Effects of Calcium Chloride and Calcium Sulfate in an Oral Bolus Given as a Supplement to Postpartum Dairy Cows*

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CLINICAL RELEVANCE

An oral calcium bolus (Bovikalc, Boehringer Ingelheim Vetmedica) supplying calcium to dairy cows in the form of calcium chloride and calcium sulfate was evaluated to determine the effect on calcium homeostasis immediately after calving. Cows in the treatment group received one bolus immediately after calving and a second bolus 12 hours later. Control cows received no calcium supplementation. Blood was analyzed for ionized calcium, and urine was collected for urinary pH determination. Postpartum supplementation with the Bovikalc bolus significantly increased serum ionized calcium levels and decreased urine pH values.

■ INTRODUCTION

The onset of lactation results in a sudden and considerable demand for calcium and imposes arduous physiologic challenges to calcium homeostasis in dairy cows. Cows that are unable to adapt to this change in calcium demand develop hypocalcemia. The lower threshold for serum ionized calcium (iCa) in a healthy, normocalcemic cow is approximately 1 mmol/L.[1] Depending on its severity, hypocalcemia may manifest as a clinical condition called *milk fever*. Clinical milk fever occurs in 5% to 10% of dairy cows, with incidences of subclinical milk fever (hypocalcemia) ranging from 23% to 39%.[2] It is well documented that milk fever increases the risk for numerous peri-

parturient disorders such as dystocia, retained placenta, displaced abomasum, mastitis, and ketosis.[3,4] In addition, hypocalcemia has been linked to impaired immune function.[5] With this in mind, the need for management strategies to combat or reduce the incidence of hypocalcemia is obvious.

Several strategies have been studied to enhance the ability of periparturient dairy cows to maintain calcium homeostasis. These include limiting total calcium intake prepartum, modifying the dietary cation–anion difference (DCAD) by feeding anionic salts prepartum,

placed abomasum. Calcium chloride gels are acidogenic and provide a highly bioavailable calcium source, but their viscosity can make them difficult to administer and cause dosage errors.

Bovikalc (Boehringer Ingelheim Vetmedica), a commercially available oral bolus, is a dietary supplement containing calcium chloride dihydrate and calcium sulfate hemihydrate. The fat-encapsulated bolus was designed to facilitate administration and reduce the risk of aspirational pneumonia. The label directions indicate cows should receive one bolus

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treating with an IV or SC calcium infusion immediately after calving, and giving an oral calcium supplement.[6]

The DCAD feeding regimen has three potential drawbacks: reduced palatability leading to decreased feed intake, increased labor needed to monitor urine pH, and exclusion of springing heifers in close-up cow groups because feeding anionic salts to primiparous animals is not recommended.[2,7,8] Postpartum oral calcium supplementation has been shown to be effective in raising plasma calcium concentrations.[9] However, drenching cows with a liquid calcium supplement at calving involves a risk of aspiration pneumonia or death if the tube is incorrectly placed in the cow's airway, and bolus administration may cause pharyngeal damage if given incorrectly. Oetzel[10] reported that treatment with calcium chloride gels significantly decreased the incidence of milk fever, parturient hypocalcemia, and dis-

immediately after calving and another 12 hours later.[11] The objective of this study was to investigate the effects of Bovikalc on calcium homeostasis and blood and urine pH during the first 24 hours after calving.

■ **MATERIALS AND METHODS**
Treatment Groups

This project was conducted under a protocol approved by the University of Missouri Institutional Animal Care and Use Committee. Twenty multiparous Holstein cows ($n = 10$/treatment) were housed in a free-stall barn with sand bedding, allowed free access to clipped exercise pasture, and fed a total mixed ration formulated to meet National Research Council requirements (Table 1). Anionic salts were not included in the dry cow diet. The dry cow diet provided 15.88% crude protein, 0.76 Mcal/lb dry matter, and a DCAD of +26.26 mEq/100 g (Table 1). DCAD values of diets

on day 1 after calving were evaluated based on suppressed dry matter intake (DMI) due to calving (Table 2). DMI was estimated for day 1 postpartum using the following equation, in which *FCM* = 4% fat-corrected milk (kg/day), *BW* = body weight in kilograms, and *WOL* = week of lactation and with the term $1-e^{[-0.192 \times (WOL + 3.67)]}$ correcting for depressed intake in early lactation[12]:

$$\frac{DMI}{(kg/day)} = (0.372 \times FCM + 0.0968 \times BW^{0.75}) \times \{1-e^{[-0.192 \times (WOL + 3.67)]}\}$$

