementation (yes or no), STEar (continuous), STCox (continuous), and rectal temperature (continuous). We selected the model that best fit the data by finding the model with the lowest value for the Akaike information criterion us-

ing a backward elimination procedure that removed all variables with $P > 0.10$.

We evaluated the factors that influenced STEar using a GENLINMIXED procedure with STEar as the independent variable. We performed model-building and selection of the model that best fit the data as described above. This initial model contained the following explanatory variables as fixed effects: lactation group (1 to 4), breed (Holstein or Jersey), time after parturition (continuous, 0 to 48 h), blood calcium (continuous), oral calcium supplementation (yes or no), ambient temperature (continuous), rectal temperature (continuous).

To define reference criteria for identifying cows with subclinical hypocalcemia based on their skin temperature (i.e., STEar, STCox) or rectal temperature, we used a receiver operating characteristic (**ROC**) analysis. The continuous variable was either skin or rectal temperature, and the classification variable was calcium status. The ROC curves compare sensitivity with 100 − specificity. To differentiate between normocalcemia and hypocalcemia, we used 4 thresholds, which were associated with a negative health or production outcome (Chapinal et al., 2011, 2012a,b; Seifi et al., 2011; Martinez et al., 2012; Roberts et al., 2012). Sensitivity was the proportion of cows diagnosed with hypocalcemia that had serum calcium concentrations below the threshold; specificity was the proportion of cows diagnosed with normocalcemia that had serum calcium concentrations above the threshold (Greiner et al., 2000). The point on the ROC curve with the highest combined sensitivity and specificity was considered the critical threshold. Interpretation of this critical threshold was based on the area under the curve (**AUC**) according to Swets (1988) as noninformative (AUC = 0.5), accurate (0.5 < AUC ≤ 0.7), very accurate (0.7 < AUC ≤ 0.9), highly accurate (0.9 < AUC < 1), and perfect (AUC = 1).

A significant difference between the levels of a classification variable was declared when $P < 0.05$; differences between $P \geq 0.05$ and $P \leq 0.10$ were considered a statistical tendency.

RESULTS

Data from 251 animals were available for final analysis. Sixty were primiparous cows (23.9%); 55 (21.9%), 67 (26.7%), and 69 (27.5%) were in second, third, and fourth or greater lactation, respectively.

The mean interval between calving and investigation of the ear temperature was 19.96 h (SD 13.91). Overall, the prevalence of subclinical hypocalcemia and clinical milk fever was 27.4% (69/251) and 7.2% (18/251), respectively. The prevalence of hypocalcemia increased with lactation number. Prevalence was 3.3% (2/60), 27.3% (15/55), 32.8% (22/67), and 69.6% (48/69) for cows in first, second, third, and fourth or greater lactation, respectively. None of the cows in first or second lactation had clinical milk fever. Prevalence of clinical milk fever was 6.0% (4/67) and 20.3% (14/69) for cows in third and fourth or greater lactation, respectively.

As indicated by the multivariate analyses, there was a positive association between each of the 3 animal temperature measures (STEar, STCox, rectal temperature) and serum calcium concentration (Tables 1, 2, and 3). Hypothermia was more pronounced in clinical milk fever compared with subclinical hypocalcemia for STEar (Figure 2, Panel A), STCox (Figure 2, Panel B), and rectal temperature (Figure 2, Panel C), respectively. We analyzed ear skin temperature, STCox, and rectal temperature using ROC curves to determine the critical thresholds (combined highest sensitivity and specificity) for distinguishing between normocalcemia and subclinical hypocalcemia, excluding animals with clinical milk fever (Table 4). The AUC for the differentiation

Table 1. Association between serum calcium concentration (mmol/L) and ear skin temperature based on multivariate analysis

Variable[1]	Estimate, mmol/L	SE	95% CI		*P*-value
			Lower CI	Upper CI	
Intercept	1.867	0.112	1.646	2.087	0.001
Lactation group					
Lactation 1	Referent				
Lactation 2	−0.047	0.063	−0.171	0.076	0.450
Lactation 3	−0.199	0.059	−0.315	−0.082	0.001
Lactation 4+	−0.483	0.062	−0.606	−0.360	0.001
Breed					
Holstein	Referent				
Jersey	−0.113	0.052	−0.216	−0.010	0.032
Skin temperature of the ear, °C	0.012	0.003	0.005	0.019	0.001

[1]Model adjusted for the random effect of the herd.

