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Blood calcium dynamics after prophylactic treatment of subclinical hypocalcemia with oral or intravenous calcium

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ABSTRACT

Total serum Ca dynamics and urine pH levels were evaluated after prophylactic treatment of subclinical hypocalcemia after parturition in 33 multiparous Jersey × Holstein crossbreed cows. Cows were blocked according to their calcemic status at the time of treatment [normocalcemic (8.0–9.9 mg/dL; n = 15) or hypocalcemic (5.0–7.9 mg/dL; n = 18)] and randomly assigned to 1 of 3 treatments: control [no Ca supplementation (n = 11)]; intravenous Ca [Ca-IV (n = 11), 500 mL of 23% calcium gluconate (10.7 g of Ca and 17.5 g of boric acid as a solubilizing agent; Durvet, Blue Springs, MO)]; or oral Ca [Ca-Oral (n = 11), 1 oral bolus (Bovikalc bolus, Boehringer Ingelheim, St. Joseph, MO) containing $CaCl_2$ and $CaSO_4$ (43 g of Ca) 2 times 12 h apart]. Total serum Ca levels were evaluated at 0, 1, 2, 4, 8, 12, 16, 20, 24, 36, and 48 h, and urine pH was evaluated at 0, 1, 12, 24, 36, and 48 h after treatment initiation. Total serum Ca levels were higher for Ca-IV than for control and Ca-Oral cows at 1, 2, and 4 h after treatment initiation, but lower than Ca-Oral cows at 20, 24, and 36 h and lower than control cows at 36 and 48 h. At 1 h after treatment initiation, when serum Ca levels for Ca-IV cows peaked (11.4 mg/dL), a greater proportion of Ca-IV (n = 8) cows had total serum Ca levels >10 mg/dL than control (n = 0) and Ca-Oral (n = 1) cows. At 24 h after treatment initiation, when Ca-IV cows reached the total serum Ca nadir (6.4 mg/dL), a greater proportion of Ca-IV (n = 10) cows had serum Ca levels <8 mg/dL than control (n = 5) and Ca-Oral (n = 2) cows. Treatment, time, and treatment × time interaction were significant for urine pH. Mean urine pH was lower for Ca-Oral cows (6.69) than for control (7.52) and Ca-IV (7.19) cows. Urine pH levels at 1 h after treatment were lower for Ca-IV cows compared with both control and Ca-Oral cows, a finding likely associated with the iatrogenic administration of boric acid added as a solubilizing agent of the intravenous Ca solution used. At 12, 24, and 36 h, urine pH levels were lower for Ca-Oral cows compared with both control and Ca-IV cows. This was expected because the oral Ca supplementation used (Bovikalc) is designed as an acidifying agent. Wide fluctuations in blood Ca were observed after prophylactic intravenous Ca supplementation. The implications for milk production and animal health, if any, of these transient changes in total serum Ca have yet to be evaluated.
Key words: hypocalcemia, hypercalcemia, calcium homeostasis

INTRODUCTION

Hypocalcemia is a common metabolic disease of dairy cows after parturition. In the United States, the incidence of clinical hypocalcemia has been reported to be 5%, whereas subclinical hypocalcemia can be as high as 54% in multiparous cows (Reinhardt et al., 2011). Hypocalcemia has important consequences because Ca is essential for skeletal and smooth muscle contraction as well as for immune function (Kimura et al., 2006). Clinical hypocalcemia has been associated with dystocia, uterine prolapse, retained placenta, endometritis, decreased fertility, mastitis, and decreased rumen and abomasum motility (Curtis et al., 1983; Borsberry and Dobson, 1989; Goff et al., 2004). Similarly, cows with subclinical hypocalcemia are at a greater risk for metritis (Martinez et al., 2012), displaced abomasum (Chapinal et al., 2011), and culling (Roberts et al., 2012), and show an impaired hepatic lipid metabolism (Chamberlin et al., 2013).