In an effort to include cows that were either hypocalcemic or approaching hypocalcemia, only cows with an iCa level of ≤1.10 mmol/L at calving (hour 0) were eligible for enrollment in the study. Eligible cows in their second or higher lactation were classified by calving date and parity and randomly assigned to one of two treatment groups. Control cows received no calcium supplementation, and treated calves received one bolus shortly after calving and a second bolus 12 hours later.

Prepartum Ionized Serum Calcium

Prepartum blood samples were collected via venipuncture of the coccygeal vein into a sodium heparin Vacutainer tube at approximately 48 and 24 hours before calving. iCa was measured on site, directly after collection, using an IDEXX VetStat Analyzer.

Postpartum Sample and Data Collection

Blood samples were collected at 0, 1, 6, 12, 13, and 24 hours postpartum. Blood was collected via venipuncture of the coccygeal vein into two separate Vacutainers. One sample was collected into a 10-ml sodium heparin Vacutainer and analyzed on site for ionized calcium and pH levels. Blood pH was determined using an electronic pH meter (Acorn pH 5 Meter, Oakton Instruments, Vernon Hills, IL).

TABLE 1. Diet Ingredients and Nutrient Analysis of Total Mixed Ration Offered to Prepartum Cows Ad Libitum

Ingredient	Amount Fed (% Dry Matter)
Brome hay	22.3
Soybean meal (48%)	10.3
Soy hulls	20.9
Corn silage	31.2
Roasted soybeans	3.1
Ground corn	11.1
Vitamin/mineral premix*	1.1

Nutrient	Dry-Matter Basis (%)
Dry matter	59.3
Neutral detergent fiber	47.39
Acid detergent fiber	31.14
Crude protein	15.88
Crude fat	3.48
DCAD (mEq/100 g)	+26.26

*Vitamin/mineral premix included vitamins A, D, and E, salt, biotin, choline, rumensin, iron, manganese, zinc, copper, cobalt, iodine, and selenium.
DCAD = dietary cation–anion difference $[(Na^+ + K^+) - (Cl^- - S^{-2})]$.[12]

Blood iCa values were "normalized" to a pH of 7.4.[13,14] The following equation was used for normalization, in which *X* = the measured pH of the sample, *[Ca++]X* = the iCa concentration in the sample at the measured pH, and *[Ca++]7.4* = the normalized concentration of ionized calcium at pH 7.4:

$$\log[Ca^{++}]7.4 = \log [Ca^{++}]X - 0.24(7.4 - X)$$

This equation assumes a normal total protein concentration and may be used for measured values between pH 7.2 and 7.6. All samples outside this range were excluded from analysis for normalized calcium (NCa).

The second blood sample was collected into serum separator tubes and centrifuged on site to achieve clean separation of serum and blood. Serum was then transferred via pipette

TABLE 2. Estimated Dry-Matter Intake of Total Mixed Ration Offered to Postpartum Cows Ad Libitum on Day 1 Postpartum

| | ESTIMATED DRY MATTER INTAKE (kg) | | |
| | | Day 1 Postpartum | |
INGREDIENT	Lactation Diet	Bolus Group	Control Group
Corn silage	5.5	3.1	3.1
Alfalfa silage	2.8	1.6	1.6
Alfalfa hay	3.2	1.8	1.8
Corn, cracked	6.8	3.8	3.8
Soybean, whole roasted	1.0	0.6	0.6
Soybean meal (48%)	0.5	0.3	0.3
Cottonseed, whole with lint	2.8	1.6	1.6
Brewer's grain, wet	1.0	0.5	0.5
Brome hay	0.8	0.4	0.4
Soy hulls	1.6	0.9	0.9
Vitamin/mineral premix*	0.9	0.4	0.4
Oral Ca^{++} bolus[†]	0	0.4	0
Total estimated dry-matter intake (kg)	26.9	15.4	15.0
DCAD (mEq/100 g)	+26.3	−26.6	+16.2

