Effect of continued metabolic acidification into the first 3 days of lactation on blood calcium status in postpartum dairy cattle: A randomized controlled trial

G. U. Maier,^{1*} Bret McNabb,¹ Richard Pereira,¹ Heejung Bang,² Sharif S. Aly,^{1,2}
and Heidi A. Rossow^{1,3}

¹Department of Population Health and Reproduction, School of Veterinary Medicine, University of California Davis, 95616

²Division of Biostatistics, Department of Public Health Sciences, University of California Davis, 95616

³Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California Davis, Tulare 93274

ABSTRACT

Although incidence of clinical hypocalcemia in postpartum dairy cows is low in US dairies, subclinical hypocalcemia after calving is common and has been associated with metabolic and infectious disease. It is widespread farm practice to feed a diet rich in anions to prepartum dairy cattle to support calcium homeostasis. However, this diet is typically discontinued at parturition, when calcium needs are still high. The objective of this trial was to determine the effects of extending metabolic acidification into the first 3 d of lactation in multiparous Holstein cows with the use of magnesium chloride (MgCl_2) hexahydrate drenches on blood ionized calcium concentrations. Adult Holstein cows at a commercial dairy in their second or higher lactation, with a urine pH of 6.8 or less on the day of calving, were randomly assigned to either treatment or control groups, resulting in 13 cows in the treatment group and 14 cows in the control group. Treatment cows received 480 g of oral MgCl_2 hexahydrate once daily for 3 d for continued acidification starting on the day of calving, whereas cows in the control group received no treatment. Urine pH was measured daily for 5 d, starting on the day of calving (0 DIM), to assess acidification status; blood was collected on day of calving (0 DIM), 2 DIM, and 4 DIM and analyzed for ionized calcium concentrations. Differences in blood ionized calcium and urine pH over time were compared using longitudinal data analysis. Urine pH was lower in treatment cows compared with control cows at 1, 2, and 3 DIM. Blood ionized calcium concentrations were different from baseline, taken at enrollment (0 DIM) and at 2 and 4 DIM in both

treatment and control cows. However, no difference was detectable between treatment and control cows at 2 or 4 DIM with respect to blood ionized calcium concentrations. Oral supplementation with MgCl_2 hexahydrate resulted in the desired acidification of urine pH in the treatment group, similar to feeding of an anionic close-up diet. Continued acidification of dairy cows until 2 DIM did not result in clinically meaningful higher blood calcium concentrations compared with controls, and further research is needed, to identify physiological reasons for this finding.

Key words: DCAD, hypocalcemia, postpartum, dairy cows

INTRODUCTION

Milk fever has been recognized as a disease of dairy cattle for over 200 years (Murray et al., 2008). The disease is caused by inadequate blood calcium concentrations necessary for proper nerve and muscle function and is of particular concern in the periparturient cow, in which the onset of lactation often overwhelms homeostatic capabilities (Goff, 2008). The reported incidence of clinical milk fever in US dairy herds is low, estimated at 2.8% based on the 2014 survey by the National Animal Health Monitoring System (USDA NAHMS, 2016). However, the prevalence of subclinical hypocalcemia is 25% and 47% in primiparous and multiparous cows, respectively (Reinhardt et al., 2011). Subclinical hypocalcemia has been defined in the literature as a serum total calcium concentration of <2.15 mmol/L (8.60 mg/dL; Martinez et al., 2012) or blood ionized calcium <1.0 mmol/L (Martinez et al., 2014). Hypocalcemia in dairy cattle is an area of ongoing research, and cut points have evolved based on new knowledge, taking into consideration factors such as DIM and parity (Neves et al., 2018). Subclinical hypocalcemia has been associated with decreased feed intake and less time spent chewing and ruminating, reduced neutrophil concentration in

Received April 4, 2020.

Accepted July 31, 2020.