Table 2. Association between serum calcium concentration (mmol/L) and skin temperature on the coxal tuber based on multivariate analysis

Variable[1]	Estimate, mmol/L	SE	95% CI		*P*-value
			Lower CI	Upper CI	
Intercept	1.379	0.312	0.764	1.994	0.001
Lactation group					
Lactation 1	Referent				
Lactation 2	−0.042	0.062	−0.164	0.079	0.495
Lactation 3	−0.200	0.058	−0.315	−0.085	0.001
Lactation 4+	−0.479	0.061	−0.599	−0.358	0.001
Breed					
Holstein	Referent				
Jersey	−0.088	0.052	−0.191	0.015	0.094
Skin temperature of the coxal tuber, °C	0.027	0.006	0.015	0.038	0.001

[1]Model adjusted for the random effect of the herd.

between normocalcemia and subclinical hypocalcemia based on STEar, STCox, and rectal temperature were 0.641, 0.668, and 0.606, respectively ($P = 0.001$).

Ambient temperature was a potential confounder for STEar (Table 5). However, serum calcium concentration was still associated with STEar when we considered ambient temperature in the same model.

There was a high correlation between STEar and manual ear score (r = 0.76; $P = 0.001$). Multivariate analysis indicated an association between manual ear score and serum calcium concentration. Compared with cows with an ear score of 1, estimated serum calcium concentration was 0.346 mmol/L (95% CI: 0.193–0.498; $P = 0.001$) and 0.432 mmol/L (95% CI: 0.291–0.573; $P = 0.001$) higher for cows with ear scores of 2 and 3, respectively.

DISCUSSION

It is well known that cows with clinical milk fever have cold extremities (Guterbock, 2004; Radostits et al., 2007). Therefore, many farmers and practitioners implement manual palpation of the ears in fresh-cow protocols to evaluate calcium status and provide calcium supplementation based on this sensorial but subjective observation.

The objective of this study was to evaluate whether this practice might be a useful predictor for identifying not only cows with clinical milk fever but also cows with subclinical hypocalcemia. Hypocalcemia was more prevalent in older cows in our study, which is in agreement with previous results. Reinhardt et al. (2011) showed that serum calcium concentration declined significantly with increasing number of lactations. In the same experiment, the concentration of 1,25-dihydroxyvitamin D [**1,25(OH_2)D**] increased from first to third lactation but plateaued beyond third lactation, which might indicate that endocrine adaptation is inadequate in these cows.

Horst et al. (1990) showed in rats that the concentration of 1,25(OH_2)D receptors in intestine and bone declined significantly with increasing age. Goff (2014) assumed for dairy cows that the inability to maintain calcium homeostasis was due to the mature skeleton

Table 3. Association between serum calcium concentration (mmol/L) and rectal temperature based on multivariate analysis

Variable[1]	Estimate, mmol/L	SE	95% CI		*P*-value
			Lower CI	Upper CI	
Intercept	−3.157	1.559	−6.229	−0.084	0.044
Lactation group					
Lactation 1	Referent				
Lactation 2	−0.031	0.063	−0.156	0.094	0.621
Lactation 3	−0.164	0.060	−0.282	−0.045	0.007
Lactation 4+	−0.460	0.064	−0.586	−0.333	0.001
Breed					
Holstein	Referent				
Jersey	−0.081	0.054	−0.189	0.026	0.137
Rectal temperature, °C	0.137	0.040	0.059	0.215	0.001

[1]Model adjusted for the random effect of the herd.