To prevent clinical and subclinical hypocalcemia, some dairy herds may benefit from implementing prophylactic strategies. One approach is to feed anionic salts prepartum. This strategy has been reported to significantly reduce the incidence of clinical hypocalcemia (Charbonneau et al., 2006). However, feeding anionic salts has decreased the incidence of subclinical hypo-

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calcemia in some studies (Oetzel et al., 1988; Moore et al., 2000), but failed to do so in others (Ramos-Nieves et al., 2009). A different strategy is the prophylactic treatment of hypocalcemia with oral or intravenous Ca supplementation immediately after calving. The benefits of supplementing Ca to cows fed a low-DCAD diet require further investigation. Melendez et al. (2002) failed to find any effect on plasma Ca, P, Mg, NEFA, BHBA, or glucose when cows fed anionic salts prepartum were supplemented immediately after calving with various sources of Ca either orally (Ca chloride, Ca propionate, and Ca propylene glycol) or intravenously (Ca gluconate). However, sampling frequency (2, 3, 6, 9, and 12 d after calving) in that study was inadequate to evaluate the short-term effects of Ca supplementation on plasma constituents. However, in a different study, lame cows and high-producing multiparous cows benefited from oral Ca supplementation even when low-DCAD diets were fed (Oetzel and Miller, 2012).

Calcium hemodynamics after prophylactic Ca supplementation may vary with route of administration. Intravenous Ca treatment of clinical hypocalcemia results in a rapid increase of serum Ca, reaching concentrations that could be toxic. Results from an uncontrolled study indicated that hypercalcemia is followed by a steep decline that leads to a transient hypocalcemic stage (Braun et al., 2009).

The effect of administering Ca as a prophylactic treatment after parturition on Ca hemodynamics is unknown. Thus, the objective of this study was to evaluate total serum Ca dynamics in dairy cows after prophylactic treatment of hypocalcemia with oral and intravenous sources of Ca.

MATERIALS AND METHODS

All procedures were approved by The Institutional Animal Care and Use Committee (IACUC) at the University of California Davis, School of Veterinary Medicine.

Cows and Herd Management

The study was conducted on a commercial freestall dairy farm in California with 8,000 Jersey and Jersey-cross lactating cows with an average milk yield per day of 27 kg. Thirty-three multiparous cows in their third or greater lactations were enrolled. The study was conducted in February 2013 over an 8-d period.

During the last 3 wk of gestation, cows were housed in a dry-lot facility until they showed primary signs of calving. Close-up cow diets were offered ad libitum, once a day, a TMR with anionic salts included in the mineral mix (Table 1). Then, cows were moved to a covered loafing maternity pen. Immediately after parturition, calves were separated from dams. Within 4 h after parturition, cows were moved to an open dry-lot pen for 2 to 3 d until milk was clear of dry-cow treatment antibiotic residues. Postpartum cows were milked twice a day and fed a TMR once a day (Table 1). At the study onset, feedbunk TMR samples of close-up and fresh cow diets were collected for wet chemistry analysis (Table 1; Dairyland Laboratories, St. Cloud, MN).

Experimental Design

Treatments were arranged in a randomized block design. Immediately after calving (1 to 3 h postcalving), total serum Ca levels were determined by using a portable blood analyzer (Vetscan 200-1000R, Abaxis Veterinary Diagnostics, Union City, CA) and used to block cows as normocalcemic (8.0–9.9 mg/dL; n = 15) or hypocalcemic (5.0–7.9 mg/dL; n = 18). Within each