*Corresponding author: gumaier@ucdavis.edu

the blood, impaired neutrophil function, and increased risk of several postpartum diseases (Hansen et al., 2003; Martinez et al., 2012). Risk of developing metritis or displaced abomasum increases in cows with subclinical hypocalcemia in the first 3 to 4 DIM following calving, compared with normocalcemic cows (Martinez et al., 2012; Neves et al., 2018). Cows with blood calcium concentrations <2.2 mmol/L in the first week postpartum had a higher incidence of displaced abomasum than cows with higher blood calcium concentrations (Chapinal et al., 2011). Cows with retained fetal membranes had lower plasma calcium concentrations (1.79 mmol/L ± 0.09) soon after calving, compared with cows without retained fetal membranes (2.19 mmol/L ± 0.07 ; Melendez et al., 2004).

Inducing mild metabolic acidosis in dairy cows in the close-up period before parturition by increasing the dietary anions SO_4^{2-} and Cl^- relative to the dietary cations Na^+ and K^+ (i.e., DCAD) remains the most accepted preventive measure for milk fever (Murray et al., 2008). Urine pH is correlated with blood pH and blood HCO_3^- and decreases with lower DCAD values (Luebke et al., 2011). Urine pH is often used as a surrogate for metabolic acidification and as a monitoring tool for dairy cows in the close-up pen due to its relative ease of sample collection and low cost. Metabolic acidosis has several effects on the function of parathyroid hormone, released during a state of hypocalcemia, and its receptor. The conformation of the parathyroid hormone receptor is more sensitive to parathyroid hormone at a lower pH, and the kidneys have higher levels of reabsorption of calcium from the glomerular filtrate and function better at converting 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D, the active form of vitamin D, which is required for intestinal uptake of calcium (Goff, 2008). A negative DCAD is typically fed during the last 3 to 4 wk of pregnancy (Goff, 2014). Once a cow has calved, she is usually moved to a different pen, where the addition of acidifying salts to the diet is discontinued and DCAD becomes positive.

The objective of our study was to evaluate the effect of an extension of negative DCAD into the first 3 d of lactation on the blood calcium concentration of postpartum multiparous dairy cows, to evaluate this technique as an approach to reducing postpartum subclinical hypocalcemia. Our hypothesis was that cows receiving continued metabolic acidification into the first 3 d of lactation would have higher blood calcium concentrations than those that did not. The primary outcome was mean blood ionized calcium concentrations at 2 and 4 DIM, and the secondary outcome was mean urine pH measured once daily between 1 and 4 DIM for cows in the treatment and control groups.

MATERIALS AND METHODS

Our study was approved by the University of California Davis Institutional Animal Care and Use Committee (protocol #18395). A sample size calculation resulted in 14 cows per group to detect a difference of 0.1 mmol/L with a standard deviation of 0.09 mmol/L of ionized calcium between groups with a power of 80% and α at 0.05. We enrolled 27 multiparous Holstein dairy cows on a commercial dairy on the day of calving (0 DIM) after they had calved and after measuring their urine pH with a digital urine pH meter (Horiba Scientific, Piscataway, NJ). Cows were randomly assigned to either treatment or control groups. Randomization was conducted using a list of random numbers generated in a spreadsheet program (Excel, Microsoft Corp., Redmond, WA). Cows in the study were not administered any routine postpartum calcium supplementation.

Cows with urine pH above 6.8 were considered not acidified and were not eligible to be enrolled in the study. Likewise, we excluded cows from enrollment that appeared visually depressed, based on demeanor, ear carriage, and appetite, or that had retained fetal membranes, as these cows were more likely to have decreased feed intake or to suffer from low blood calcium concentrations during the study period, which would have confounded results. After determining eligibility of a cow to be enrolled in the study, the investigator was told by the assistant whether the cow was in the treatment or control group based on the random numbers list, but no special concealment measure for allocation was put in place. At enrollment, the investigator determined the BCS of the enrolled study cows using the 1-to-5 BCS system, with 1 being emaciated and 5 being obese (Edmonson et al., 1989). Rectal temperature was measured using a digital thermometer on the day of calving. For practical reasons, study cows were moved to the early-lactation cow pen, where cows spend the first 14 to 18 DIM, and received the same diet as other postpartum cows in the herd. Compositions of pre- and postpartum diets are described in Table 1.