*Vitamin/mineral premix included vitamins A, D, and E, salt, dicalcium phosphate, limestone, yeast, potassium, magnesium sulfate, biotin, choline, rumensin, iron, manganese, zinc, copper, cobalt, iodine, and selenium.
[†]Bovikalc; inclusion rate represents two doses of a 190-g bolus; DCAD (per bolus) = −862.5 mEq/100 g.
DCAD = dietary cation–anion difference $[(Na^+ + K^+) − (Cl^- − S^{-2})]$.[12]

into sterile storage vials and stored at −20°C until the conclusion of the trial. At that time, serum was analyzed to determine total blood calcium (TCa) values using atomic absorption spectrometry (S Series AA Spectrometer, Thermo Electron Corporation, Beverly, MA).

Cows were manually stimulated to urinate, and midstream samples were collected at 0, 1, 6, 12, 13, and 24 hours postpartum. Urine pH was measured on site using the same electronic pH meter as for blood pH. Urine samples were stored at −20°C until the conclusion of the trial. At that time, all urine samples were analyzed to determine urinary calcium values using the same atomic absorption spectrometry as for TCa.

Rectal temperatures were measured with a digital thermometer (Fast Read series, Wal-

greens), and clinical scores for appetite, muscle tremors, ataxia, and fecal consistency were recorded at 0, 6, 12, and 24 hours postpartum according to the scoring system listed in the box on page 135. All personnel involved with clinical scoring and analysis of blood and urine samples were blinded to treatment group assignment.

Statistical Analysis

A correlation analysis was performed using the SAS PROC CORR (SAS Institute, Cary, NC) on blood and urinary calcium measurements, blood and urine pH, and clinical evaluations. The data were analyzed using a repeated measurement design as outlined by Littell and associates.[15] The SAS PROC MIXED contained the random effects of block and treat-

ment by block and the fixed effects of treatment, time, and treatment by time. The autoregressive [AR(1)] covariance structure type was used. Mean differences were determined using Fisher's least significant difference (LSD), which was produced by the least squares (LS) means statement. A P value of $\leq .05$ was considered significant. A statistical trend was defined as P values > .05 but $\leq .10$.

■ RESULTS

Blood Calcium

There was no difference in iCa values across groups over time before calving ($P = .72$). The mean iCa levels for bolus and control cows were 1.22 and 1.24 mmol/L (SE, 0.02) at 48 hours prepartum and 1.18 and 1.19 mmol/L (SE, 0.02) at 24 hours prepartum, respectively. At calving, mean iCa concentration for both bolus and control cows were below normocalcemic levels (0.94 and 0.95 mmol/L, respectively; SE, 0.05). Blood iCa was different between treatment groups over time (24 hours) after calving ($P = .02$; Figure 1). Blood iCa tended to be higher ($P <$ .10; SE, 0.06) for the bolus group at 1 hour postpartum and was significantly higher ($P < .02$; SE, 0.06) for the bolus group versus the control group at 13 hours.

The overall mean NCa level was not different between treatment groups ($P = .40$), with LS means of 1.06 (SE, 0.05) and 1.01 mmol/L (SE, 0.05) for bolus and control cows, respectively. However, NCa levels were different between treatment groups over time after calving ($P = .007$; Figure 2). Blood NCa values tended to be higher for the bolus group compared with the control group at 1 hour postpartum ($P < .10$).

