Interpretative Summary: **Effect of oral calcium supplementation on early lactation health and milk yield in commercial dairy herds.** *By Oetzel and Miller, page 000.* Low blood calcium often occurs in older cows around calving and impairs subsequent health and milk yield. Second lactation or greater cows in 2 commercial dairies with effective milk fever prevention were divided into control (no oral calcium boluses) and supplemented groups (cows given 2 oral calcium boluses after calving). Lame cows supplemented with oral calcium had improved early lactation health, and cows with higher milk yield in the previous lactation had improved early lactation milk yield when given oral calcium boluses. Targeted groups of cows can benefit from oral calcium bolus supplementation, even in herds with very little hypocalcemia.

ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

Effect of Oral Calcium Bolus Supplementation on Early Lactation Health and Milk Yield in Commercial Dairy Herds

G. R. Oetzel,[*,1] and B. E. Miller[†]

[*]School of Veterinary Medicine, University of Wisconsin, Madison 53706

[†]Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO 64506

[1]Corresponding author: groetzel@wisc.edu

Garrett R. Oetzel, Department of Medical Sciences, School of Veterinary Medicine, 2015 Linden Drive, Madison, WI 53706; Voice: 608/265-5476; FAX: 608/265-8020

accepted for publication by the Journal of Dairy Science 7/24/12 and now in press

ABSTRACT

The objective of this study was to evaluate the effect of supplementation with oral calcium (Ca) boluses after calving on early lactation health and milk yield. Second lactation or greater cows (n = 927) from 2 large dairies in Wisconsin were enrolled during the summer of 2010. Both herds fed supplemental anions during the pre-fresh period and treated less than 1% of fresh cows for clinical milk fever. Cows were scored prior to calving for lameness and body condition, then randomly assigned to either control or oral Ca bolus supplemented groups. Control cows received no oral Ca boluses around calving. Cows in the oral Ca bolus group received 2 oral Ca boluses (Bovikalc, Boehringer Ingelheim, St. Joseph, MO) - one bolus 0 to 2 h after calving and the second 8 to 35 h after calving. The oral Ca bolus administration schedule allowed fresh cows to be restrained in headlocks only once daily. Whole blood samples were collected just before the second oral Ca bolus was given and were analyzed for ionized Ca (Ca^{2+}) concentration. Early lactation health events were recorded and summed for each cow. There were only 6 cases (0.6% of calvings) of clinical milk fever during the trial, and only 14% of cows tested were hypocalcemic (Ca^{2+} less than 1.0 mmol/L) at 8 to 35 h after calving. Mean Ca^{2+} concentrations were not different between the control and oral Ca bolus supplemented groups. Blood samples from the cows given oral Ca boluses were collected an average of 20.6 hours after administration of the first bolus. Subpopulations of cows with significant responses to oral Ca bolus supplementation were identified based on significant interactions between oral Ca bolus supplementation and covariates in mixed multiple regression models. Lame cows supplemented with oral Ca boluses averaged 0.34 fewer health events in the first 30 days in milk compared to lame cows that were not supplemented with oral Ca boluses. Cows with a higher previous lactation mature equivalent milk production (greater than 105% of herd rank) and supplemented with oral Ca boluses produced 2.9 kg more milk at their first test after calving

compared to cows with higher previous lactation milk yield who were not supplemented. Results of this study indicate that lame and higher producing cows respond favorably to supplementation with oral Ca boluses. Supplementing targeted subpopulations of cows with oral Ca boluses was beneficial even for dairies with a very low incidence of hypocalcemia.

Key Words: oral calcium chloride, oral calcium bolus, hypocalcemia, dairy cow

INTRODUCTION

Milk fever (parturient hypocalcemia) is an important metabolic disorder of dairy cattle around the time of calving. The mean incidence of clinical milk fever in published field studies was about 3.5% for North American and Australasian studies and about 6.2% for European studies (DeGaris and Lean, 2008). About 50% of second and greater lactation cows have blood Ca concentrations that fall below the threshold for subclinical hypocalcemia after calving (Reinhardt et al., 2011). Hypocalcemia may lead to reduced feed intake, poor rumen and intestine motility, increased risk for displaced abomasum, reduced milk yield, increased susceptibility to infectious diseases, and increased risk for early lactation removal from the herd (Curtis et al., 1983; Goff, 2008; Seifi et al., 2011). Mechanisms that may explain the detrimental effects of hypocalcemia include impaired energy balance, which is reflected in higher serum non-esterified fatty acid concentrations (Reinhardt et al., 2011) and direct impairment of immune cell responses to an activating stimulus (Kimura et al., 2006).

Identification of cows with subclinical hypocalcemia is impractical because these cows, by definition, do not display overt clinical signs. Thus, prevention is the only option for managing subclinical hypocalcemia. One prevention strategy is to supplement anions prior to calving. Charbonneau et al. (2006) conducted a large meta-analysis of previously published studies and determined that feeding a typical dose of anions prior to calving results in a 5-fold reduction in

the risk for clinical milk fever. Fewer studies have evaluated the impact of anion supplementation on subclinical hypocalcemia. Beede et al. (1992) reported that feeding anionic salts before calving in a large field study (n = 510 cows, all parities) reduced the subclinical hypocalcemia (defined as $Ca^{2+} \leq 1.00$ mmol/L on the day of calving) from 50% in the control cows to 19% in the cows receiving the anionic salts. Other, smaller studies have shown similar reductions in subclinical hypocalcemia when anions were supplemented (Oetzel et al., 1988) or smaller numerical reductions in the incidence of subclinical hypocalcemia that were not statistically significant (Goff and Horst, 1997; Ramos-Nieves et al., 2009).

Another approach to prevention of subclinical hypocalcemia is oral Ca supplementation around calving. Calcium chloride may be particularly beneficial as an oral supplement because it provides highly available oral Ca (Goff and Horst, 1993; 1994) and because it is a more potent acidifier than other anion sources (Goff et al., 2004; Gelfert et al., 2010). Systemic acidification has been associated with beneficial effects on Ca metabolism beyond the expected contribution of Ca absorbed from the GI tract. The underlying mechanism for the benefits of systemic acidification is the correction of metabolic alkalosis, which blunts the response of the cow to parathyroid hormone (Goff et al., 1991; Phillippo and Reid, 1994; Goff, 2008).

Thilsing-Hansen et al. (2002) summarized oral Ca supplementation trials and found oral Ca from a variety of formulations to be consistently beneficial. Using oral Ca chloride as the source of oral Ca has been shown to increase blood Ca concentrations, reduce the risk for clinical and subclinical hypocalcemia, and reduce the risk for displaced abomasum (Oetzel, 1996; Dhiman and Sasidharan, 1999). However, Ca chloride may be caustic to oral mucosa, and large, repeated doses could induce an uncompensated metabolic acidosis, especially if the cow is already being fed an acidogenic diet (Goff and Horst, 1993).

The effect of oral Ca supplementation in cows that received an acidogenic diet prior to calving has been minimally studied. Melendez et al. (2002) evaluated oral Ca chloride in a herd feeding anionic salts and found no effect of oral Ca supplementation on Ca^{2+} at 24 h post-calving. The authors concluded that oral Ca supplementation around calving may not be necessary when negative DCAD diets are fed. However, relatively few cows were enrolled in this study (30 controls and 30 cows supplemented with oral Ca chloride) and it was not possible to identify subpopulations of cows that may have responded well to the oral Ca chloride supplementation. Neither milk yield nor cow health was evaluated in this study.

Calcium chloride, in combination with Ca sulfate, has been formulated into a solid bolus coated with fat (Bovikalc, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO). One bolus provides 43 g of Ca (71% from Ca chloride and 29% from Ca sulfate). Compared to oral Ca gel formulations, the bolus has the advantages of protecting the cow from the sharp taste of the Ca chloride and eliminating the risk for aspiration pneumonia (Pehrson and Jonsson, 1991). In a two-part field study, Pehrson and Jonsson (1991) gave 4 total oral Ca boluses (1 before calving, one at calving, and 2 after calving) and reported that the boluses reduced the risk for clinical milk fever 4-fold compared to cows administered a placebo bolus. They concluded that the oral Ca boluses were at least as effective as a Ca chloride gel in preventing clinical milk fever and that the more sustained release of Ca from the bolus formulation could be responsible for its beneficial effects.

Sampson et al. (2009) supplemented multiparous cows (n = 20) with 2 oral Ca boluses after calving. They reported significantly increased Ca^{2+} concentrations 1 h after administration of the first oral Ca bolus (which was given at calving), and 1 h after administration of the second bolus (which was given 12 h after calving). Urinary pH was significantly reduced from about 8.0 to

111 6.8 in cows given the oral Ca boluses; the authors concluded that acidification likely contributed
112 to the ability of the boluses to support blood Ca^{2+} concentrations after calving. No larger scale
113 studies have been conducted with oral Ca boluses, and no studies have evaluated milk production
114 or cow health outcomes.

115 The objectives of this study were to conduct a large field study in commercial dairy herds
116 with effective anionic salts feeding programs already in place to 1) evaluate the effects of
117 supplementation with an oral Ca bolus containing Ca chloride and Ca sulfate on detailed
118 measures of early lactation health and milk yield and 2) determine if groups of cows could be
119 identified within these herds that have significantly different responses to oral Ca bolus
120 supplementation.