Cows were enrolled twice daily, either in the morning between 0600 and 0700 h or in the afternoon between 1500 and 1600 h, so that the first sample (0 DIM) was collected in a window between 0 and 14 h after calving. Subsequent samples were collected only in the mornings and correspond to the following postcalving time windows: between 14 and 38 h at 1 DIM, between 38 and 62 h at 2 DIM, between 62 and 86 h at 3 DIM, and between 86 and 110 h at 4 DIM. Cows in the treatment group were administered 480 g of MgCl_2 hexahydrate (Sierra Chemical, West Sacramento, CA) dissolved in approximately 3 L of water and delivered via Magrath cattle pump (Springer Magrath Co., Glencoe, MN) with

Table 1. Pre- and postpartum diet composition for 27 multiparous Holstein cows that received either oral MgCl₂ hexahydrate for 3 d postpartum (treatment) or no treatment (control)

Item, % DM basis	Prepartum	Early lactation
Nutrient		
DM	57.6	53.1
CP	15.4	16.9
ADF	26.6	24.5
NDF	38.4	35.9
Lignin	4.8	4.8
Fat	2.6	3.5
Starch	13.9	17.7
Ash	9.6	9.2
NE _L , MJ/kg	6.6	6.8
NE _M , MJ/kg	7.1	7.6
NE _G , MJ/kg	4.3	4.6
DCAD, ¹ mEq/100 g	-12.7	21.8
Sodium	0.18	0.49
Chloride	1.3	0.74
Potassium	1.5	1.55
Sulfur	0.35	0.29
Magnesium	0.38	0.36
Calcium	1.2	1.03
Ingredient		
Corn silage	38	30
Alfalfa hay	27	19
Corn	4.6	14
Wheat straw	4.0	1.6
Beet pulp pellets	5.4	5.3
Soy hulls pellets	3.8	4.1
Wheat mill run	3.4	3.2
Canola meal	3.2	4.3
Dry corn distillers grains	2.5	1.2
Molasses cane	0.13	0.51
Whole cottonseed		7.1
Soybean meal		5.4
Soy Best ²		1.6
Blood meal		0.75
Supplement ^{3,4}	8.85	2.65

¹DCAD was calculated as (Na + K) – (S + Cl), using the DCAD calculator, Arm and Hammer Animal and Food Production (<https://ahfoodchain.com/calculators/dcad-calculator>).

²Soy Best (Grain States Soya Inc., West Point, NE).

³Prepartum supplement: Calcium carbonate, calcium chloride, magnesium sulfate 7H₂O, calcium sulfate dihydrate, magnesium oxide, vitamin E, white salt, Rumensin 90.7 (Elanco, Greenfield, IN), Prequel 21 (Virtus Nutrition LLC, Corcoran, CA), Alimet (Novus International Inc., Saint Charles, MO), Celmanax SCP (Arm and Hammer Animal Nutrition, Princeton, NJ), Diamond V XPC (Diamond V, Cedar Rapids, IA), Clarify 0.67% (Central Garden and Pet Company, Schaumburg, IL), Biochlor (Arm and Hammer Animal Nutrition), Animate (Phibro Animal Health Corporation, Teaneck, NJ), Reashure Choline (Balchem Corporation, New Hampton, NY), PDS L55 Mintrex Premix (Progressive Dairy Solutions Inc., Oakdale, CA).

⁴Early-lactation supplement (first 14–18 DIM): Magnesium oxide, Vitamin E, oyster shell (ground), sodium bicarbonate, white salt, Zinpro4 Plex C (Diamond V, Cedar Rapids, IA), Rumensin 90.7 (Elanco), Prequel 21 (Virtus Nutrition LLC), Alimet (Novus International Inc.), Celmanax SCP (Arm and Hammer Animal Nutrition), Diamond V XPC (Diamond V), Clarify 0.67% (Central Garden and Pet Company), PDS L45 TM (Progressive Dairy Solutions Inc.).

a 152-cm orogastric tube, according to manufacturer's instructions, once at enrollment (morning or afternoon) and 2 more times in the morning starting on the day

of calving. Cows in the control group did not receive acidification interventions.

