

## ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

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

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### 11 Effect of Oral Calcium Bolus Supplementation on Early Lactation Health 12 and Milk Yield in Commercial Dairy Herds

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### ABSTRACT

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

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

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

### 47 INTRODUCTION

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

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

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

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

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

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

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

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

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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  $\text{Ca}^{2+}$  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

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

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

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

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

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157 normal management activities of large dairies and required cows to be restrained in headlocks  
158 only once daily. The schedule was also chosen to include the time period cows are expected to  
159 be experiencing the most profound hypocalcemia, which is about 12 to 24 h post-calving (Goff,  
160 2008; Ramos-Nieves et al., 2009). Mean time between calving and administration of the second  
161 bolus was 20.6 h ( $\pm$  0.2 SEM).

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

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

### 175 **Study outcomes**

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



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

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

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

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

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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  $\text{Ca}^{2+} \leq 1.00$  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

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

### 242 ***Statistical analysis***

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

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

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

262 The effect of blanket supplementation with 2 oral Ca boluses for all study cows was  
263 evaluated using a two-step statistical method. The method was the same whether the outcome  
264 was continuous or categorical (using multivariate linear regression), binary (using multivariate  
265 Poisson regression), or pregnancy status by 305 DIM (using survival analyses). First, a large  
266 model for each study outcome was fitted using every potential covariate applicable to that  
267 outcome, plus the interactions of each covariate with oral Ca bolus supplementation and with  
268 herd. The specific terms available to the large models were herd (as a fixed effect), oral Ca bolus  
269 supplementation, lactation group (second, third, or fourth and greater), pre-fresh lameness status  
270 (0 if locomotion score 1 or 2, 1 if locomotion score 3 or 4), pre-fresh body condition score ( $\leq$   
271 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  
272 calf or calves, 1 if 1 or 2 calves born dead), calving ease score category (1 if ease score 1, 2 if  
273 ease score 2, 3 if ease score 3 or 4), calving month (June, July, or August), previous lactation  
274 mature equivalent milk production (expressed as percent rank within herd - the values for all

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275 cows in each herd were ranked from lowest to highest and then assigned a percent rank, with a  
276 value of 100% representing the average mature equivalent milk production in the herd at that  
277 time), previous lactation length (days), previous lactation days dry, and previous lactation  
278 gestation length (days). Days in milk at first breeding, AI synchronization status (synchronized  
279 = 1, not synchronized = 0), and first breeding month (instead of calving month) were included in  
280 the analyses for first service conception, pregnancy by 150 DIM, and days open for cows  
281 pregnant by 150 DIM. The interactions between all variables and herd, plus all interactions  
282 between these variables and oral Ca bolus supplementation were also eligible for inclusion in the  
283 large models. Terms were removed from the models in a stepwise, backwards fashion until all  
284 were  $P < 0.05$  in the model. Single variables were removed from the model only after there were  
285 no interaction terms including that variable remaining in the model.

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

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

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

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

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

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

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

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

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

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

346 **RESULTS AND DISCUSSION**

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

353 The study was conducted only in summer months; however, it is unlikely that the low  
354 observed incidence of hypocalcemia was a seasonal effect. Østergaard et al. (2003) attempted to  
355 find published evidence for seasonality of clinical milk fever but was unsuccessful. An  
356 evaluation of calvings and cases of clinical milk fever from six years of data in the Dairy Comp  
357 305 archives for both study herds (80 cases of milk fever and 18,887 fresh events for second and  
358 greater lactation cows) revealed no difference between the risk for milk fever for cows calving in  
359 the summer months compared to cows who calved any other time of the year (relative risk for  
360 milk fever with summer calvings was 0.63, with a 95% confidence interval of 0.36 to 1.13).

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

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



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

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

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

## ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

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

393 A new analysis was then conducted, starting with a new stepwise, backwards elimination in  
394 the large model, to determine how the new dichotomous variable (previous lactation mature  
395 equivalent milk yield  $> 105\%$  or  $\leq 105\%$  of herd rank) affected oral Ca bolus response. Cows  
396 above the cutpoint had significantly ( $P = 0.024$ ) increased milk yield when supplemented with  
397 oral Ca bolus compared to cows above the cutpoint who were not supplemented. This  
398 dichotomized variable was then included in a final, small model. The response to oral Ca bolus  
399 supplementation in cows with high previous lactation milk yield was  $+2.9$  kg of milk at first DHI  
400 test after calving ( $P = 0.009$ , Table 7). This increase in milk yield (7.2%) was of greater  
401 magnitude than the 3.6% increase in milk yield reported for cows supplemented with anionic  
402 salts (Beede et al., 1992). The inclusion of first lactation animals in the analysis of Beede et al.  
403 (1992) could explain a portion of the lower overall milk production response. Smaller-scale  
404 studies have shown no effect of oral Ca supplementation on subsequent milk yield (Goff et al.,  
405 1996; Dhiman and Sasidharan, 1999; Melendez et al., 2002); these studies did not attempt to  
406 identify subpopulations of cows that might have a different response to oral Ca supplementation.