Table 1. Ingredients and nutrient composition of close-up and fresh cow rations

Item	Close-up	Fresh
Ingredient (% of DM)		
Alfalfa hay	25.2	19.6
Almond hulls	—	7.6
Canola meal	9.2	8.8
Close-up mineral[1]	5.5	—
Corn gluten feed	—	9.6
Corn silage	15.1	10.0
Lactating cow mineral[1]	—	2.4
Prequel Ω3 bypass[2]	1.0	—
Rolled corn	17.4	18.0
Water	0.4	—
Wet distillers grain	7.4	—
Wheat silage	14.7	12.1
Wheat straw	4.1	—
Whey	—	3.8
Whole cottonseed	—	8.2
Nutrient composition[3] (DM basis)		
CP (%)	15.3	18.0
ADF (%)	19.9	19.3
NDF (%)	30.0	30.2
Lignin (%)	3.5	4.0
Starch (%)	23.2	21.3
Ether extract (%)	3.4	5.6
Ash (%)	10.1	9.2
Ca (%)	1.97	1.13
P (%)	0.45	0.5
Mg (%)	0.52	0.37
K (%)	1.57	1.46
S (%)	0.52	0.32
Na (%)	0.46	0.52
Cl (%)	1.6	0.75
DCAD[4] (mEq/100 g)	−17.40	18.84

[1]Nutrius LLC (Kingsburg, CA).

[2]Virtus Nutrition LLC (Corcoran, CA).

[3]Wet chemistry analysis (Dairyland Laboratories, St Cloud, MN).

[4]DCAD calculations were performed according to the following equation: DCAD (mEq/100 g) = [(Na + K) − (Cl + S)].

block, cows were randomly assigned to 1 of 3 treatments: control (n = 11), intravenous Ca (**Ca-IV**, n = 11), or oral Ca (**Ca-Oral**, n = 11). Initial treatment was applied within 1 to 5 h after calving. Cows in control group received no Ca supplementation. Cows assigned to the Ca-IV treatment received 500 mL of 23% Ca gluconate (10.7 g of Ca and 17.5 g of boric acid as a solubilizing agent; Durvet, Blue Springs, MO) in the right subcutaneous abdominal vein administered slowly over 15 min. During intravenous Ca administration, cows were observed for irregular breathing and muscle spasm. Cows assigned to Ca-Oral received an oral bolus (Bovikalc bolus, Boehringer Ingelheim, St. Joseph, MO) containing $CaCl_2$ and $CaSO_4$ (43 g of Ca) 2 times 12 h apart.

Description of all cows included in the study is presented in Table 2. No twinning occurred and no calving assistance was required for cows enrolled in the study. Two Ca-IV cows were initially enrolled in the study but had to be replaced. One cow had severe mastitis 24 h after calving, and another cow was mistakenly given a Ca bolus 12 h after intravenous Ca treatment. Data from these cows were not included in the final data set.

Sample Collection and Laboratory Analysis

Blood samples (7 mL) were collected from the coccygeal vein using an evacuated tube without anticoagulant (Vacutainer, Becton Dickinson Co., Franklin Lakes, NJ) and allowed to clot for 20 min. Samples were centrifuged at 1,400 × *g* for 15 min (Premiere centrifuge model xc-2000, Max RCF 1790g; C and A Scientific, Manassa, VA). Blood was collected at 0, 1, 2, 4, 8, 12, 16, 20, 24, 36, and 48 h relative to treatment application. Serum was separated and stored frozen at −20°C until analysis. Upon completion of the trial, serum samples were sent overnight on dry ice for analysis (Marshfield Lab, Marshfield, WI) of total Ca using the enzymatic rate/automated chemistry analyzer method (arsenazo III; Michaylova and Ilkova, 1971) with a Beckman Coulter analyzer (Beckman Coulter Inc., Brea, CA).

Urine was sampled by midstream catch at 0, 1, 12, 24, 36, and 48 h after treatment application. Urine pH was measured on-farm with Oakton pH Testr 20 (Oakton Instruments, Vernon Hills, IL), calibrated once a day with pH 4.0, 7.0 and 10.0 standard solutions.