We calculated DCAD for treatment and control groups using the formula $DCAD = (\% \text{ sodium} \times 43.5) + (\% \text{ potassium} \times 25.6) - (\% \text{ chlorine} \times 28.2) - (\% \text{ sulfur} \times 62.5)$ (Lean et al., 2006). The target DCAD for the treatment group was <0 mEq/100 g of DM. In both treatment and control groups, we collected urine samples via perineal stimulation on the day of calving (morning or afternoon, 0 DIM) and on the following 4 d (1–4 DIM) in the morning and measured urine pH via a handheld pH meter (Horiba Scientific). Prior to each day of use, we calibrated the pH meter at pH 4, 7, and 10. On the day of calving (0 DIM), and at 2 and 4 DIM, we collected 10 mL of blood from a coccygeal vessel into an evacuated collection tube containing heparin and analyzed these samples via a point-of-care handheld blood analyzer (iStat, Abaxis, Union City, CA) for ionized calcium within 2 h of collection. Cows that required calcium supplementation (intravenous calcium gluconate) during the study period (0–4 DIM) because of clinical signs of hypocalcemia were censored: only data collected up to calcium supplementation were analyzed. A total of 17 cows were enrolled during December 2014, and 10 cows were enrolled in March 2015.

We performed the statistical analysis with SAS software (SAS version 9.4, SAS Institute Inc., Cary, NC). Linear mixed models with urine pH or blood ionized calcium concentrations as the response variable included sampling time points (0, 1, 2, 3, and 4 DIM for urine pH and 0, 2, and 4 DIM for blood ionized calcium concentration), treatment group, and the interaction of sampling time points and treatment group, with repeated measures by cow. Baseline values for urine pH or blood ionized calcium concentration as well as parity for the model with ionized calcium as the outcome were offered to models as possible confounders and remained in the models if $P < 0.05$. Residuals were visually inspected for deviation from normality on Q-Q plots. Results for the main parameters of interest (treatment × group interaction) were considered statistically significant at the $P < 0.025$ level, to adjust for the fact that we tested for 2 outcomes. Model fit was assessed using the Akaike information criterion for the covariance structures compound symmetry, first-order autoregressive, unstructured, and Toeplitz. The Kenward-Roger method was used to estimate degrees of freedom. Contrasts were used to compare least squares means between different sampling time points using the diff function in SAS. For these subanalyses, 95% confidence intervals (CI) were reported and were considered statistically significant if they did not include 0. The numbers of subclinically hypocalcemic cows between groups were compared with a chi-squared test for a

difference in proportions or Fisher's exact test for small cell counts (50% of cells with expected counts less than 5). We used a cutoff of <1.0 mmol/L for subclinical hypocalcemia (Martinez et al., 2014).

RESULTS

The DCAD for the early-lactation cow TMR was 22 mEq/100 g of DM: $(0.49\% \text{ sodium} \times 43.5) + (1.55\% \text{ potassium} \times 25.6) - (0.74\% \text{ chlorine} \times 28.2) - (0.29\% \text{ sulfur} \times 62.5)$. Assuming a DMI of 19.3 kg of early-lactation cow diet, adding 480 g of MgCl_2 hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) with a DM content of 225 g lowered DCAD for the same diet to -2.4 mEq/100 g of DM: $(0.48\% \text{ sodium} \times 43.5) + (1.53\% \text{ potassium} \times 25.6) - (1.59\% \text{ chlorine} \times 28.2) - (0.29\% \text{ sulfur} \times 62.5)$.

Baseline characteristics and urine pH of study cows are listed in Table 2. No cows had to be excluded based on high urine pH. Study groups were similar in terms of lactation number, BCS, rectal temperature, blood ionized calcium, or urine pH. One cow in the treatment group developed mild diarrhea at 2 DIM and did not receive a third MgCl_2 hexahydrate treatment. Another cow in the treatment group developed clinical signs of milk fever at 3 DIM and was treated with and responded to intravenous calcium gluconate after a blood sample was obtained. No blood sample was obtained at 4 DIM for this cow. Furthermore, we were unable to obtain urine from one cow in the treatment group at 4 DIM. The data for the 2 cows that were excluded after study start were used until the day of study exclusion. Apart from these exceptions, the results from all enrolled cows were available and used in the analysis.