121 ## MATERIALS AND METHODS

122 ### *Study population*

123 The study was conducted on 2 large commercial dairy farms in Wisconsin during the summer
124 of 2010. To be selected for the study the herds had to meet the following criteria: milk at least
125 1,500 cows, have headlocks in the pre-fresh and post-fresh cow pens, utilize Dairy Comp 305
126 (Valley Agricultural Software, Tulare, CA) records for on-farm management, be willing to
127 administer the boluses according to the project protocol, and be willing to collect and properly
128 handle whole blood samples after calving. A description of the study herds has been previously
129 published (McArt et al., 2011). Herd A in the current study corresponds to Herd C in the
130 previous description, and Herd B in the current study corresponds to Herd D. A more detailed
131 description of typical diets fed to the herds during the trial is presented in Table 1. Herd A
132 contributed 327 cows (40% of total cows) to the study and Herd B contributed 555 cows (60% of
133 total cows). Both cooperating dairies signed a consent form agreeing to the project protocol and

were given a document containing information on disease definitions used in the trial. The study protocol was reviewed and approved by the University of Wisconsin-Madison School of Veterinary Medicine Animal Care and Use Committee (#V01479-0-05-10).

Cows were randomly assigned to either the control or oral Ca bolus groups prior to calving. A random numbers generator was used to determine the treatment assignment for the first cow enrolled; remaining treatment assignments were made sequentially (every other cow). Treatment assignments were indicated by color-coded neck chain tags placed on the cows.

At the same time the cows were assigned to treatment they were also evaluated for locomotion score and body condition score. Locomotion score was determined using the 1 to 4 point scoring system described by Nordlund et al. (2004), in which cows were categorized as non-lame (score 1), slightly lame, moderately lame, or severely lame (score 4). Post-fresh body condition score was determined using a 1 to 5 point scoring system with 0.25 unit increments as described by Ferguson et al. (1994), where a higher score represents greater body condition. Two trained evaluators assigned all of the pre-fresh scores, with one evaluator assigning each locomotion score and one evaluator assigning each body condition score. Scores were taken an average of 15.6 d (± 0.3 SEM) prior to calving.

Cows were enrolled in the study immediately after calving; pre-fresh data were collected from some cows that did not meet final criteria for enrollment. Cows in the control group received no oral Ca boluses after calving and cows in the oral Ca bolus group received 2 boluses. The first bolus was administered within 2 h after calving. The second bolus was given when the cow was next locked up in the post-fresh pen after calving and had to be administered between 8 to 35 h after calving. If the cow reached the post-fresh pen before 8 h post-calving she was given her second bolus the next day. The bolus administration schedule was chosen to fit within the

normal management activities of large dairies and required cows to be restrained in headlocks only once daily. The schedule was also chosen to include the time period cows are expected to be experiencing the most profound hypocalcemia, which is about 12 to 24 h post-calving (Goff, 2008; Ramos-Nieves et al., 2009). Mean time between calving and administration of the second bolus was 20.6 h (± 0.2 SEM).

There were 1780 calvings during the study period; 1,127 of these involved second or greater lactation cows. To be eligible for the trial, multiparous cows needed valid on-farm record information, could not start the lactation with an abortion (defined as gestation length < 260 d), could not have a calving ease score of 5 (indicative of a C-section or fetotomy), had to remain in the herd until at least 2 days in milk, had to have been randomly assigned to treatment prior to calving, and (for cows assigned to the oral Ca bolus group) had to receive both of the boluses within the specified time periods. Of 1127 possible cows, 927 were entered into the trial and 200 were excluded. Table 2 presents a summary description of cows excluded from the study.

Of the 927 cows enrolled, 431 were in the oral Ca bolus group and 496 were controls. The slight imbalance occurred because more of the cows initially assigned to the oral Ca bolus group were excluded from the study. More criteria had to be met for a cow to fulfill the requirements for being in the oral Ca bolus group (i.e., correct bolus administration both times) compared to the very minimal requirements for inclusion in the control group.

Study outcomes

Whole blood samples were collected for Ca^{2+} analysis at 8 to 35 h after calving. Between 1 and 3 mL of whole blood was collected from the coccygeal vein or artery into syringes containing dry lithium heparin (Portex Pro-Vent Plus, Smith's Medical, New York). After collection, air was excluded from the syringes using the provided filter tip and the syringes were refrigerated within 30 m. A portable blood analyzer (VetStat Electrolyte and Blood Gas

Analyzer, Idexx Laboratories, Westbrook, ME) was used to measure Ca^{2+}. Whole blood from the syringes was analyzed for Ca^{2+} concentration within 72 h of collection (average time from collection to analysis was 28.1 h, ± 0.6 SEM). This storage and sampling protocol was validated by collecting a blood sample from 16 parturient cows into dry lithium heparin syringes and measuring the Ca^{2+} concentration from this syringe at 0, 24, 48, and 72 h after collection. The mean concentration of Ca^{2+} changed minimally (0.01 mmol/L) after 72 h of storage.

The Ca^{2+} results from the portable analyzer were validated by comparing them to in-house laboratory results. Ionized Ca results samples collected from 20 parturient cows were analyzed on both the portable analyzer and on an in-house blood gas plus Ca^{2+} analyzer (Nova Stat Profile pHOx Plus, Nova Biomedical Corp, Waltham, MA). Results were compared by linear regression and agreement between the 2 different analyzers was excellent ($R^2 = 0.92$). A difference between intercepts for the 2 different analyzers was corrected by adding 0.09 mmol/L to each Ca^{2+} result from the portable analyzer.

Whole blood BHBA concentration was determined 6 times for each cow between 3 and 16 DIM on Mondays, Wednesdays, and Fridays using a hand-held meter (Precision Xtra, Abbott Diabetes Care, Alameda, CA). Iwersen et al. (2009) reported that this meter has excellent agreement with laboratory analysis of serum for BHBA ($R^2 = 0.90$). Ketosis was defined as BHBA ≥ 1.2 mmol/L on any BHBA test (McArt et al., 2011). Cows were required to have 5 or 6 negative BHBA tests before they were classified as negative for ketosis.

First test daily milk weights came either from DHI test weights (Herd A) or from daily milk weights collected by the parlor meters (Herd B). About 8% of the parlor milking weights were missing for Herd B. In order to minimize the number of missing first test day milk weights that would result from a cow missing any one of her three milking weights on test day, missing milk

204 weights were imputed using a method based on hot-deck imputation. A randomly selected
205 milking weight from one of the five milking weights before or after the missing weight was used
206 to fill in the missing value. A milking weight was not imputed (i.e., it was left as a missing
207 value) if the 10 milking weights around it were all missing as well.

208 Early lactation health outcomes recorded were metritis, ketosis, displaced abomasum,
209 mastitis, pneumonia, herd removal, or death. Clinical milk fever, hypocalcemia (defined as
210 blood $Ca^{2+} \leq 1.00$ mmol/L at 8 to 35 h after calving), and retained placenta were summarized but
211 not evaluated statistically because they could be diagnosed before both oral Ca boluses were
212 administered. Health events were considered only if they occurred in the first 30 days in milk.
213 Health outcomes were recorded by the cooperating dairy producers. Early lactation cow health
214 was analyzed as individual events and as a single, continuous variable that represented the sum
215 of individual health events during the first 30 days in milk. Up to 6 events per cow were
216 possible; the actual range was 0 to 4 events per cow.

217 Other study outcomes included post-fresh locomotion score, post-fresh body condition score,
218 and reproduction outcomes (first service conception, pregnancy by 150 days in milk, days open
219 for cows pregnant by 150 days in milk, and days from voluntary waiting period (**VWP**) to
220 conception by 305 DIM). Post-fresh locomotion and body condition scores were determined
221 between 40 and 60 DIM using the same methods as described before, with the addition of a third
222 trained evaluator who assigned about 65% of the post-fresh scores.

223 ***Study covariates***

224 Covariates for early lactation outcomes were factors that could be known about the cow at
225 the time of calving. These included parity, pre-fresh locomotion score, pre-fresh body condition
226 score, twin calves or single birth, stillborn or live calf, previous gestation length (i.e., length of
227 the gestation preceding enrollment in the study), previous lactation length, previous dry period

length, and calving ease score (1 to 5 scale, with 1 representing no calving assistance and 5 representing extreme assistance). The time interval between calving and blood sample collection was included as an additional covariate for the Ca^{2+} analysis, DIM at first DHI test was included as an additional covariate for the first test milk yield analysis, and DIM at scoring was included as an additional covariate for post-fresh locomotion score and body condition score analyses. Additional covariates included in the analyses for first service conception, pregnancy by 150 DIM, and days open for cows pregnant by 150 DIM included days in milk at first breeding, AI synchronization status (synchronized = 1, not synchronized = 0), and first breeding month. Categorical covariates were compressed into combined categories as needed in order to avoid missing combinations of covariates and to make the standard errors as equivalent as possible across group means. Continuous covariates were plotted against study outcomes, and the resulting plots were inspected for evidence of breakpoints in the relationship along the spectrum of values for the continuous covariates. No logical breakpoints were apparent, so no continuous covariates were compressed into categorical variables for the initial evaluations.

Statistical analysis

Descriptive statistics were generated with the MEANS and FREQ procedures of SAS 9.3 (SAS Inst. Inc., Cary, NC). Continuous study outcomes (Ca^{2+} 8 to 35 h after calving, first test milk yield, sum of health events in the first 30 days in milk, post-fresh locomotion score, post-fresh body condition score, and days open for cows pregnant by 150 days in milk) were analyzed using multivariate linear regression with the MIXED procedure of SAS. Study outcomes with a binary response (metritis, ketosis, displaced abomasum, mastitis, pneumonia, herd removal, death, first service conception, and pregnant by 150 days in milk) were analyzed using mixed effects multivariate Poisson regression with the GENMOD procedure of SAS (Frome and Checkoway, 1985; Spiegelman and Hertzmark, 2005). Poisson regression has the advantage of

directly estimating disease rates and relative risk, which is the most intuitive approach to modeling and presenting these data (Ospina et al., 2012).