Statistical Analyses

The general linear models procedure of SAS (PROC GLM; SAS Institute Inc., Cary, NC) was used to evaluate whether pretreatment initiation lactation number, DIM at dry-off, days in close-up, 305-d mature-equivalent milk yield, total serum Ca levels, and urine pH were similar across treatment groups. Standard errors were obtained with the STDERR option of the LSMEANS statement. General linear mixed models with repeated measures (Littell et al., 1998) were used for the analyses of blood Ca and urine pH using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) using Kenward-Rogers adjustment for calculation of denominator degrees of freedom. The covariance structure for repeated measurement was chosen based on the Akaike's information criterion (Littell et al., 1998). Total serum Ca and urine pH levels at 0 h relative to treatment application, respectively, were offered as a covariate for each of the models. The model included the effects of treatment, time, and treatment × time interaction. Block and cow were included as random terms and used as the error term to test for the effects of treatments. Variables were declared significant at $P < 0.05$. Significant interactions of treatment × time were explored using the SLICE option of the LSMEANS statement of the MIXED procedure of SAS. Comparisons across treatments at each significant time point were conducted with the PDIFF option of the LSMEANS statement. Proportion of cows with serum Ca >10 mg/dL at 1 h after treatment initiation and <8 mg/dL at 24 h was evaluated using the Fisher exact test with the FREQ procedure of SAS.

Table 2. Mean values of parameters describing cows before treatment initiation

	Treatment[1]				
Item	Control	Ca-IV	Ca-Oral	SEM	*P*-value
Lactation number	3.40	3.60	3.60	0.99	0.89
DIM at dry-off	303.00	324.00	306.00	12.42	0.43
Days in close-up	18.67	21.51	22.68	1.59	0.29
305-d mature-equivalent milk yield (kg)	9,078.24	9,497.20	9,958.00	555.89	0.54
Total serum calcium (mg/dL)	7.16	7.50	7.29	0.30	0.72
Urine pH	6.59	6.81	6.51	0.20	0.47

[1]Control = no treatment; Ca-IV = 500 mL of 23% calcium gluconate (Durvet, Blue Springs, MO) at postpartum; Ca-Oral = BoviKalc bolus (Boehringer Ingelheim, St. Joseph, MO) twice 12 h apart at postpartum.

RESULTS AND DISCUSSION

Total Serum Calcium

To evaluate the effect of treatment on total serum Ca, data were adjusted by total serum Ca at 0 h relative to treatment. This covariate, although not significant ($P = 0.07$), was kept in the model. Time and treatment × time interaction were significant ($P < 0.001$; Figure 1) for total serum Ca. The significant time × treatment interaction reflects the increase in serum Ca concentrations for Ca-IV cows, peaking at 1 h after treatment (11.4 mg/dL), followed by a decline that reached its nadir at 24 h after treatment (6.4 mg/dL). Calcium-IV cows had higher serum Ca than control and Ca-Oral cows at 1, 2, and 4 h after treatment. However, serum Ca was lower for Ca-IV cows than for Ca-Oral cows at 20, 24 and 36 h and lower than for control cows at 36 and 48 h (Figure 1).

At 1 h after treatment initiation, a greater ($P < 0.001$) proportion of Ca-IV (n = 8) cows had serum Ca levels >10 mg/dL than control (n = 0) and Ca-Oral (n = 1) cows. Total serum Ca concentrations reached values up to 14.5 mg/dL in Ca-IV cows. However, the present study might have failed to capture the highest total serum Ca concentration for Ca-IV cows, which likely occurred within minutes after treatment application (Braun et al., 2009). At 24 h, when Ca-IV cows reached the total serum Ca nadir, a greater ($P = 0.03$) proportion of Ca-IV (n = 10) cows had serum Ca levels <8 mg/dL than control (n = 5) and Ca-Oral (n = 2) cows.