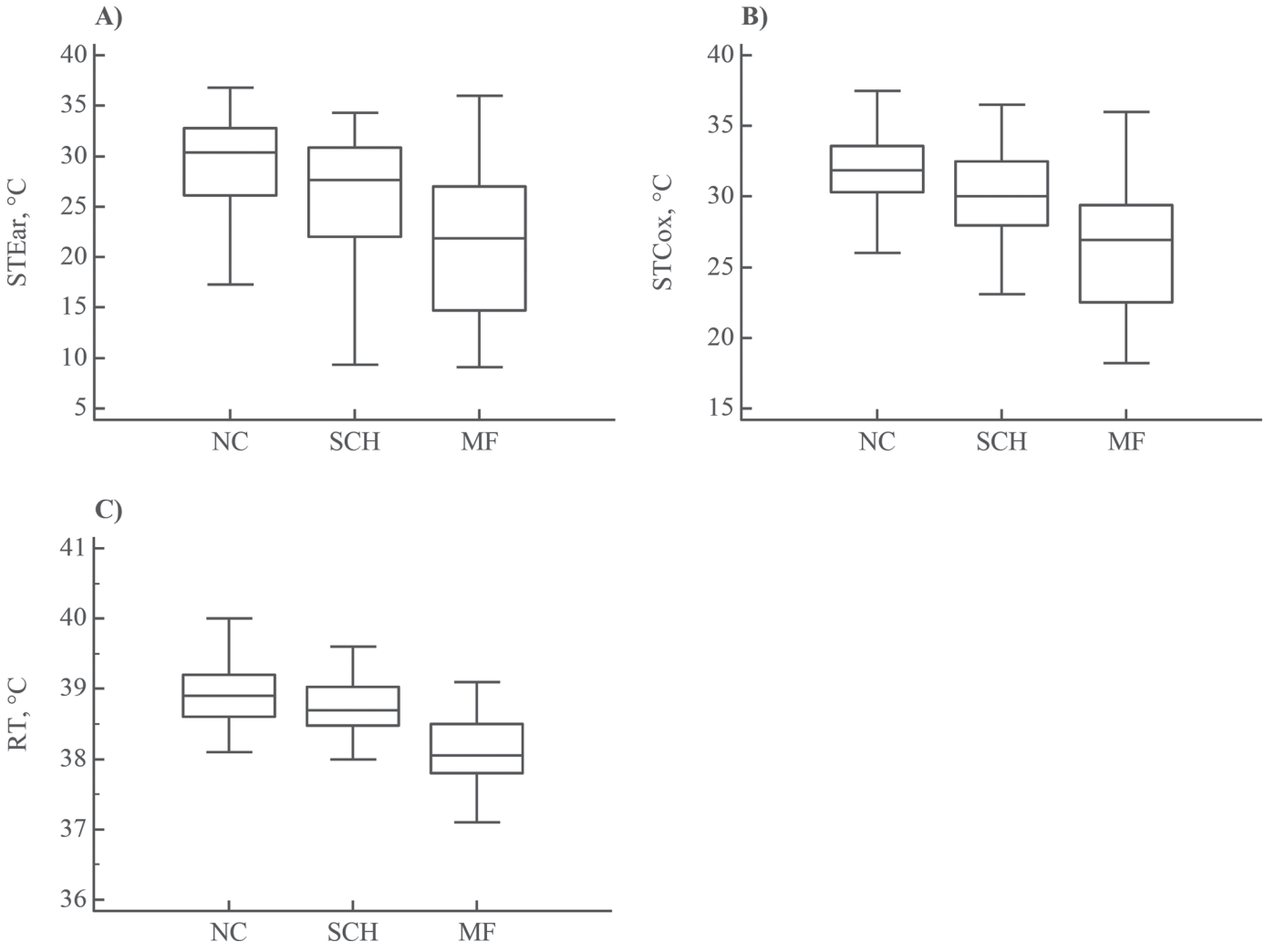

Figure 2. Association between ear skin temperature (STEar; panel A), skin temperature on the coxal tuber (STCox; panel B), rectal temperature (RT; panel C) and calcium status [normocalcemia (NC) n = 164; subclinical hypocalcemia (SCH) n = 69; milk fever (MF) n = 18]. The central box represents the interquartile range from the first to third quartile. A segment inside the box shows the highest and lowest case within 1.5 times the interquartile range, respectively.

and reduced response to parathyroid hormone in older animals; bone remodeling was reduced, and active osteoclasts and osteoblasts were scarce. It has also been demonstrated that parathyroid hormone receptors in the kidneys are downregulated with age in rats (Hanai et al., 1990). This might also occur in osteoclasts and osteoblasts of cows, being another reason for maladaptation of mineral metabolism after parturition (Goff, 2014).

Wittek and Jonnson (2011) assumed that postpartum calcium homeostasis and the ability to mobilize calcium might be dependent on immunological processes. They observed a difference in the expression of different immunological profiles of cytokines and chemokines from cows in first lactation compared with cows in a higher lactation. However, further research regarding the role of the immune system in the etiology of hypocalcemia is indicated.