Large models for the analysis of pregnancy status through 305 DIM were conducted using a semiparametric proportional hazards model (Cox, 1972) in the PHREG procedure of SAS. The time series variable for the model was the number of days from the end of the VWP (65 days for Herd A and 78 days for Herd B) until conception or censoring. Cows were dropped from the survival analysis (non-informative censoring) when they were removed from the herd (either sold or died) or declared ineligible for additional breedings ("do not breed" designation in the herd records). A small number of cows (n = 23) were still open and eligible for additional breedings by 305 DIM.

The effect of blanket supplementation with 2 oral Ca boluses for all study cows was evaluated using a two-step statistical method. The method was the same whether the outcome was continuous or categorical (using multivariate linear regression), binary (using multivariate Poisson regression), or pregnancy status by 305 DIM (using survival analyses). First, a large model for each study outcome was fitted using every potential covariate applicable to that outcome, plus the interactions of each covariate with oral Ca bolus supplementation and with herd. The specific terms available to the large models were herd (as a fixed effect), oral Ca bolus supplementation, lactation group (second, third, or fourth and greater), pre-fresh lameness status (0 if locomotion score 1 or 2, 1 if locomotion score 3 or 4), pre-fresh body condition score ($\leq$ 2.75, 3.00, 3.25, 3.50, 3.75 or $\geq$ 4.00), twin (0 if single birth, 1 if twin birth), stillbirth (0 if live calf or calves, 1 if 1 or 2 calves born dead), calving ease score category (1 if ease score 1, 2 if ease score 2, 3 if ease score 3 or 4), calving month (June, July, or August), previous lactation mature equivalent milk production (expressed as percent rank within herd - the values for all

cows in each herd were ranked from lowest to highest and then assigned a percent rank, with a value of 100% representing the average mature equivalent milk production in the herd at that time), previous lactation length (days), previous lactation days dry, and previous lactation gestation length (days). Days in milk at first breeding, AI synchronization status (synchronized = 1, not synchronized = 0), and first breeding month (instead of calving month) were included in the analyses for first service conception, pregnancy by 150 DIM, and days open for cows pregnant by 150 DIM. The interactions between all variables and herd, plus all interactions between these variables and oral Ca bolus supplementation were also eligible for inclusion in the large models. Terms were removed from the models in a stepwise, backwards fashion until all were $P < 0.05$ in the model. Single variables were removed from the model only after there were no interaction terms including that variable remaining in the model.

Survival analysis of pregnancy status by 305 DIM required the additional step of testing the reduced large model to determine if the assumption of proportional hazards was valid. This was done by including time-dependent covariates (log transformed days since VWP) in the large model (Allison, 1995). Because the time-dependent covariates were significant, time-dependent covariates for every variable (plus their interactions) were made available and the model with the extra terms was reduced again by stepwise, backwards elimination.

The purpose of the large models was to establish whether there was an effect of oral Ca bolus supplementation after all possible covariates (and interactions) had been considered. However, the large models were not useful for determining effect sizes. Almost all of the large models contained continuous covariates after the backwards elimination procedure, and least squares means calculated from such models are specific to a discrete value for each continuous covariate in the model. Many of the models contained 3 or 4 continuous covariates, which rendered it

impractical to estimate the oral Ca bolus effect at every possible combination of these covariates. Therefore, a second analysis was conducted for each study outcome using small models that consisted of herd (as a fixed effect), oral Ca bolus supplementation, and the interaction of herd and oral Ca bolus supplementation. The interaction term was removed if $P < 0.05$. Because the small models contained no continuous covariates, they were used to determine the estimates of effect sizes.

The effect of the oral Ca boluses was considered significant for an outcome only if oral Ca bolus supplementation was $P < 0.05$ in both the large and small models for that outcome. Because there were no continuous covariates present in the small models, overall least squares means could be calculated for control vs. oral Ca bolus supplemented cows. Least squares means and *P* values from the small models were then reported for each outcome.

For the pregnancy status by 305 DIM survival analysis, the small model consisted of a Kaplan-Meier analysis (Kaplan and Meier, 1958) using the LIFETEST procedure of SAS. The model included oral Ca bolus supplementation and days from the end of VWP. From this procedure, the effect size of oral Ca supplementation was evaluated by calculating mean days from the end of the VWP until conception for both groups. Days open could be estimated by adding the average VWP to the mean value for days from the end of the VWP until conception.

After all of the large and small models were completed and the effects of blanket supplementation for all cows were evaluated, the large models were examined to find interactions ($P < 0.05$) between oral Ca bolus supplementation and any covariate. These interactions were the starting point for determining if subpopulations of cows responded differently to oral Ca bolus supplementation. When such interactions were found for a continuous covariate, a cutpoint was derived after visual inspection of a plot of the covariate vs.

the outcome, and the covariate was dichotomized based on this cutpoint. The dichotomized covariate was then re-evaluated in a large model as a binary outcome. If the interaction of oral Ca bolus supplementation with the dichotomized covariate remained $P < 0.05$ (or if the interaction with any binary covariate was $P < 0.05$), then the interaction was evaluated in a small model that contained only oral Ca bolus supplementation, herd, the covariate of interest, the interaction of this covariate with oral Ca bolus supplementation, the interaction of this covariate with herd, and the interaction of herd with oral Ca bolus supplementation. Interaction terms with herd were removed from the model if $P > 0.05$. Least squares means were computed for the oral Ca bolus supplemented vs. control cows.

Two interactions with oral Ca bolus supplementation were significant at every evaluation point in the analyses described above. Specifically, they were 1) the interaction between pre-fresh lameness and oral Ca bolus supplementation for the sum of health events before 30 DIM and 2) previous lactation mature equivalent milk production and oral Ca bolus supplementation for milk production at the first DHI test. Because some cows would be represented in both subpopulations and because the most practical application of these results would be to target both subpopulations together for oral Ca bolus supplementation, the 2 covariates were combined into a single covariate. The analyses described above (large models followed by small models) were then repeated using the new, combined covariate as though it was a single covariate. Results were interpreted by evaluating the interaction between the combined covariate and oral Ca bolus supplementation. Least squares means were then reported for the combined subpopulation of cows that would be targeted for oral Ca bolus supplementation.

Residual versus predicted value plots from the analysis of the continuous outcomes were visually evaluated as a test for the assumption of normal distribution of the data. There was no

appearance of heteroscedasticity or atypical distribution. Significance was claimed at $P < 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Table 3 presents continuous outcomes and covariates by herd, and Table 4 presents binary outcomes and covariates by herd. Only 6 cows (0.6%) were treated for clinical milk fever during the study, and only 14.2% of cows had hypocalcemia (blood $Ca^{2+} \leq 1.00$ mmol/L) when evaluated at 8 to 35 h post-calving. These results are extremely low compared to previously published data (DeGaris and Lean, 2008; Reinhardt et al., 2011) and indicate excellent overall effectiveness of the anionic salts feeding program that was in place for both herds.

The study was conducted only in summer months; however, it is unlikely that the low observed incidence of hypocalcemia was a seasonal effect. Østergaard et al. (2003) attempted to find published evidence for seasonality of clinical milk fever but was unsuccessful. An evaluation of calvings and cases of clinical milk fever from six years of data in the Dairy Comp 305 archives for both study herds (80 cases of milk fever and 18,887 fresh events for second and greater lactation cows) revealed no difference between the risk for milk fever for cows calving in the summer months compared to cows who calved any other time of the year (relative risk for milk fever with summer calvings was 0.63, with a 95% confidence interval of 0.36 to 1.13).

Oral Ca bolus supplementation did not affect ($P > 0.05$) any study outcomes when all study cows were considered together. This result was expected, given the very low incidence of hypocalcemia during the study period. Melendez et al. (2002) also gave oral Ca supplements to multiparous cows fed a low DCAD diet and found no effect of these supplements on plasma concentrations of total Ca, P, Mg, NEFA, BHBA, or glucose.

There was no effect of oral Ca bolus supplementation on days from the end of the VWP to

conception in the large survival model (data not shown) or in the small survival models. Kaplan-Meier curves from the small models are presented in Figure 2 (data from all cows) and Figure 3 (data from cows in the subpopulation targeted for oral Ca bolus supplementation). Hernandez et al. (1999) also reported no effect of oral Ca supplementation on first service conception; however, this was a small study that only enrolled cows with retained fetal membranes. Chapinal et al. (2012) reported that cows with low serum Ca either the week before or the week after calving had reduced odds for conceiving at first service. They did not evaluate reproductive performance beyond the first service.

Two variables had significant interactions with oral Ca bolus supplementation that were present throughout the process of evaluating the large and small models. The first was the interaction between oral Ca bolus supplementation and pre-fresh lameness (locomotion score 3 or 4) for the sum of health events by 30 DIM (Table 5, $P = 0.005$). The other variable to have a significant interaction with oral Ca bolus supplementation was previous lactation mature equivalent milk production rank with first test milk yield (Table 6, $P = 0.015$). Pre-fresh lameness had a significant interaction with first test milk yield in the large model; however, this interaction was not significant in the small model.

Using the small models, least squares means were calculated for oral Ca bolus supplemented and control cows for first test milk yield across a range of values for previous lactation milk yield. These least squares means were then plotted (Figure 1). Based on an evaluation of this plot, cutpoints between 100 and 115 previous lactation milk yield percent rank were considered. These cutpoints were evaluated for their effect on both first test milk yield and the sum of health events in early lactation. The cutpoint was kept as low as possible in order to maximize the number of cows that could benefit from oral Ca bolus supplementation, still preserve a

significant increase in milk yield, and not increase the number of health events before 30 DIM. A cutpoint of > 105% of herd rank was the lowest cutpoint at which oral Ca bolus supplementation significantly increased milk yield without impairing health.