Similar findings were reported after treating clinical hypocalcemia (n = 30) with various intravenous sources of Ca (Braun et al., 2009). Serum Ca rapidly increased, peaking 10 min after treatment; however, from 5 to 24 h after treatment, cows were in a transient hypocalcemic stage (Braun et al., 2009). The short-lived hypercalcemia observed after intravenous Ca supplementation alters the ability of the animal to maintain Ca homeostasis. According to Goff (1999), hypercalcemia causes a decrease in blood parathyroid hormone levels and an increase in thyrocalcitonin release, which decreases renal and bone Ca reabsorption, decreases calcitrol conversion to 1,25-dihydroxyvitamin D, increases aciduria, and depletes blood of cations. Braun et al. (2009) observed a prompt suppression in parathyroid hormone

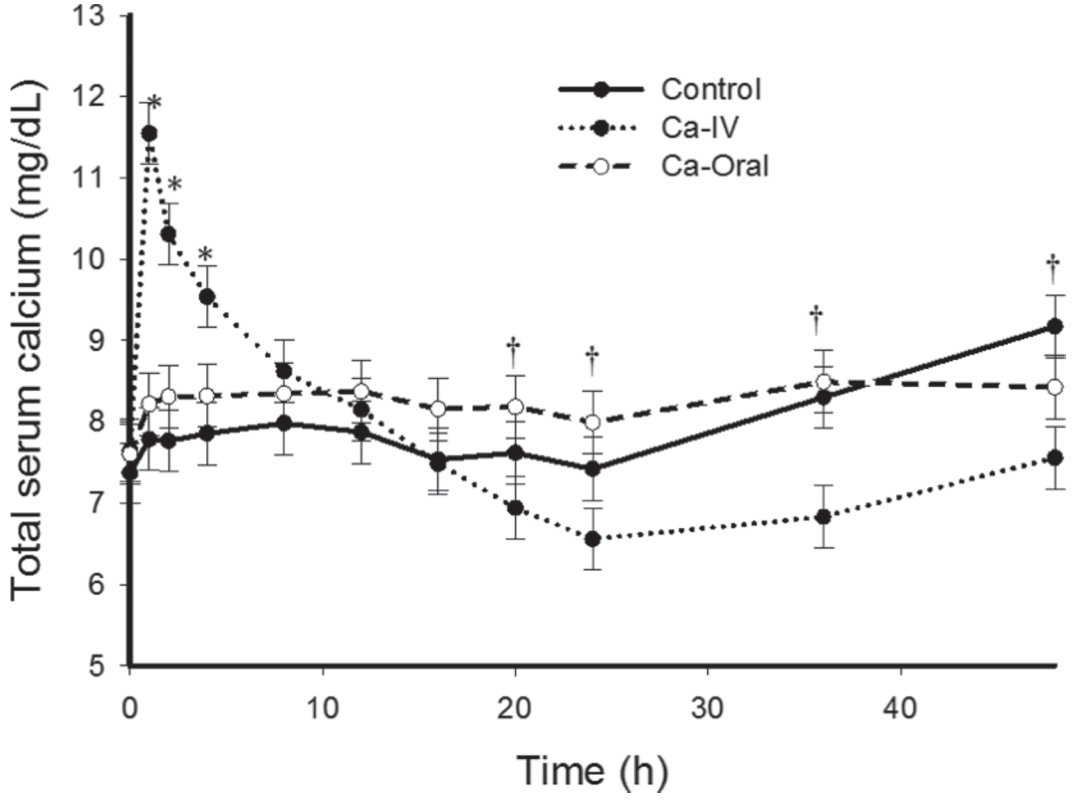

Figure 1. Total serum Ca levels (LSM ± SEM) after postpartum prophylactic treatment of subclinical hypocalcemia in control (no Ca supplementation; n = 11), intravenous Ca [Ca-IV, 500 mL of 23% calcium gluconate (Durvet, Blue Springs, MO) at postpartum; n = 11], and oral Ca [Ca-Oral, 2 Bovikalc (Boehringer Ingelheim, St. Joseph, MO) boluses 12 h apart at postpartum; n = 11)]. We detected a significant ($P < 0.001$) effect of time and treatment × time interaction. Significant treatment × time differences at each time point are indicated by † ($P < 0.05$) and * ($P < 0.001$).