Table 2. Mean (SD) or median (interquartile range) and range of values for baseline characteristics on day of calving in 27 multiparous Holstein cows that received either oral MgCl_2 hexahydrate for 3 d postpartum (treatment) or no treatment (control)

Baseline characteristic	Treatment (n = 13)	Control (n = 14)
Lactation number ¹	3.0 (2.0–4.0)	3.5 (2.0–5.0)
Range	2–5	2–6
BCS ^{1,2}	3.0 (2.75–3.5)	3.0 (3.0–3.25)
Range	2.5–3.5	2.75–3.75
Rectal temperature, ³ °C	38.7 (0.6)	38.7 (0.6)
Range	37.6–39.9	37.1–39.4
Whole blood ionized calcium, ³ mmol/L	1.10 (0.10)	1.05 (0.11)
Range	0.92–1.24	0.80–1.22
Urine pH ³	6.00 (0.37)	5.84 (0.33)
Range	5.47–6.60	5.39–6.40

¹Median (interquartile range).

²BCS was measured on a scale from 1 to 5, with 1 being emaciated and 5 being obese.

³Mean (SD).

Figure 1. Mean urine pH ($\pm 95\%$ CI) for 27 multiparous Holstein dairy cows on day of calving and during the first 4 d postpartum. The treatment group (n = 13) was treated with 480 g of MgCl_2 hexahydrate as an oral drench for the first 3 d (0, 1, and 2 DIM), whereas cows in the control group (n = 14) received no treatment. Data points marked with * differ between groups (95% CI for difference excludes 0).

Change of mean urine pH over the study period is depicted in Figure 1. Mean urine pH at enrollment was $5.84 (\pm 0.09)$ in control cows and $6.00 (\pm 0.1)$ in treatment cows (95% CI for difference: -0.32 – 0.64). On subsequent study days, urine pH in control and treatment cows, respectively, was $7.22 (\pm 0.2)$ and $6.38 (\pm 0.2)$ at 1 DIM (95% CI for difference: 0.36 – 1.31), $7.91 (\pm 0.1)$ and $6.77 (\pm 0.3)$ at 2 DIM (95% CI for difference: 0.67 – 1.64), $7.95 (\pm 0.07)$ and $6.78 (\pm 1.6)$ at 3 DIM (95% CI for difference: 0.69 – 1.66), and $7.97 (\pm 0.05)$ and $7.64 (\pm 0.2)$ at 4 DIM (95% CI for difference: -0.80 – 0.20), where the interaction between study group and study day had a *P*-value of 0.001. The covariance structure with the lowest Akaike information criterion was first-order autoregressive, which was used in the final model. Baseline urine pH was excluded from the model (*P* = 0.46).

The change in blood ionized calcium for the 2 groups is illustrated in Figure 2. Mean blood ionized calcium on day of calving was $1.05 (\pm 0.02)$ for controls and $1.09 (\pm 0.03)$ for treatment cows (95% CI for difference: -0.03 – 0.11). At 2 DIM, ionized calcium concentrations were $1.12 (\pm 0.02)$ for controls and $1.17 (\pm 0.02)$ for treatment cows (95% CI for difference: -0.01 – 0.11). Finally, at 4 DIM, ionized calcium was $1.17 (\pm 0.02)$ for controls and $1.18 (\pm 0.02)$ for treatment cows (95% CI for difference: -0.05 – 0.06). Ionized calcium concentrations increased over time. Blood ionized calcium concentrations were different from baseline (1.07 ± 0.02) in both treatment and control cows at 2 DIM (1.14 ± 0.01 , 95% CI for difference: 0.03 – -0.12) and 4 DIM ($1.17 \pm$

Figure 2. Mean ionized blood calcium concentration in mmol/L ($\pm 95\%$ CI) in 27 multiparous Holstein dairy cows on day of calving and during the first 4 d postpartum. The treatment group ($n = 13$) was treated with 480 g of MgCl_2 hexahydrate as an oral drench for the first 3 d (0, 1, and 2 DIM), whereas cows in the control group ($n = 14$) received no treatment.