A new analysis was then conducted, starting with a new stepwise, backwards elimination in the large model, to determine how the new dichotomous variable (previous lactation mature equivalent milk yield > 105% or ≤ 105% of herd rank) affected oral Ca bolus response. Cows above the cutpoint had significantly ($P = 0.024$) increased milk yield when supplemented with oral Ca bolus compared to cows above the cutpoint who were not supplemented. This dichotomized variable was then included in a final, small model. The response to oral Ca bolus supplementation in cows with high previous lactation milk yield was +2.9 kg of milk at first DHI test after calving ($P = 0.009$, Table 7). This increase in milk yield (7.2%) was of greater magnitude than the 3.6% increase in milk yield reported for cows supplemented with anionic salts (Beede et al., 1992). The inclusion of first lactation animals in the analysis of Beede et al. (1992) could explain a portion of the lower overall milk production response. Smaller-scale studies have shown no effect of oral Ca supplementation on subsequent milk yield (Goff et al., 1996; Dhiman and Sasidharan, 1999; Melendez et al., 2002); these studies did not attempt to identify subpopulations of cows that might have a different response to oral Ca supplementation.

Although there was a significant interaction between oral Ca bolus supplementation and lameness for the sum of early lactation health events, there was no significant effect of oral Ca bolus supplementation on any of the individual disease or reproductive outcomes (Table 8). Oetzel (1996) reported decreased displaced abomasum following oral Ca administration around calving; however, the underlying incidence of hypocalcemia (53%) was much greater in this study compared to 14% in the current one. Melendez et al. (2002) and Dhiman and Sasidharan

(1999) reported no effect of oral Ca supplementation around calving on later concentrations of BHBA, and Goff et al. (1996) reported no effect of oral Ca supplementation on rates of primary ketosis. These findings are in agreement with the current study, which demonstrated no effect of oral Ca supplementation on the incidence of cows with blood BHBA ≥ 1.2 mmol/L.

No other variables were found to have a significant interaction with oral Ca bolus supplementation. Thus, the final target group for oral Ca bolus supplementation was cows that were lame in the pre-fresh period and cows with higher previous lactation mature equivalent milk production (> 105% of herd rank). The lame and higher previous milk production cows were combined and designated with single new binary variable in the dataset. This subpopulation represented 444 cows, or about 48% of the eligible cows. After combining the 2 targeted groups, the same analyses using the smaller models was done for all outcomes, this time looking at the interaction between oral Ca bolus and the new variable that represented the combined subpopulations. These results are reported in Tables 7 and 8. The combined lame and and high previous lactation milk production cows gave 3.1 kg more milk ($P = 0.002$) compared to the control cows. This same group of cows had 0.04 fewer health events in the first 30 days in milk. This difference was not significant ($P = 0.628$), although it is noteworthy that cows supplemented with oral Ca boluses gave more milk without a concomitant increase in health events. The size of the milk yield increase following oral Ca bolus supplementation to the targeted subpopulation of cows in the current study was very similar to early lactation milk yield losses of 3.2 kg/d for cows with serum Ca ≤ 2.1 mmol/L during wk -1 relative to calving and 4.8 kg/d for cows with serum Ca ≤ 2.1 mmol/L during wk +1 relative to calving reported by Chapinal et al. (2012).

Oral Ca bolus supplementation did not improve Ca^{2+} concentrations in the sample collected

436 at 8 to 35 h after calving ($P = 0.271$). The timing of the collection of the Ca^{2+} sample (20.6 h
437 after administration of the first bolus, and just before the administration of the second bolus) was
438 such that an effect of oral Ca supplementation was not expected. Sampson et al. (2009) reported
439 that oral Ca boluses increased blood Ca^{2+} concentrations at only 2 time points - 1 h after
440 administration of both the first oral Ca bolus (given at calving) and 1 h after administration of the
441 second bolus (given 12 h after calving). Sampson et al. (2009) also reported a significant
442 decrease in urinary pH at 24 h after calving, which could explain more prolonged benefits to oral
443 Ca bolus administration beyond the short time period of increased blood Ca^{2+}.

444 It was not feasible in the current study to document blood Ca^{2+} changes soon after the second
445 bolus was administered, because the cows could be restrained in headlocks just once daily and
446 were restrained for the shortest time possible at each lockup. Ramos-Nieves et al. (2009)
447 reported that cows have the highest proportion of subclinical hypocalcemia about 16 h after
448 calving, which is close to the mean time (20.6 h post-calving) that the second bolus was
449 administered in the current study.

450 The mechanism for the beneficial effect or oral Ca bolus supplementation cannot be
451 determined from the results of this study. Lame cows could be prone to injury after calving,
452 especially if they are weakened by hypocalcemia. Transient correction of hypocalcemia could
453 prevent injury and decrease the total number of health events in lame cows. Stimulation of
454 additional dry matter intake following correction of transient hypocalcemia is a plausible
455 explanation for the increased milk yield. This explanation is consistent with the observation that
456 feeding low DCAD diets before calving was associated with higher dry matter intake in early
457 lactation (Eppard et al., 1996; Joyce et al., 1997). However, dry matter intake in individual cows
458 was not measured in the current study.

Over-conditioned cows are reported to be at increased risk for hypocalcemia (Heuer et al., 1999). However, pre-fresh body condition score did not have a significant interaction with oral Ca bolus supplementation in the current study. The number of overly conditioned animals (body condition score ≥ 4.00) was only about 4% for the 2 study herds.

Increasing lactation number has been associated with increased risk for hypocalcemia (Reinhardt et al., 2011). However, parity did not have a significant interaction with oral Ca bolus supplementation in the current study. This finding suggests that oral Ca supplementation should not be restricted to very old (e.g., lactation 3 or greater) cows only.

Results of this study did not reveal any detrimental effects of providing additional anions (in this case, chloride and sulfate anions from the oral Ca boluses) to cows that had received a low DCAD diet prior to calving. This was not surprising, considering that cows have lowered feed intake on the day of calving and are typically switched from the low DCAD diet soon after calving. Cows in the current study were all consuming a higher DCAD (lactating) diet when they received their second oral Ca bolus. It appeared that the benefits of the additional acidification from oral Ca bolus administration (Sampson et al., 2009) caused more benefits via improved Ca metabolism than potentially detrimental effects from systemic acidification. Supporting higher blood Ca concentrations during the critical first 2 days after calving may be of primary importance to multiparous cows.

CONCLUSIONS

Supplementing all second and greater lactation cows with 2 oral Ca boluses in herds with very effective anionic salts feeding programs neither harmed nor benefitted early lactation health or milk yield. Cows that were lame before calving and were supplemented with oral Ca boluses had improved early lactation health compared to lame cows that were not supplemented. Cows

that had high previous lactation mature equivalent milk production and were supplemented with oral Ca boluses had increased early lactation milk yield compared to high-producing cows that were not supplemented. This subpopulation of lame and higher producing cows represented 48% of the multiparous cows in the 2 study herds. Supplementation of the combined subpopulation of cows with oral Ca boluses resulted in increased milk yield without affecting early lactation health. These results indicate that dairy herds already experiencing a very low incidence of hypocalcemia can target a subpopulation of cows that will respond favorably to supplementation with oral Ca boluses.

ACKNOWLEDGMENTS

Financial support for this research was provided by Boehringer Ingelheim Vetmedica, Inc. The authors express gratitude to Lindley Reilly, Eric Rooker, Angela Niles, Tom Bennett, Chris Eisele, Tyler Franks, Catherine Bargren, Diego Calderon, Cristian Vergara, Loïc Commun, and Marsha Bush (University of Wisconsin-Madison) for their invaluable assistance with data collection and analysis, to Nick Keuler (University of Wisconsin-Madison) for statistical consultation, to Jessica McArt, Paula Ospina, and Daryl Nydam (Cornell University) for advice regarding experimental design and statistical analysis, and especially to the owners and employees of the collaborating dairies for administering the boluses, collecting the blood samples, and granting us access to their facilities, cows, and herd records.

REFERENCES

Allison, P. D. 1995. Survival Analysis using the SAS System: A Practical Guide. SAS Institute Inc., Cary, NC.

Beede, D. K., C. A. Risco, G. A. Donovan, C. Wang, L. F. Archbald, and W. K. Sanchez. 1992. Nutritional management of the late pregnant dry cow with particular reference to dietary cation-anion difference and calcium supplementation. The Bovine Proceedings 24:51-55.

Chapinal, N., M. E. Carson, S. J. LeBlanc, K. E. Leslie, S. Godden, M. Capel, J. E. Santos, M. W. Overton, and T. F. Duffield. 2012. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J. Dairy Sci. 95:1301-1309.

Charbonneau, E., D. Pellerin, and G. R. Oetzel. 2006. Impact of lowering dietary cation-anion difference in nonlactating dairy cows: A meta-analysis. J. Dairy Sci. 89:537-548.

Cox, D. 1972. Regression models and life-tables. J. R. Stat. Soc. B 34:187-200.

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, P. A. Powers, M. C. Smith, M. E. White, R. B. Hillman, and E. J. Pearson. 1983. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. J. Am. Vet. Med. Assoc. 183:559-561.

DeGaris, P. J., and I. J. Lean. 2008. Milk fever in dairy cows: a review of pathophysiology and control principles. Vet. J. 176:58-69.

Dhiman, T. R., and V. Sasidharan. 1999. Effectiveness of calcium chloride in increasing blood calcium concentrations of periparturient dairy cows. J. Anim. Sci. 77:1597-1605.

Eppard, P. J., J. J. Veenhuizen, W. J. Cole, P. G. Comens-Keller, G. F. Hartnell, R. L. Hintz, L. Munyakazi, P. K. Olsson, R. H. Sorbet, T. C. White, C. A. Baile, R. J. Collier, J. P. Goff, and R. L. Horst. 1996. Effect of bovine somatotropin administered to periparturient dairy cows on the incidence of metabolic disease. J. Dairy Sci. 79:2170-2181.