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

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413 (1999) reported no effect of oral Ca supplementation around calving on later concentrations of  
414 BHBA, and Goff et al. (1996) reported no effect of oral Ca supplementation on rates of primary  
415 ketosis. These findings are in agreement with the current study, which demonstrated no effect of  
416 oral Ca supplementation on the incidence of cows with blood BHBA  $\geq 1.2$  mmol/L.

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

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

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436 at 8 to 35 h after calving ( $P = 0.271$ ). The timing of the collection of the  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$ .

444 It was not feasible in the current study to document blood  $\text{Ca}^{2+}$  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 of oral Ca bolus supplementation cannot be  
451 determined from the results of this study. Lamé 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 lamé 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.

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459 Over-conditioned cows are reported to be at increased risk for hypocalcemia (Heuer et al.,  
460 1999). However, pre-fresh body condition score did not have a significant interaction with oral  
461 Ca bolus supplementation in the current study. The number of overly conditioned animals (body  
462 condition score  $\geq 4.00$ ) was only about 4% for the 2 study herds.

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

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

### 477 **CONCLUSIONS**

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

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482 that had high previous lactation mature equivalent milk production and were supplemented with  
483 oral Ca boluses had increased early lactation milk yield compared to high-producing cows that  
484 were not supplemented. This subpopulation of lame and higher producing cows represented  
485 48% of the multiparous cows in the 2 study herds. Supplementation of the combined  
486 subpopulation of cows with oral Ca boluses resulted in increased milk yield without affecting  
487 early lactation health. These results indicate that dairy herds already experiencing a very low  
488 incidence of hypocalcemia can target a subpopulation of cows that will respond favorably to  
489 supplementation with oral Ca boluses.

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**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.

Feed ingredient or nutrient	Herd A		Herd B	
	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, <sup>1</sup> %	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, <sup>2</sup> mEq/kg	-109	283	-18	285

<sup>1</sup> NFC = Non-fiber carbohydrates, calculated as 100 - CP - Ether Extract - NDF - Ash.

<sup>2</sup> DCAD = Dietary cation-anion difference, calculated as mEq [(Na + K) - (Cl + S)].

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**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

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**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.

Item	Herd A			Herd B		
	n	mean	SEM	n	mean	SEM
Continuous or categorical outcomes:						
Ca <sup>2+</sup> 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 <sup>1</sup> ≤ 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

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

ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

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

Item	Herd A			Herd B		
	1 <sup>1</sup>	0 <sup>2</sup>	%	1	0	%
Binary outcomes:						
clinical milk fever	0	372	0.0	6	549	1.1
hypocalcemia <sup>3</sup>	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 <sup>4</sup> ≤ 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 <sup>5</sup>	41	325	11.2	63	469	11.8

<sup>1</sup> 1 = count of cows with the condition.

<sup>2</sup> 0 = count of cows without the condition.

<sup>3</sup> Hypocalcemia was defined as blood Ca<sup>2+</sup> ≤ 1.00 mmol/L at 8 to 35 h after calving.

<sup>4</sup> 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.

<sup>5</sup> Lameness cows had a pre-fresh locomotion score of 3 or 4, using a 1 to 4 point scale.

ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

**Table 5.** Estimates and Type 3 tests of fixed effects from a reduced mixed regression model for the sum of health events<sup>1</sup> in the first 30 DIM. All possible covariates plus interactions with herd and oral Ca bolus<sup>2</sup> 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. <sup>3</sup> df	Den. <sup>3</sup> df	$P$ -value <sup>4</sup>
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)	— <sup>5</sup>	— <sup>5</sup>			
Herd			1	867	0.002
Herd A	-0.406	0.322			
Herd B	— <sup>5</sup>	— <sup>5</sup>			
Lactation group			2	867	0.032
Lactation = 2	-0.170	0.068			
Lactation = 3	-0.051	0.073			
Lactation ≥ 4	— <sup>5</sup>	— <sup>5</sup>			
Pre-fresh lameness			1	867	0.046
Not lame (locomotion score 1 or 2)	0.064	0.121			
Lame (locomotion score 3 or 4)	— <sup>5</sup>	— <sup>5</sup>			
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	— <sup>5</sup>	— <sup>5</sup>			
Calving month			2	867	< 0.001
June	— <sup>5</sup>	— <sup>5</sup>			
July	0.037	0.070			
August	-0.276	0.072			
Prev. lact. milk yield, <sup>6</sup> % 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	— <sup>5</sup>	— <sup>5</sup>			



ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

(Table 5 is continued on the next page)

ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

**Table 5**, continued.

Effect and effect level	Estimate	SE	Num. <sup>3</sup> df	Den. <sup>3</sup> df	<i>P</i> -value <sup>4</sup>
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	— <sup>5</sup>	— <sup>5</sup>			
Oral Ca bolus * Pre-fresh lameness			1	867	0.005
No oral Ca boluses and Not lame	-0.474	0.169			
All other combinations	— <sup>5</sup>	— <sup>5</sup>			

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

<sup>2</sup> Bovikal<sup>®</sup>, 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).