levels after treatment, followed by a slow increase and a subsequent decrease. The negative consequences of longer term hypocalcemia and hypercalcemia are well documented (Goff, 1999, 2004; Kimura et al., 2006, Chapinal et al., 2011). Nonetheless, the effects of this short, transient disruption in Ca homeostasis at parturition after intravenous administration of Ca are not known and have yet to be studied.

Total serum Ca levels for Ca-Oral were consistently numerically above those of control cows from 1 to 24 h after treatment but no statistical differences were detected ($P = 0.22$; Figure 1). The average difference in serum calcium between Ca-Oral and control cows was 0.35 mg/dL. However, the current study had 80% power to declare significant differences above 0.60 mg/dL. Similarly, Melendez et al. (2002), with 30 cows per treatment group, found no significant effect of oral Ca bolus supplementation on total Ca levels at 2, 3, 6, 9, and 12 d after parturition. In contrast, Sampson et al. (2009), with 10 cows per treatment group, found that postpartum oral Ca bolus supplementation increased serum Ca levels 1 h after the administration of the second Ca bolus. This increase in Ca was significant ($P < 0.05$) for ionized Ca but was only a tendency ($P < 0.09$) for total Ca. In a recent large study including more than 800 cows, Oetzel and Miller (2012) evaluated the effects of oral Ca supplementation 8 to 35 h postcalving but detected no significant increase in ionized serum Ca concentration. Nevertheless, this study indicated that oral Ca bolus supplementation might benefit high-producing dams and lame cows, respectively, by increasing milk yield and reducing health events.

Urine pH

To evaluate the effect of treatment on urine pH, data were adjusted by urine pH at 0 h relative to treatment. This covariate was significant ($P = 0.03$) and therefore was kept in the model. Treatment, time, and treatment × time interaction were significant predictors of urine pH ($P < 0.001$; Figure 2). Mean urine pH was lower for cows treated with Ca-Oral (6.69) compared with both control (7.52) and Ca-IV (7.19) cows. We observed an effect of time and a treatment × time interaction ($P < 0.001$). Mean urine pH levels at 1 h were lower for Ca-IV compared with both control and Ca-Oral. Although a direct association was not established, the aciduria observed for Ca-IV 1 h after treatment initiation might