524 Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition
525 score in Holstein cows. J. Dairy Sci. 77:2695-2703.
526 Frome, E. L., and H. Checkoway. 1985. Epidemiologic programs for computers and calculators.
527 Use of Poisson regression models in estimating incidence rates and ratios. Am. J. Epidemiol.
528 121:309-323.
529 Gelfert, C. C., L. M. Loeffler, S. Fromer, M. Engel, K. Manner, and R. Staufenbiel. 2010.
530 Comparison of the impact of different anionic salts on the acid-base status and calcium
531 metabolism in non-lactating, non-pregnant dairy cows. Vet. J. 185:305-309.
532 Goff, J. P. 2008. The monitoring, prevention, and treatment of milk fever and subclinical
533 hypocalcemia in dairy cows. Vet. J. 176:50-57.
534 Goff, J. P., and R. L. Horst. 1993. Oral administration of calcium salts for treatment of
535 hypocalcemia in cattle. J. Dairy Sci. 76:101-108.
536 Goff, J. P., and R. L. Horst. 1994. Calcium salts for treating hypocalcemia: carrier effects, acid-
537 base balance, and oral versus rectal administration. J. Dairy Sci. 77:1451-1456.
538 Goff, J. P., and R. L. Horst. 1997. Effects of the addition of potassium or sodium, but not
539 calcium, to prepartum rations on milk fever in dairy cows. J. Dairy Sci. 80:176-186.
540 Goff, J. P., R. L. Horst, P. W. Jardon, C. Borelli, and J. Wedam. 1996. Field trials of an oral
541 calcium propionate paste as an aid to prevent milk fever in periparturient dairy cows. J.
542 Dairy Sci. 79:378-383.
543 Goff, J. P., R. L. Horst, F. J. Mueller, J. K. Miller, G. A. Kiess, and H. H. Dowlen. 1991.
544 Addition of chloride to a prepartal diet high in cations increases 1,25-dihydroxyvitamin D
545 response to hypocalcemia preventing milk fever. J. Dairy Sci. 74:3863-3871.

546 Goff, J. P., R. Ruiz, and R. L. Horst. 2004. Relative acidifying activity of anionic salts
547 commonly used to prevent milk fever. J. Dairy Sci. 87:1245-1255.
548 Hernandez, J., C. A. Risco, and J. B. Elliot. 1999. Effect of oral administration of a calcium
549 chloride gel on blood mineral concentrations, parturient disorders, reproductive
550 performance, and milk production of dairy cows with retained fetal membranes. J. Am. Vet.
551 Med. Assoc. 215:72-76.
552 Heuer, C., Y. H. Schukken, and P. Dobbelaar. 1999. Postpartum body condition score and results
553 from the first test day milk as predictors of disease, fertility, yield, and culling in
554 commercial dairy herds. J. Dairy Sci. 82:295-304.
555 Iwersen, M., U. Falkenberg, R. Voigtsberger, D. Forderung, and W. Heuwieser. 2009.
556 Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. J. Dairy
557 Sci. 92:2618-2624.
558 Joyce, P. W., W. K. Sanchez, and J. P. Goff. 1997. Effect of anionic salts in prepartum diets
559 based on alfalfa. J. Dairy Sci. 80:2866-2875.
560 Kaplan, E. L., and P. Meier. 1958. Nonparametric estimation from incomplete observations. J.
561 Am. Stat. Assoc. 53:457-481.
562 Kimura, K., T. A. Reinhardt, and J. P. Goff. 2006. Parturition and hypocalcemia blunts calcium
563 signals in immune cells of dairy cattle. J. Dairy Sci. 89:2588-2595.
564 McArt, J. A., D. V. Nydam, P. A. Ospina, and G. R. Oetzel. 2011. A field trial on the effect of
565 propylene glycol on milk yield and resolution of ketosis in fresh cows diagnosed with
566 subclinical ketosis. J. Dairy Sci. 94:6011-6020.

Melendez, P., A. Donovan, C. A. Risco, M. B. Hall, R. Littell, and J. Goff. 2002. Metabolic responses of transition Holstein cows fed anionic salts and supplemented at calving with calcium and energy. J. Dairy Sci. 85:1085-1092.

Nordlund, K. V., N. B. Cook, and G. R. Oetzel. 2004. Investigation strategies for laminitis problem herds. J. Dairy Sci. 87 (E. Suppl.):E27-E35.

Oetzel, G. R. 1996. Effect of calcium chloride gel treatment in dairy cows on incidence of periparturient diseases. J. Am. Vet. Med. Assoc. 209:958-961.

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.

Ospina, P. A., D. V. Nydam, and T. J. Diciccio. 2012. Technical note: The risk ratio, an alternative to the odds ratio for estimating the association between multiple risk factors and a dichotomous outcome. J. Dairy Sci. 95:2576-2584.

Østergaard, S., J. T. Sorensen, and H. Houe. 2003. A stochastic model simulating milk fever in a dairy herd. Prev. Vet. Med. 58:125-143.

Pehrson, B., and M. Jonsson. 1991. Prevention of milk fever by oral administration of encapsulated Ca-salts. Bov. Pract. 26:36-37.

Phillippo, M., and G. W. Reid. 1994. Parturient hypocalcemia in dairy cows: effects of dietary acidity on plasma minerals and calciotrophic hormones. Res. Vet. Sci. 56:303-309.

Ramos-Nieves, J. M., B. J. Thering, M. R. Waldron, P. W. Jardon, and T. R. Overton. 2009. Effects of anion supplementation to low-potassium prepartum diets on macromineral status and performance of periparturient dairy cows. J. Dairy Sci. 92:5677-5691.

589 Reinhardt, T. A., J. D. Lippolis, B. J. McCluskey, J. P. Goff, and R. L. Horst. 2011. Prevalence
590 of subclinical hypocalcemia in dairy herds. Vet. J. 188:122-124.
591 Sampson, J. D., J. N. Spain, C. Jones, and L. Carstensen. 2009. Effects of calcium chloride and
592 calcium sulfate in an oral bolus given as a supplement to postpartum dairy cows. Vet. Ther.
593 10:131-139.
594 Seifi, H. A., S. J. Leblanc, K. E. Leslie, and T. F. Duffield. 2011. Metabolic predictors of post-
595 partum disease and culling risk in dairy cattle. Vet. J. 188:216-220.
596 Spiegelman, D., and E. Hertzmark. 2005. Easy SAS calculations for risk or prevalence ratios and
597 differences. Am. J. Epidemiol. 162:199-200.
598 Thilsing-Hansen, T., R. J. Jørgensen, and S. Østergaard. 2002. Milk fever control principles: a
599 review. Acta Vet. Scand. 43:1-19.

Table 1. Summary of formulated feed ingredient amounts and formulated nutrient composition of example diets fed on 2 commercial dairies to the pre- and post-fresh cows during the study.

	Herd A		Herd B	
Feed ingredient or nutrient	Pre-fresh	Post-fresh	Pre-fresh	Post-fresh
Ingredient (% of DM)				
Alfalfa hay	13.0	9.8	—	—
Alfalfa silage	—	17.4	7.1	24.5
Corn silage	29.8	36.5	16.3	41.6
Grass hay	14.0	—	10.7	—
Wheat straw	14.0	2.2	22.7	—
Ground corn	—	8.7	13.3	1.9
Whey permeate	—	2.2	—	—
Corn gluten feed (dry)	—	8.5	10.7	6.5
Corn distillers grains (dry)	—	—	9.2	8.8
Corn distillers grains (wet)	—	—	—	2.3
Brewers grains (wet)	—	—	7.1	6.5
Pre-fresh concentrate mix	29.2	—	2.9	—
Post-fresh concentrate mix	—	14.8	—	7.9
DM (% as fed)	45.2	43.0	59.0	41.5
Nutrient composition (DM basis)				
CP, %	13.7	17.2	15.0	17.5
Ether extract, %	3.5	4.7	4.3	4.9
NDF, %	44.3	30.3	43.1	33.5
Starch, %	14.8	19.8	19.6	21.8
NFC,[1] %	30.5	39.3	29.4	36.0
Ash, %	8.04	8.50	8.23	8.10
Ca, %	0.93	0.92	0.88	.79
P, %	0.34	0.43	0.41	.44
Mg, %	0.39	0.37	0.34	.37
Na %	0.09	0.48	0.14	.46
K, %	1.08	1.54	1.27	1.44
Cl, %	0.73	0.58	0.88	.45
S, %	0.35	0.25	0.25	.25
DCAD,[2] mEq/kg	-109	283	-18	285

[1] NFC = Non-fiber carbohydrates, calculated as 100 - CP - Ether Extract - NDF - Ash.
[2] DCAD = Dietary cation-anion difference, calculated as mEq [(Na + K) - (Cl + S)].

Table 2. Reasons for excluding cows from the trial. Experimental cows were selected from multiparous cows that calved during a 3-month time period on 2 commercial dairies.

Reason	Cows (n)
Invalid record information	4
Aborted (< 260 d gestation length)	31
Calving ease score of 5 (C-section or fetotomy)	1
Removed before 2 DIM	9
Calved without random treatment assignment (no tag)	69
Received the first oral Ca bolus but not the second	85
Received the second oral Ca bolus but not the first	1

Table 3. Summary of continuous and categorical study outcomes and covariates by herd; data are from 927 second and greater lactation cows in 2 commercial dairies.