Using multivariate analysis, we compared serum calcium concentrations with temperatures measured on the ear surface, but there was only a moderate relationship between serum calcium concentration and STEar. Hypothermia seemed to be more pronounced in cows with clinical milk fever, but a major confounder was ambient temperature. This finding was consistent with a previous study, which found that the temperature of hooves in horses and cows depended markedly on ambient temperature (Gloster et al., 2011). It was not possible to sample healthy cows under the same environmental conditions as a control group, because a valid cow-side test is not available. Such a comparison might have improved the results of our study.

Table 4. Critical thresholds for ear skin temperature, skin temperature on the coxal tuber, and rectal temperature to differentiate between normocalcemia and subclinical hypocalcemia based on receiver operating characteristic analyses and considering 4 calcium thresholds to define subclinical hypocalcemia

Calcium threshold, mmol/L	Prevalence, %	Temperature variable[1]	Threshold, °C	Sensitivity	Specificity	AUC[2]	*P*-value
2.0	29.6	STEar	27.0	49.3	73.8	0.641	0.001
		STCox	30.0	52.2	78.7	0.668	0.001
		RT	39.0	75.4	42.7	0.606	0.009
2.1	45.1	STEar	27.9	47.6	71.8	0.615	0.001
		STCox	30.9	54.3	63.3	0.597	0.010
		RT	38.5	28.6	85.2	0.576	0.046
2.2	65.2	STEar	29.4	53.3	66.7	0.600	0.007
		STCox	30.8	50.0	70.4	0.600	0.009
		RT	39.3	21.7	90.1	0.536	0.350
2.3	84.5	STEar	28.3	42.1	77.8	0.607	0.026
		STCox	30.5	40.6	77.8	0.580	0.090
		RT	39.1	31.0	83.3	0.557	0.224

[1]RT = rectal temperature; STCox = skin temperature on the coxal tuber; STEar = skin temperature on the ear.
[2]AUC = area under the curve.

Cows with hypocalcemia also had a lower skin surface temperature on the coxal tubers (Radostits et al., 2007) and a lower rectal temperature (Larsen et al., 2001).

Maladaptation of mineral metabolism to periparturient calcium loss via colostrum and milk production leads to calcium deficiency and diminished muscle contraction, finally resulting in recumbency (Goff, 2004). One explanation for the reduced skin and core temperature observed in our study might be this decreased muscle contraction, causing lower thermal energy production. Furthermore, dry matter intake is reduced in periparturient cows (Grummer et al., 2004), which might decrease the heat of fermentation and therefore core temperature.

Another reason for a decreased body temperature is the decrease in serum progesterone concentration around calving (Suthar et al., 2012), which can be used to predict birth in dairy cows (Burfeind et al., 2011).

As stated by Lima and Bakker (2005), sensorial assessment of surface temperature in humans can be used to diagnose reduced peripheral perfusion in shock-like situations. Due to a decrease in cardiac output, clinical milk fever might also go along with reduced blood circulation to the extremities and result in colder ears.

The objective of this study was to evaluate whether STEar could be used to identify cows with hypocalcemia, but hypothermia was a poor cow-side predictor for diagnosis of subclinical hypocalcemia, as evidenced by the results from the ROC curve analyses. Hypothermia was more pronounced in cows with clinical milk fever. Therefore, measuring ear temperature has a certain but limited value for determining calcium status. Overall, it cannot be recommended as a diagnostic test for subclinical hypocalcemia. In the absence of a validated cow-side blood test for hypocalcemia, further development and research approaches are warranted to improve the current concept of using risk factors as decision criteria for individual calcium supplementation in fresh cows (Oetzel and Miller, 2012).

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Table 5. Association between ear skin temperature and serum calcium concentration considering ambient temperature

			95% CI		
Variable[1]	Estimate, °C	SE	Lower CI	Upper CI	*P*-value
Intercept	6.683	1.942	2.856	10.509	0.001
Ambient temperature, °C	0.783	0.059	0.667	0.899	0.001
Calcium, mmol/L	3.918	0.734	2.472	5.364	0.001

[1]Model adjusted for the random effect of the herd.

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