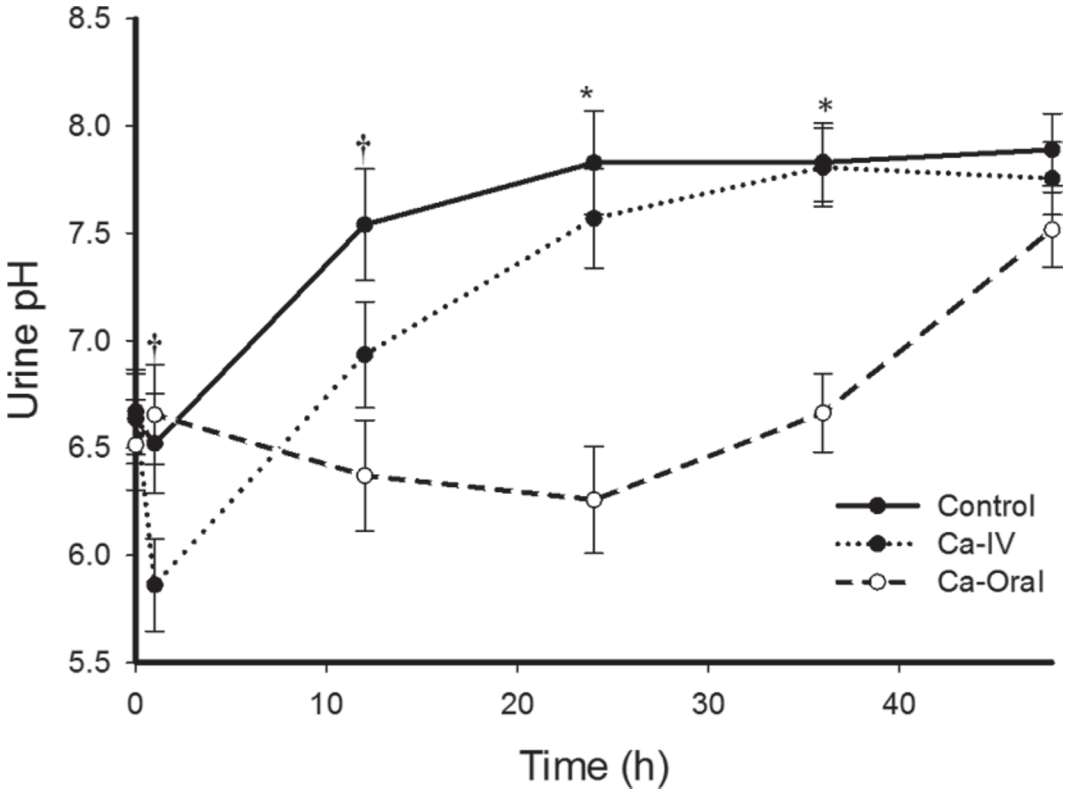

Figure 2. Urine pH levels (LSM ± SEM) after postpartum prophylactic treatment of subclinical hypocalcemia in dairy cows in control (no Ca supplementation; n = 11), intravenous Ca [Ca-IV, 500 mL of 23% calcium gluconate (Durvet, Blue Springs, MO) at postpartum; n = 11], and oral Ca [Ca-Oral, 2 Bovikalc (Boehringer Ingelheim, St. Joseph, MO) boluses 12 h apart at postpartum; n = 11)]. We detected significant ($P < 0.001$) effects of treatment, time, and treatment × time interaction. Significant treatment × time differences at each time point are indicated by † ($P < 0.05$) and * ($P < 0.001$).

be associated with the iatrogenic administration of 17.5 g of boric acid added as a solubilizing agent of the intravenous Ca solution used. The lower urine pH for Ca-Oral was expected because oral Ca boluses (e.g., Bovikalc) are designed as anionic acidifiers and sources of Ca in a buffered fat coating.

Urine pH levels at 12, 24, and 36 h after treatment were lower for Ca-Oral compared with both control and Ca-IV. Urine pH for Ca-Oral was $\leq$6.5 at 1, 12, and 24 h after treatment initiation. However, Sampson et al. (2009) reported that cows fed close-up diets with no anionic salts had alkaline urine pH at calving (7.6) and only 12 h after the administration of the second Ca bolus treatment did urine pH reach acidic levels (6.8).

Mean urine pH for cows in control group was 6.6 at 0 h. After removing cows from their dietary source of supplemental anions, urine pH returned to alkaline levels (7.5) at 12 h after treatment initiation. Initial urine pH values indicated that the DCAD program was partly successful, with only 14 out of 33 cows reaching pH levels <6.5 at time of treatment application.

CONCLUSIONS

Prophylactic treatment of subclinical hypocalcemia at parturition with intravenous Ca (Ca-IV) resulted in total serum Ca that reached hypercalcemic levels (peaking 1 h after treatment initiation) that later declined to hypocalcemic levels (nadir at 24 h). Total serum Ca was significantly higher for Ca-IV cows at 1, 2, and 4 h after treatment than for control and Ca-Oral cows. However, total serum Ca was lower for Ca-IV cows than for Ca-Oral cows at 20, 24, and 36 h, and was lower than for control cows at 36 and 48 h. Further research is needed to evaluate the implications, for health and production, of this transient change in Ca homeostasis in Ca-IV cows.

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