	Herd A			Herd B		
Item	n	mean	SEM	n	mean	SEM
Continuous or categorical outcomes:						
Ca^{2+} 8 to 35 h post-calving, mmol/L	318	1.13	0.01	525	1.16	0.01
first test milk yield, kg	364	44.4	0.6	527	37.2	0.5
sum of health events[1] ≤ 30 dim	372	0.59	0.04	555	1.01	0.04
post-fresh locomotion score, 1 to 4 scale	324	1.79	0.04	466	1.71	0.03
post-fresh body condition score, 1 to 5 scale	325	2.51	0.02	466	2.56	0.02
days open for cows pregnant by 150 DIM	207	90.4	1.7	298	99.5	1.2
Continuous or categorical covariates:						
lactation number	372	2.79	0.05	555	3.05	0.04
previous lactation length, d	371	361.0	4.1	554	353.1	3.1
previous gestation length, d	372	277.6	0.3	555	277.4	0.2
previous lactation 305-d mature equivalent milk yield, % rank within herd	371	100.6	0.8	554	99.7	0.7
previous days dry	372	48.6	0.8	555	58.8	1.0
pre-fresh locomotion score, 1 to 4 scale	366	1.60	0.04	532	1.63	0.03
pre-fresh body condition score, 1 to 5 scale	364	3.18	0.02	529	3.26	0.02
calving ease score, 1 to 5 scale	372	1.19	0.03	555	1.28	0.03
days in milk at first test	364	19.4	0.5	527	19.4	0.4
days in milk at first breeding	332	73.5	0.3	409	84.3	0.2

[1] Health events included were metritis, ketosis, displaced abomasum, mastitis, pneumonia, herd removal, or death (only for events in the first 30 DIM).

Table 4. Summary of binary study outcomes and covariates by herd; data are from 927 second and greater lactation cows in 2 commercial dairies.

	Herd A			Herd B		
Item	1[1]	0[2]	%	1	0	%
Binary outcomes:						
clinical milk fever	0	372	0.0	6	549	1.1
hypocalcemia[3]	55	263	17.3	65	460	12.4
retained placenta	30	342	8.1	63	492	11.4
metritis ≤ 14 DIM	52	320	14.0	103	452	18.6
ketosis[4] ≤ 16 DIM	98	84	53.8	284	158	64.3
displaced abomasum ≤ 30 DIM	12	360	3.2	31	524	5.6
mastitis ≤ 30 DIM	38	334	10.2	76	479	13.7
pneumonia ≤ 30 DIM	5	367	1.3	22	533	4.0
herd removal ≤ 30 DIM	14	358	3.8	44	511	7.9
died ≤ 30 DIM	5	367	1.3	18	537	3.2
first service conception	129	203	38.9	172	237	42.1
pregnant by 150 DIM	207	104	66.6	298	118	71.6
Binary covariates:						
twin calves	26	346	7.0	46	509	8.3
stillborn (1 or more calves dead)	11	361	3.0	24	531	4.3
pre-fresh lameness[5]	41	325	11.2	63	469	11.8

[1] 1 = count of cows with the condition.

[2] 0 = count of cows without the condition.

[3] Hypocalcemia was defined as blood Ca^{2+} ≤ 1.00 mmol/L at 8 to 35 h after calving.

[4] Ketosis was defined as one or more blood BHBA tests ≥ 1.2 mmol/L. Cows were tested six times between 3 and 16 DIM and were classified if they had ≥ 5 total BHBA tests or ≥ 1 positive test.

[5] Lame cows had a pre-fresh locomotion score of 3 or 4, using a 1 to 4 point scale.

Table 5. Estimates and Type 3 tests of fixed effects from a reduced mixed regression model for the sum of health events[1] in the first 30 DIM. All possible covariates plus interactions with herd and oral Ca bolus[2] supplementation were considered and removed by backwards elimination until $P < 0.05$ in the model. Data are from 927 second and greater lactation cows in 2 commercial dairies.

Effect and effect level	Estimate	SE	Num.[3] df	Den.[3] df	*P*-value[4]
Intercept	1.292	0.289	—	—	< 0.001
Oral Ca bolus supplementation			1	867	0.120
control (no oral Ca boluses)	0.368	0.159			
supplemented (2 oral Ca boluses given)	—[5]	—[5]			
Herd			1	867	0.002
Herd A	-0.406	0.322			
Herd B	—[5]	—[5]			
Lactation group			2	867	0.032
Lactation = 2	-0.170	0.068			
Lactation = 3	-0.051	0.073			
Lactation ≥ 4	—[5]	—[5]			
Pre-fresh lameness			1	867	0.046
Not lame (locomotion score 1 or 2)	0.064	0.121			
Lame (locomotion score 3 or 4)	—[5]	—[5]			
Pre-fresh body condition score			4	867	0.382
≤ 2.75 body condition score	0.006	0.149			
3.00 body condition score	0.163	0.133			
3.25 body condition score	0.033	0.121			
3.50 body condition score	0.167	0.128			
≥ 3.75 body condition score	—[5]	—[5]			
Calving month			2	867	< 0.001
June	—[5]	—[5]			
July	0.037	0.070			
August	-0.276	0.072			
Prev. lact. milk yield,[6] % rank within herd	-0.005	0.002	1	867	0.003
Previous lactation length, d	0.0003	0.0005	1	867	0.005
Previous dry period length, d	0.004	0.001	1	867	0.009
Herd * Previous lactation length			1	867	0.049
Herd A and Previous lactation length	0.0014	0.0007			
Herd B and Previous lactation length	—[5]	—[5]			

(Table 5 is continued on the next page)

Table 5, continued.

Effect and effect level	Estimate	SE	Num.[3] df	Den.[3] df	*P*-value[4]
Herd ⁎ Pre-fresh body condition score			4	867	0.021
Herd A and ≤ 2.75 body condition score	-0.464	0.230			
Herd A and 3.00 body condition score	-0.689	0.212			
Herd A and 3.25 body condition score	-0.416	0.203			
Herd A and 3.50 body condition score	-0.573	0.218			
All other combinations	—[5]	—[5]			
Oral Ca bolus ⁎ Pre-fresh lameness			1	867	0.005
No oral Ca boluses and Not lame	-0.474	0.169			
All other combinations	—[5]	—[5]			

[1] Health events included were metritis, ketosis, displaced abomasum, mastitis, pneumonia, herd removal, or death (only for events in the first 30 DIM).

[2] Bovikalc, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO. Cows in the oral Ca bolus group received 2 oral Ca boluses (1 bolus at calving and 1 bolus 8 to 35 h after calving).

[3] Num. = numerator; Den. = denominator.

[4] Except for the intercept, *P*-values reported are the type 3 test of fixed effects for the entire variable.

[5] Reference group.

[6] 305 d mature equivalent milk production; 100% represented the average cow in the herd at the time the cow calved.

Table 6. Type 3 tests of fixed effects from a reduced mixed regression model for first test milk yield (kg/cow/d). All possible covariates plus interactions with herd and oral Ca bolus[1] supplementation were considered and removed by backwards elimination until $P < 0.05$ in the model. Data are from 927 second and greater lactation cows in 2 commercial dairies.

Effect and effect level	Estimate	SE	Num.[3] df	Den.[3] df	*P*-value[4]
Intercept	-2.00	22.52	—	—	0.929
Oral Ca bolus supplementation			1	831	0.057
Control (no oral Ca boluses)	5.42	4.14			
Supplemented (2 oral Ca boluses given)	—[5]	—[5]			
Herd			1	831	0.045
Herd A	-73.39	34.54			
Herd B	—[5]	—[5]			
Pre-fresh lameness			1	831	0.478
Not lame (locomotion score 1 or 2)	-1.30	1.40			
Lame (locomotion score 3 or 4)	—[5]	—[5]			
Pre-fresh body condition score			4	831	0.337
≤ 2.75 body condition score	-3.41	1.70			
3.00 body condition score	-3.84	1.48			
3.25 body condition score	-2.42	1.37			
3.50 body condition score	-2.54	1.47			
≥ 3.75 body condition score	—[5]	—[5]			
Calving month			2	831	0.271
June	—[5]	—[5]			
July	1.42	1.06			
August	0.24	1.06			
Prev. lact. milk yield,[6] % rank within herd	0.260	0.029	1	831	< 0.001
Previous lactation length, d	-0.0095	0.0041	1	831	0.023
Previous gestation length, d	0.040	0.081	1	831	0.005
Days in milk at first test	0.449	0.033	1	831	< 0.001
Herd * Previous gestation length			1	831	0.028
Herd A and Previous gestation length	0.275	0.125			
Herd B and Previous gestation length	—[5]	—[5]			

(Table 6 is continued on the next page)

Table 6, continued

Effect and effect level	Estimate	SE	Num.[3] df	Den.[3] df	*P*-value[4]
Herd ⁎ Calving month			2	831	0.003
Herd A and July calving month	-3.77	1.67			
Herd A and August calving month	0.94	1.64			
All other combinations	—[5]	—[5]			
Herd ⁎ Pre-fresh body condition score			4	831	0.028
Herd A and ≤ 2.75 body condition score	5.01	2.63			
Herd A and 3.00 body condition score	7.17	2.41			
Herd A and 3.25 body condition score	6.80	2.30			
Herd A and 3.50 body condition score	4.50	2.48			
All other combinations	—[5]	—[5]			
Oral Ca bolus ⁎ Prev. lact. milk yield			1	831	0.015
No oral Ca boluses and Prev. milk yield	-0.092	0.038			
Oral Ca boluses and Prev. milk yield	—[5]	—[5]			
Oral Ca bolus ⁎ Pre-fresh lameness			1	831	0.042
No oral Ca bolus and Not lame	4.00	1.97			
All other combinations	—[5]	—[5]			

[1] Health events included were metritis, ketosis, displaced abomasum, mastitis, pneumonia, herd removal, or death (only for events in the first 30 DIM).

[2] Bovikalc, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO. Cows in the oral Ca bolus group received 2 oral Ca boluses (1 bolus at calving and 1 bolus 8 to 35 h after calving).

[3] Num. = numerator; Den. = denominator.