TCa levels were not different across treatment groups ($P = .41$), with LS means of 2.19 and 2.12 mmol/L for the bolus and control treatment groups, respectively. Serum TCa values for bolus cows tended to be high-

Assignment of Clinical Scores

Appetite
1 = Normal
2 = Interested, but not eating
3 = No interest; not eating
4 = Gaunt and dehydrated

Muscle Tremors
1 = Normal
2 = Mild
3 = Moderate
4 = Severe

Ataxia
1 = Normal
2 = Mild
3 = Moderate
4 = Severe

Fecal Consistency
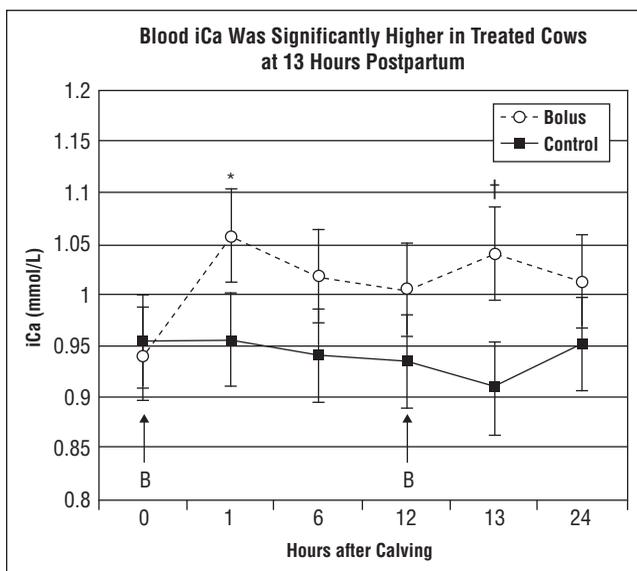1 = Watery
2 = Loose
3 = Normal
4 = Thick
5 = Firm

Figure 1. *Postpartum serum ionized calcium (iCa) across treatment groups over time (*P = .02*). (*B = *time of calcium bolus administration.)* *P < .10. †P < .05.*
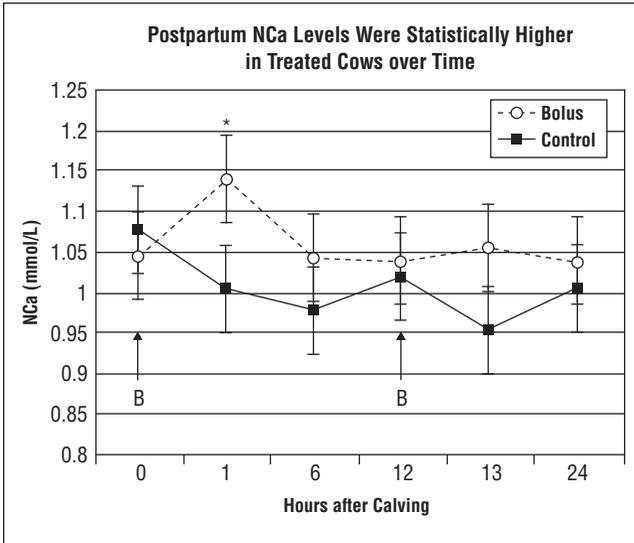
Figure 2. *Normalized calcium (NCa) values to pH 7.4 were statistically different owing to a treatment by time interaction (*P = .007*). (*B = indicates time of calcium bolus administration.) **P < .10.*
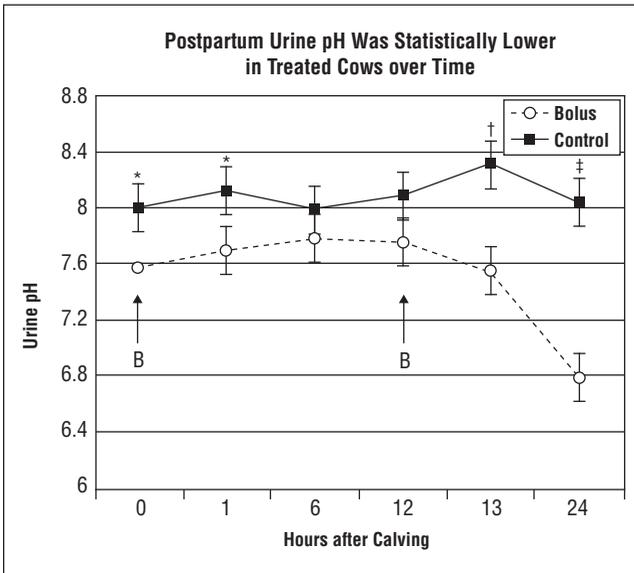