0.01, 95% CI for difference: 0.06–0.15), but not between treatment groups (95% CI for difference between groups for all measurements: -0.01 – 0.08), and the slopes for the change in ionized calcium concentrations over time between study groups were not statistically different ($P = 0.16$). A model that included baseline calcium concentrations did not converge. Lactation number ($P = 0.02$) was included in the final model to adjust for its effect. The unstructured covariance structure for the repeated measures analysis had the lowest Akaike information criterion and was used in the final model. At 0 DIM, 2 cows were subclinically hypocalcemic (ionized calcium concentration < 1 mmol/L) in the treatment group compared with 3 in the control group. At 2 DIM the control group had 1 subclinically hypocalcemic cow. All other ionized calcium concentrations measured were ≥ 1 mmol/L, and a Fisher's exact test showed no association between group and subclinical hypocalcemia ($P = 0.52$).

DISCUSSION

Cows in our study responded to treatment with oral MgCl_2 hexahydrate with a lowered urine pH during the treatment period (0–2 DIM). The urine pH of cows in the treatment group quickly returned to the urine pH level of control cows by 4 DIM, after acidification with MgCl_2 hexahydrate had been discontinued. No difference in blood ionized calcium was associated with this difference in urine pH, indicating that acidifying cows in the early postpartum period may not contribute to a reduction in early postpartum subclinical hypo-

calcemia, although a modified study design, in which continued acidification is achieved through diet, may lead to different results. The blood ionized calcium level tended to be higher in treatment cows at 2 DIM (95% CI for difference: -0.01 – 0.11), but the absolute difference of 0.04 mmol/L blood ionized calcium between groups may not be clinically relevant. Clinically relevant differences in blood ionized calcium may be an order of magnitude larger, as demonstrated in a study that found differences between normocalcemic cows and cows with experimentally induced subclinical hypocalcemia with respect to appetite, metabolism, and immune function, where mean blood ionized calcium differed by 0.49 mmol/L (Martinez et al., 2014).

Acidogenic diets have been thought to be less palatable than those without addition of acidogenic salts and lead to depressed DMI (Oetzel et al., 1988). We expected that the delivery of MgCl_2 hexahydrate via oral drench once daily could circumvent the problem of lowered palatability and subsequent lowered DMI. However, a recent study showed that the decrease in DMI in diets with negative DCAD is attributable to metabolic acidosis and not to the addition of acidogenic salts containing chloride (Zimpel et al., 2018). Cows in our study that received the treatment may have had lower DMI than those in the control group because of their acid/base status, which could have negated any advantages in calcium mobilization brought about through lower blood pH. The DCAD achieved in our study was -2.4 mEq/100 g of DM, compared with the -5.0 to 15.0 mEq/100 g of DM recommended for prepartum cows by the National Research Council (NRC 2001). Calculation of DCAD was based on an assumed feed intake of 19.3 kg for the early-lactation cow diet fed in this herd. Actual intake on the day of calving may have been lower than on subsequent days, and, hence, DCAD for the first treatment was likely more negative than we calculated.

Because magnesium can act as a cathartic in cattle, we wanted to limit the amount of MgCl_2 hexahydrate administered to cows to a level that did not cause diarrhea in study cows. Only one of the cows developed mild diarrhea during the trial. Larger doses of MgCl_2 hexahydrate may have led to a more negative DCAD but also could have caused gastrointestinal upset in more study cows, which could have interfered with calcium absorption from the diet. The ideal prepartum DCAD for optimal performance is unknown (Zimpel et al., 2018), but the DCAD achieved in this experiment may have been inadequate in improving calcium mobilization at a clinically relevant level.