[4] Except for the intercept, *P*-values reported are the type 3 test of fixed effects for the entire variable.

[5] Reference group.

[6] 305 d mature equivalent milk production; 100% represented the average cow in the herd at the time the cow calved.

Table 7. Least squares means for cows supplemented with an oral Ca bolus[1] or control cows (no oral Ca bolus supplementation) for continuous or categorical study outcomes from 927 second and greater lactation cows in 2 commercial dairies. Results are from small models that included oral Ca bolus supplementation, herd, and their interaction (if $P < 0.05$ for the interaction term).

	Oral Ca bolus			Control				
Outcome	n	lsmean	SE	n	lsmean	SE	Diff.[2]	*P*-value[3]
Ionized Ca, 8 to 35 h post-calving, mmol/L								
all cows	418	1.15	0.01	425	1.14	0.01	0.01	0.271
lame[4] or prev. milk > 105%[5]	199	1.15	0.01	209	1.12	0.01	0.02	0.123
First test milk yield, kg								
all cows	412	41.1	0.5	497	40.5	0.5	0.6	0.404
prev. milk > 105%	168	46.1	0.8	244	43.3	0.7	2.9	0.009
lame or prev. milk > 105%	198	45.2	0.7	233	42.2	0.7	3.1	0.002
Sum of health events ≤ 30 dim								
all cows	431	0.84	0.04	496	0.76	0.04	0.07	0.182
lame	52	0.89	0.12	51	1.23	0.12	-0.34	0.040
lame or prev. milk > 105%	204	0.72	0.06	238	0.76	0.05	-0.04	0.628
Post-fresh locomotion score								
all cows	381	1.74	0.04	409	1.82	0.05	-0.06	0.256
lame or prev. milk > 105%	187	1.78	0.06	202	1.82	0.05	-0.04	0.776
Post-fresh body condition score								
all cows	381	2.49	0.02	410	2.57	0.02	-0.08	0.089[6]
lame or prev. milk > 105%	187	2.46	0.03	202	2.48	0.03	-0.01	0.756
Days open for cows pregnant by 150 DIM								
all cows	239	96.6	1.5	266	93.5	3.1	2.0	0.124
lame or prev. milk > 105%	119	99.8	2.1	124	93.0	2.0	6.8	0.053
Days from VWP to conception for cows to 305 DIM[7]								
all cows	295	70.9	4.2	343	68.8	3.7	2.1	0.930
lame or prev. milk > 105%	146	69.0	5.4	167	68.0	5.1	0.9	0.796

[1] Bovikalc, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO. Cows in the supplemented group received 2 oral Ca boluses (1 bolus at calving and 1 bolus 8 to 35 h after calving).

[2] Difference in least squares means, oral Ca bolus - control.

[3] For interaction terms, *P* = the higher of the specific comparison or the interaction term.

[4] Lame cows had a pre-fresh locomotion score of 3 or 4, using a 1 to 4 point scale.

[5] Previous lactation mature equivalent milk yield, % of herd rank.

[6] The *P* value for post-fresh body condition score is from the large model, which was $P > 0.05$ and was greater than the *P* value from the small model.

[7] Estimate of mean days from the end of the voluntary waiting period to pregnancy using Kaplan-Meier analysis. Mean voluntary waiting period was 72.5 days for all cows and 72.7 days for cows in the subpopulation targeted for oral Ca bolus supplementation.

Table 8. Least squares means for cows supplemented with an oral Ca bolus[1] or control cows with no oral Ca bolus supplementation for binary outcomes (health events ≤ 30 DIM and reproductive outcomes) from 927 second and greater lactation cows in 2 commercial dairies. Results are from small models that included oral Ca bolus supplementation, herd, and their interaction (if $P < 0.05$ for the interaction term).

	Oral Ca bolus[1]		Control		Oral Ca vs. control		
Outcome	n	risk (%)	n	risk (%)	risk ratio	95% CI	P-value[2]
Metritis, %							
all cows	431	17.2%	496	15.2%	1.13	0.85 to 1.51	0.394
lame[3] or prev. milk > 105%[4]	206	14.0%	238	14.9%	0.94	0.61 to 1.47	0.794
Ketosis[5], %							
all cows	290	60.5%	334	57.4%	1.40	0.93 to 1.19	0.422
lame or prev. milk > 105%	129	55.7%	165	54.2%	1.03	0.85 to 1.25	0.834
Displaced abomasum, %							
all cows	431	4.4%	496	4.1%	1.09	0.61 to 1.96	0.764
lame or prev. milk > 105%	206	4.0%	238	5.0%	0.81	0.35 to 1.87	0.619
Mastitis, %							
all cows	431	12.9%	496	10.6%	1.22	0.84 to 1.77	0.297
lame or prev. milk > 105%	206	13.7%	238	9.8%	1.39	0.83 to 2.34	0.512
Pneumonia, %							
all cows	431	2.2%	496	2.4%	0.91	0.43 to 1.93	0.813
lame or prev. milk > 105%	206	1.2%	238	3.6%	0.32	0.09 to 1.11	0.073
Removal from the herd %							
all cows	431	6.5%	496	4.6%	1.41	0.85 to 2.33	0.182
lame or prev. milk > 105%	206	5.1%	238	4.3%	1.17	0.54 to 2.55	0.686
Died, %							
all cows	431	2.7%	496	1.5%	1.78	0.79 to 4.02	0.167
lame or prev. milk > 105%	206	2.5%	238	1.4%	1.76	0.52 to 6.04	0.877
First service conception, %							
all cows	343	38.6%	398	42.0%	0.92	0.77 to 1.10	0.347
lame or prev. milk > 105%	168	33.8%	193	39.6%	0.85	0.65 to 1.11	0.434
Pregnancy by 150 DIM, %							
all cows	337	70.5%	390	67.8%	1.04	0.95 to 1.15	0.416
lame or prev. milk > 105%	164	72.1%	186	66.1%	1.09	0.95 to 1.26	0.391

[1] Bovikalc, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO. Cows in the supplemented group received 2 oral Ca boluses (1 bolus at calving and 1 bolus 8 to 35 h after calving).

[2] For interaction terms, P = the higher of the specific comparison or the interaction term.

[3] Lame cows had a pre-fresh locomotion score of 3 or 4, using a 1 to 4 point scale.

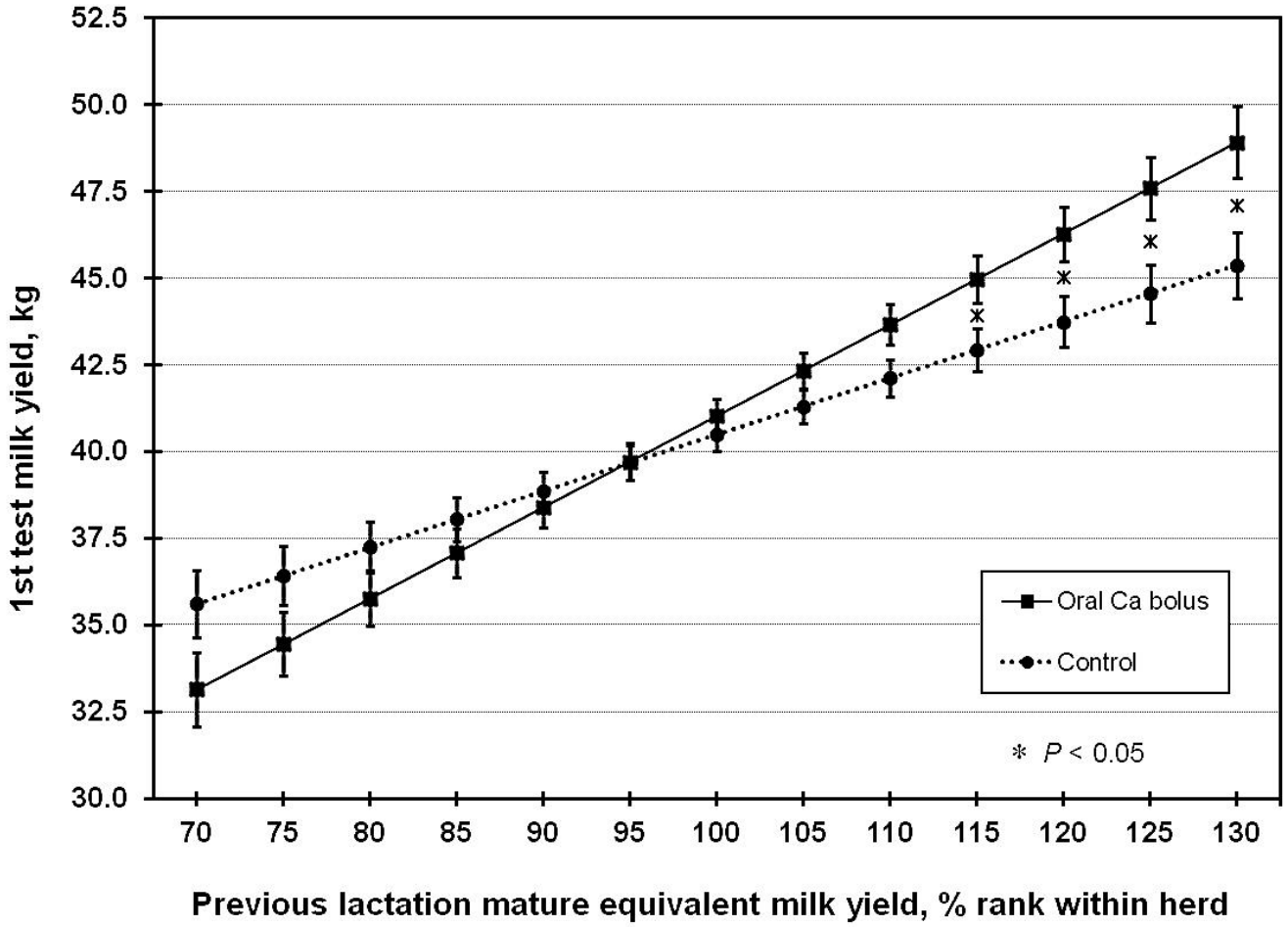
[4] Previous lactation mature equivalent milk production, % of herd rank.