Figure 3. *Postpartum urine pH was statistically different owing to a treatment by time interaction (*P = .003*). (*B = time of calcium bolus administration.) **P < .10. †P < .05. ‡P < .001.*

er as a result of treatment over time (P = .09).

The Pearson correlation coefficients of the three different blood calcium levels (i.e., iCa, TCa, and NCa) were evaluated using the SAS PROC CORR. Serum TCa values were positively correlated to iCa and NCa, with r values of 0.697 and 0.656, respectively (P < .001). Blood NCa was positively correlated to iCa, with an r value of 0.881 (P < .001).

Blood pH, Urinary pH, and Urinary Calcium

The overall LS mean for postpartum blood pH did not differ across treatment groups (P = .27), with values of 7.48 (SE, 0.03) and 7.53 (SE, 0.03) for the bolus and control groups, respectively. Blood pH after calving also did not differ between treatment groups over time (P = .33).

Urine pH after calving was more acidic as a result of treatment (P = .0024; LS mean values of 7.58 [SE, 0.09] and 8.05 [SE, 0.09] for bolus and control groups, respectively). Postpartum urine pH was also different between treatment groups over time (P = .003; Figure 3). LS mean urine pH for the bolus group dropped from 7.58 (SE, 0.16) at calving to 6.79 (SE, 0.15) at 24 hours postpartum.

The overall mean for urinary calcium did not differ between treatment groups (P = .37), with LS means of 0.38 (SE, 0.11) and 0.20 (SE, 0.14) mmol/L for the

TABLE 3. Least Squares Means of Clinical Evaluations across Treatment Groups

Variable	Bolus	Control	SE	P Value
Appetite*	1.82	1.80	0.19	0.93
Ataxia*	1.15	1.03	0.11	0.44
Fecal consistency*	2.95	3.00	0.14	0.76
Muscle tremors*	1.10	1.10	0.08	1.00
Rectal temperature (˚C)	38.48	38.47	0.11	0.97

*See box on page 135 for parameters used to assign clinical scores.

bolus and control groups, respectively. Treatments did not affect urinary calcium over time ($P = .5$).

Clinical Evaluations

Clinical evaluations of appetite ($P = .93$), ataxia ($P = .44$), fecal consistency ($P = .76$), muscle tremors ($P = 1.00$), and rectal temperature ($P = .97$) were not different between treatment groups (Table 3). Rectal temperatures tended toward a difference between treatment groups over time. Rectal temperatures

Blood iCa levels were different between treatment groups over time after calving ($P = .02$; Figure 1). Bolus administration increased iCa levels when measured 1 hour after bolus administration. Bolus cows had an 8.2% increase in iCa levels after calving, with an average value of 1.03 mmol/L between 1 hour and 24 hours. Blood iCa values of control cows did not increase after calving, with an average value of 0.94 mmol/L between 1 hour and 24 hours. These data are similar to increases in serum calcium concentrations at 1 to 3 hours

> *Bolus cows had an 8.2% increase in iCa levels after calving, with an average value of 1.03 mmol/L between 1 hour and 24 hours.*

for bolus cows had very little variation between calving and 24 hours postpartum; however, rectal temperatures for control cows numerically decreased by 0.7˚C from calving to 24 hours postpartum ($P = .08$).

■ DISCUSSION

Houe[2] and others defined the lower limit for blood iCa in normocalcemic cows to be approximately 1.0 mmol/L. In the present study, blood iCa concentrations fell to hypocalcemic levels for bolus and control cows at calving (0.94 and 0.95 mmol/L, respectively; SE, 0.05).

and 1 to 2 days after calcium chloride gel administration as reported by Goff and Horst[9] and Oetzel,[10] respectively.