We did not observe a reduction in blood calcium concentration during the first 2 DIM in study cows as

has been seen elsewhere (Martinez et al., 2012), and few animals had blood ionized calcium concentrations <1.0 mmol/L during the study period. Because one of the criteria for enrollment was a urine pH of ≤ 6.8 , it is conceivable that cows in this study were able to meet their increased postpartum calcium demands because of prepartum acidification. A larger effect may be seen in cows that are less acidified at calving and are less able to mobilize calcium and therefore more prone to subclinical hypocalcemia. It is also possible that the blood calcium nadir in this herd was at 1 DIM and differences between groups were missed because we did not sample on that day. The cow that showed clinical signs of hypocalcemia and responded to treatment with intravenous calcium gluconate was in her fifth lactation and more prone to hypocalcemia than lower-lactation cows, as lactation number is one of the risk factors for clinical postpartum hypocalcemia (Goff, 2008). This cow's blood ionized calcium level was 1.16 mmol/L, which is within the normal range. The sample was taken on the same day but before the calcium gluconate infusion. We did not monitor blood magnesium concentrations, but hypermagnesemia can impair nerve conduction, which could have been an unintended side effect of the treatment.

Limitations of this study are the use of an oral drench to achieve negative DCAD versus addition of acidogenic salts to the diet, because for practical reasons dairy farmers would likely prefer adding acidogenic salts to a diet as opposed to the drenching of each cow multiple times. For logistical reasons, separating study cows from other animals in the herd during the immediate postpartum period was not feasible, and the present solution, although not practical on a large scale, was able to achieve acidification in the study cows. Further limitations are our inability to measure DMI in study animals, limited blood sampling time points, and no placebo treatment for control cows, again for logistical reasons. Additional handling stress may have negatively affected cows in the treatment group. Measuring DMI could have determined whether the lack of differences in blood ionized calcium concentrations was due to differences in DMI. Finally, the researcher conducting the field work was not blinded to treatment allocation in this study, which may have introduced some bias, although care was taken to avoid any differential treatment of study groups.

CONCLUSIONS

To our knowledge, this is the first study evaluating the effect of continued metabolic acidification on ionized blood calcium concentrations in Holstein cows in the first few days of lactation. Although no difference

was seen in blood ionized calcium concentrations between treatment and control groups, further research into the possible benefits of this technique in a more controlled environment or with additional sampling time points may be warranted.

ACKNOWLEDGMENTS

We thank the participating dairy for their support. Funding for this project was provided by the University of California Davis Veterinary Medical Teaching Hospital Resident Research Grant. Funding for H. Bang was partly supported by the National Institutes of Health through grant UL1 TR001860. The authors have not stated any conflicts of interest.