[5] Ketosis was defined as one or more blood BHBA tests ≥ 1.2 mmol/L. Cows were tested six times between 3 and 16 DIM and were classified if they had ≥ 5 total BHBA tests or ≥ 1 positive test.

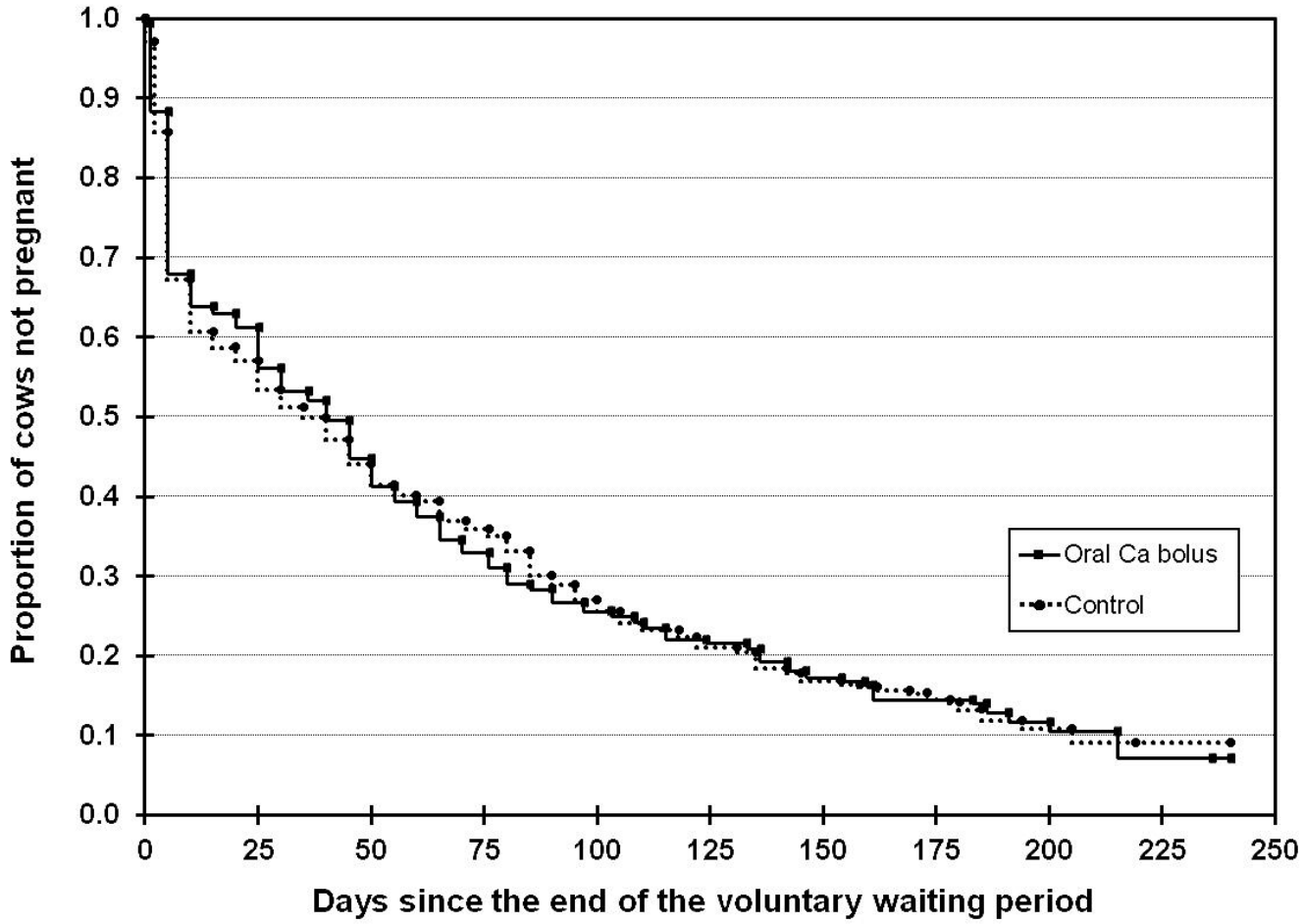
Figure 1. Interaction between between oral Ca bolus supplementation and previous lactation mature equivalent milk yield (expressed as % rank within herd) for first test milk yield after calving. Oral Ca bolus supplemented cows received 2 boluses (Bovikalc, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) after calving. Control cows were not supplemented with oral Ca boluses. Data are from 927 second and greater lactation cows in 2 commercial dairies.

Figure 2. Kaplan-Meier plot for the effect of oral Ca bolus supplementation on pregnancy to 305 DIM. Oral Ca bolus supplemented cows received 2 boluses (Bovikalc, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) after calving. Control cows were not supplemented with oral Ca boluses. Data are from 638 second and greater lactation cows in 2 commercial dairies that were eligible to be bred at the end of the voluntary waiting period.

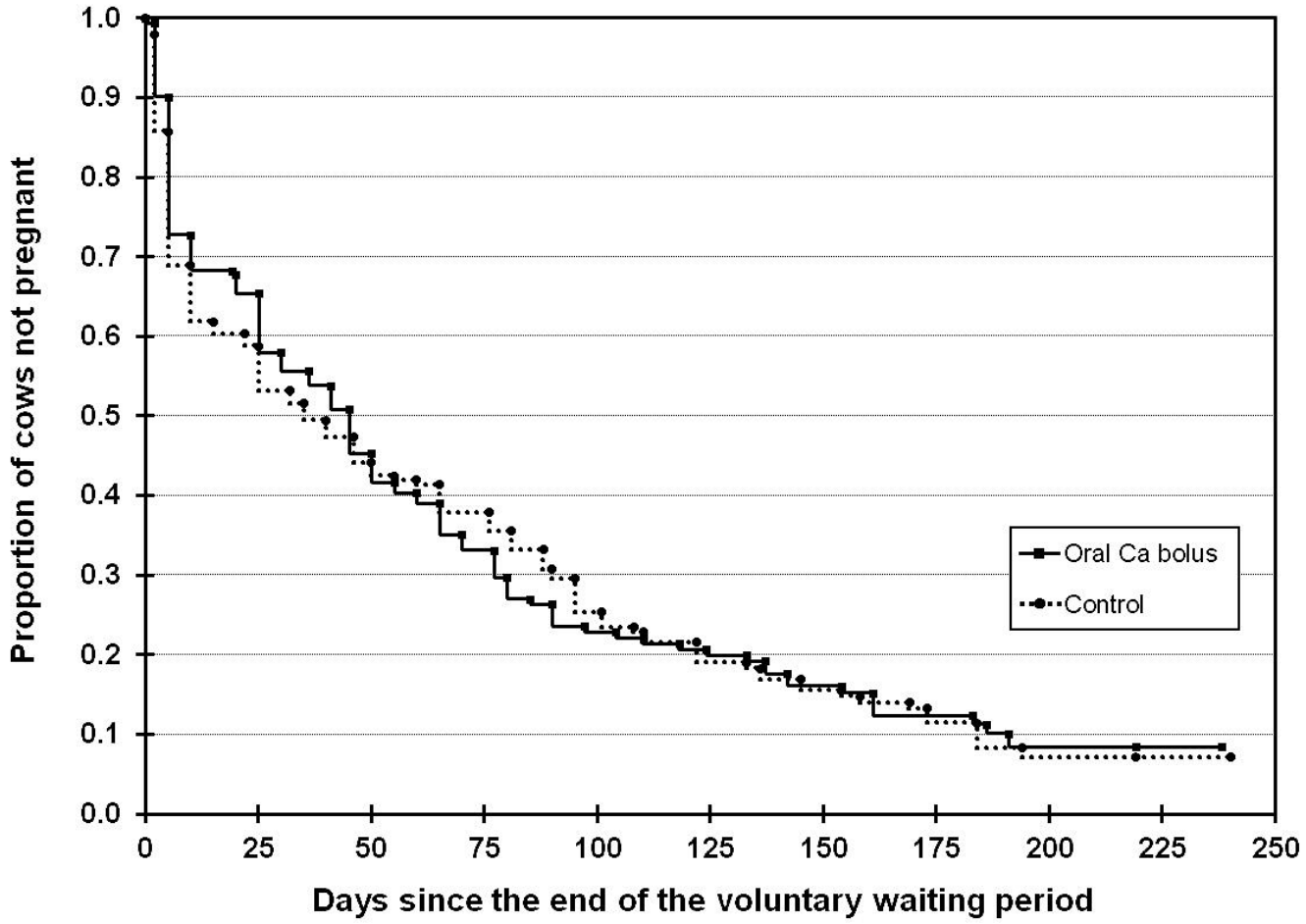
Figure 3. Kaplan-Meier plot for the effect of oral Ca bolus supplementation on pregnancy to 305 DIM. Oral Ca bolus supplemented cows received 2 boluses (Bovikalc, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO) after calving. Control cows were not supplemented with oral Ca boluses. Data are from 313 second and greater lactation cows in 2 commercial dairies that were eligible to be bred at the end of the voluntary waiting period and were in the subpopulation targeted for oral Ca bolus supplementation (previous lactation mature equivalent milk production > 105% of herd average at calving or pre-fresh lameness or both).



JDS-12-5510, Oetzel and Miller, Figure 1



JDS-12-5510, Oetzel and Miller, Figure 2



JDS-12-5510, Oetzel and Miller, Figure 3