Serum calcium exists in three fractions: bound (40%), chelated (13%), and the remainder (47%), which is free or ionized.[16] iCa is the physiologically active form and provides a more valid assessment during times of abnormal calcium status, such as parturition. iCa levels are not widely reported in the literature because of the labor needed for sample collection and handling. Samples must be drawn anaerobically to minimize carbon dioxide loss,

which would elevate pH. Samples should also be transported on ice and analyzed within hours to reduce lactate production. As this process is not feasible in many settings, TCa is more commonly reported. Some investigators normalize iCa values to a pH of 7.4, which tends to improve correlation—especially when samples cannot be analyzed within 2 hours of sampling.[1,17] Extended exposure to the air decreases the carbon dioxide concentration of samples, thereby increasing sample pH. This increase in sample pH alters the equilibrium of iCa and protein-bound calcium, decreasing the iCa concentration.[1] In our study, normalization of iCa values to pH 7.4 did not cause correlations to increase. This lack of change in correlation can be attributed to proper anaerobic sample collection and prompt on-farm sample analysis.

have been associated with decreased feed intake and indicate excessive levels of anion salts in the diet.[2]

Earlier studies suggested that systemic acidification occurs in less than 36 hours after the addition of anionic salts to the diet.[19] Diets formulated for a DCAD of –10 to –20 mEq/100 g of feed on a dry-matter basis have been the most effective in curbing milk fever.[21] Increased body acidity is thought to affect the role of parathyroid hormone (PTH) in the cow. With systemic acidification, PTH sensitivity is increased in target tissues, increasing response to PTH and calcium retention in the animal and enhancing calcium mobilization from bone to blood.[22]

The bolus group received supplementation immediately and 12 hours after calving. The bolus contained calcium chloride and calcium

The administration of Bovikalc favorably altered calcium homeostasis and urine pH on day 1 postpartum.

Blood pH remained constant for both groups postpartum, reflecting tight homeostatic regulation.[18] However, urine pH dropped significantly for bolus cows, with a notable decrease in urine pH at 24 hours (6.79 and 8.04 for bolus and control groups, respectively; Figure 3). This large drop in urine pH for bolus cows reflects similar decreases in urine pH reported in numerous studies associated with feeding a negative DCAD diet prepartum.[8,19,20] Urine pH has often been used as a tool to monitor the effectiveness of anion additions in the close-up dry cow diet to prevent milk fever. Normal urine pH values of cows consuming a standard dry cow diet will be above 7.8. When a diet effectively decreases systemic pH, urine pH values will decrease to 6.2 to 6.8. Conversely, urine pH levels below 5.5

sulfate and changed the DCAD of the total dry matter consumed by treatment animals on day 1 postpartum (Table 2). It was estimated that DCAD values of the diets for day 1 after calving were –26.6 and +16.2 mEq/100 g for bolus and control cows, respectively. This estimated change in DCAD levels between treatment groups is supported by the significant change in urinary pH at 24 hours in the bolus group (Figure 3).

The urine pH values of the bolus group at 24 hours and the decrease in dietary DCAD values suggest that the Bovikalc bolus was effective in lowering the acid–base status of the bolus cows. This change in acid–base status could lead to restoration of PTH sensitivity in target tissues and greater mobilization of calcium, thus increasing free iCa levels.[19]

■ CONCLUSION

The administration of Bovikalc to supply calcium in the forms of calcium chloride and calcium sulfate favorably altered calcium homeostasis and urine pH on day 1 postpartum. Blood iCa levels rose above 1.00 mmol/L 1 hour after cows received the first bolus and remained at that level through 24 hours postpartum. This change corresponded to changes in urine pH. Alteration of urine pH was likely associated with the acidogenic nature of the bolus, effectively altering PTH sensitivity and increasing calcium mobilization and iCa levels compared with control cows.

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