REFERENCES

- Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. Santos, and S. J. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. *J. Dairy Sci.* 94:4897–4903. <https://doi.org/10.3168/jds.2010-4075>.
- Edmonson, A. J., I. J. Lean, L. D. Weaver, T. B. Farver, and G. Webster. 1989. A body condition scoring chart for Holstein Dairy Cows. *J. Dairy Sci.* 72:68–78. [https://doi.org/10.3168/jds.S0022-0302\(89\)79081-0](https://doi.org/10.3168/jds.S0022-0302(89)79081-0).
- Goff, J. P. 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. *Vet. J.* 176:50–57. <https://doi.org/10.1016/j.tvjl.2007.12.020>.
- Goff, J. P. 2014. Calcium and magnesium disorders. *Vet. Clin. North Am. Food Anim. Pract.* 30:359–381. <https://doi.org/10.1016/j.cvfa.2014.04.003>.
- Hansen, S. S., P. Norgaard, C. Pedersen, R. J. Jorgensen, L. S. Mellau, and J. D. Enemark. 2003. The effect of subclinical hypocalcaemia induced by Na₂EDTA on the feed intake and chewing activity of dairy cows. *Vet. Res. Commun.* 27:193–205. <https://doi.org/10.1023/A:1023340506782>.
- Lean, I. J., P. J. DeGaris, D. M. McNeil, and E. Block. 2006. Hypocalcemia in dairy cows: Meta-analysis and dietary cation anion difference theory revisited. *J. Dairy Sci.* 89:669–684. [https://doi.org/10.3168/jds.S0022-0302\(06\)72130-0](https://doi.org/10.3168/jds.S0022-0302(06)72130-0).
- Luebke, M. K., G. E. Erickson, T. J. Klopfenstein, M. A. Greenquist, and J. R. Benton. 2011. Effect of dietary cation-anion difference on urinary pH, feedlot performance, nitrogen mass balance, and manure pH in open feedlot pens. *J. Anim. Sci.* 89:489–500. <https://doi.org/10.2527/jas.2009-2458>.
- Martinez, N., C. A. Risco, F. S. Lima, R. S. Bisinotto, L. F. Greco, E. S. Ribeiro, F. Maunsell, K. Galvao, and J. E. Santos. 2012. Evaluation of periparturient calcium status, energetic profile, and neutrophil function in dairy cows at low or high risk of developing uterine disease. *J. Dairy Sci.* 95:7158–7172. <https://doi.org/10.3168/jds.2012-5812>.
- Martinez, N., L. D. Sinedino, R. S. Bisinotto, E. S. Ribeiro, G. C. Gomes, F. S. Lima, L. F. Greco, C. A. Risco, K. N. Galvao, D. Taylor-Rodriguez, J. P. Driver, W. W. Thatcher, and J. E. Santos. 2014. Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. *J. Dairy Sci.* 97:874–887. <https://doi.org/10.3168/jds.2013-7408>.
- Melendez, P., G. A. Donovan, C. A. Risco, and J. P. Goff. 2004. Plasma mineral and energy metabolite concentrations in dairy cows fed an anionic prepartum diet that did or did not have retained fetal membranes after parturition. *Am. J. Vet. Res.* 65:1071–1076. <https://doi.org/10.2460/ajvr.2004.65.1071>.
- Murray, R. D., J. E. Horsfield, W. D. McCormick, H. J. Williams, and D. Ward. 2008. Historical and current perspectives on the treat-

- ment, control and pathogenesis of milk fever in dairy cattle. *Vet. Rec.* 163:561–565. <https://doi.org/10.1136/vr.163.19.561>.
- Neves, R. C., B. M. Leno, K. D. Bach, and J. A. A. McArt. 2018. Epidemiology of subclinical hypocalcemia in early-lactation Holstein dairy cows: The temporal associations of plasma calcium concentration in the first 4 days in milk with disease and milk production. *J. Dairy Sci.* 101:9321–9331. <https://doi.org/10.3168/jds.2018-14587>.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. National Academy Press, Washington, DC.
- Oetzel, G. R., J. D. Olson, C. R. Curtis, and M. J. Fettman. 1988. Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows. *J. Dairy Sci.* 71:3302–3309. [https://doi.org/10.3168/jds.S0022-0302\(88\)79935-X](https://doi.org/10.3168/jds.S0022-0302(88)79935-X).
- Reinhardt, T. A., J. D. Lippolis, B. J. McCluskey, J. P. Goff, and R. L. Horst. 2011. Prevalence of subclinical hypocalcemia in dairy herds. *Vet. J.* 188:122–124. <https://doi.org/10.1016/j.tvjl.2010.03.025>.
- USDA NAHMS. 2016. *Dairy 2014: Dairy Cattle Management Practices in the United States, 2014*. USDA-APHIS-VS-CEAH-NAHMS, Fort Collins, CO.
- Zimpel, R., M. B. Poindexter, A. Vieira-Neto, E. Block, C. D. Nelson, C. R. Staples, W. W. Thatcher, and J. E. P. Santos. 2018. Effect of dietary cation-anion difference on acid-base status and dry matter intake in dry pregnant cows. *J. Dairy Sci.* 101:8461–8475. <https://doi.org/10.3168/jds.2018-14748>.

ORCIDS

- G. U. Maier <https://orcid.org/0000-0003-1894-2170>
 Bret McNabb <https://orcid.org/0000-0001-6373-1597>
 Richard Pereira <https://orcid.org/0000-0003-2028-8761>
 Heejung Bang <https://orcid.org/0000-0002-5755-9951>
 Sharif S. Aly <https://orcid.org/0000-0003-0330-5013>
 Heidi A. Rossow <https://orcid.org/0000-0002-3753-4263>