<sup>3</sup> Num. = numerator; Den. = denominator.

<sup>4</sup> Except for the intercept, *P*-values reported are the type 3 test of fixed effects for the entire variable.

<sup>5</sup> Reference group.

<sup>6</sup> 305 d mature equivalent milk production; 100% represented the average cow in the herd at the time the cow calved.

ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

**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<sup>1</sup> 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. <sup>3</sup> df	Den. <sup>3</sup> df	<i>P</i> -value <sup>4</sup>
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)	— <sup>5</sup>	— <sup>5</sup>			
Herd			1	831	0.045
Herd A	-73.39	34.54			
Herd B	— <sup>5</sup>	— <sup>5</sup>			
Pre-fresh lameness			1	831	0.478
Not lame (locomotion score 1 or 2)	-1.30	1.40			
Lame (locomotion score 3 or 4)	— <sup>5</sup>	— <sup>5</sup>			
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	— <sup>5</sup>	— <sup>5</sup>			
Calving month			2	831	0.271
June	— <sup>5</sup>	— <sup>5</sup>			
July	1.42	1.06			
August	0.24	1.06			
Prev. lact. milk yield, <sup>6</sup> % 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	— <sup>5</sup>	— <sup>5</sup>			

(Table 6 is continued on the next page)

ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

**Table 6**, continued

Effect and effect level	Estimate	SE	Num. <sup>3</sup> df	Den. <sup>3</sup> df	<i>P</i> -value <sup>4</sup>
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	— <sup>5</sup>	— <sup>5</sup>			
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	— <sup>5</sup>	— <sup>5</sup>			
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	— <sup>5</sup>	— <sup>5</sup>			
Oral Ca bolus * Pre-fresh lameness			1	831	0.042
No oral Ca bolus and Not lame	4.00	1.97			
All other combinations	— <sup>5</sup>	— <sup>5</sup>			

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

<sup>2</sup> Bovikal<sup>®</sup>, 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).

<sup>3</sup> Num. = numerator; Den. = denominator.

<sup>4</sup> Except for the intercept, *P*-values reported are the type 3 test of fixed effects for the entire variable.

<sup>5</sup> Reference group.

<sup>6</sup> 305 d mature equivalent milk production; 100% represented the average cow in the herd at the time the cow calved.

ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

**Table 7.** Least squares means for cows supplemented with an oral Ca bolus<sup>1</sup> 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).

Outcome	Oral Ca bolus			Control			Diff. <sup>2</sup>	P-value <sup>3</sup>
	n	lsmean	SE	n	lsmean	SE		
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 <sup>4</sup> or prev. milk > 105% <sup>5</sup>	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 $\leq$ 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 <sup>6</sup>
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 <sup>7</sup>								
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

<sup>1</sup> Bovicalc, 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).

<sup>2</sup> Difference in least squares means, oral Ca bolus - control.

<sup>3</sup> For interaction terms,  $P$  = the higher of the specific comparison or the interaction term.

<sup>4</sup> Lame cows had a pre-fresh locomotion score of 3 or 4, using a 1 to 4 point scale.

<sup>5</sup> Previous lactation mature equivalent milk yield, % of herd rank.

<sup>6</sup> 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.

<sup>7</sup> 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.

ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

**Table 8.** Least squares means for cows supplemented with an oral Ca bolus<sup>1</sup> or control cows with no oral Ca bolus supplementation for binary outcomes (health events  $\leq$  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).

Outcome	Oral Ca bolus <sup>1</sup>		Control		Oral Ca vs. control		P-value <sup>2</sup>
	n	risk (%)	n	risk (%)	risk ratio	95% CI	
Metritis, %							
all cows	431	17.2%	496	15.2%	1.13	0.85 to 1.51	0.394
lame <sup>3</sup> or prev. milk > 105% <sup>4</sup>	206	14.0%	238	14.9%	0.94	0.61 to 1.47	0.794
Ketosis <sup>5</sup> , %							
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

<sup>1</sup> Bovicalc, 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).

<sup>2</sup> For interaction terms,  $P$  = the higher of the specific comparison or the interaction term.

<sup>3</sup> Lameness had a pre-fresh locomotion score of 3 or 4, using a 1 to 4 point scale.

<sup>4</sup> Previous lactation mature equivalent milk production, % of herd rank.

<sup>5</sup> Ketosis was defined as one or more blood BHBA tests  $\geq$  1.2 mmol/L. Cows were tested six times between 3 and 16 DIM and were classified if they had  $\geq$  5 total BHBA tests or  $\geq$  1 positive test.

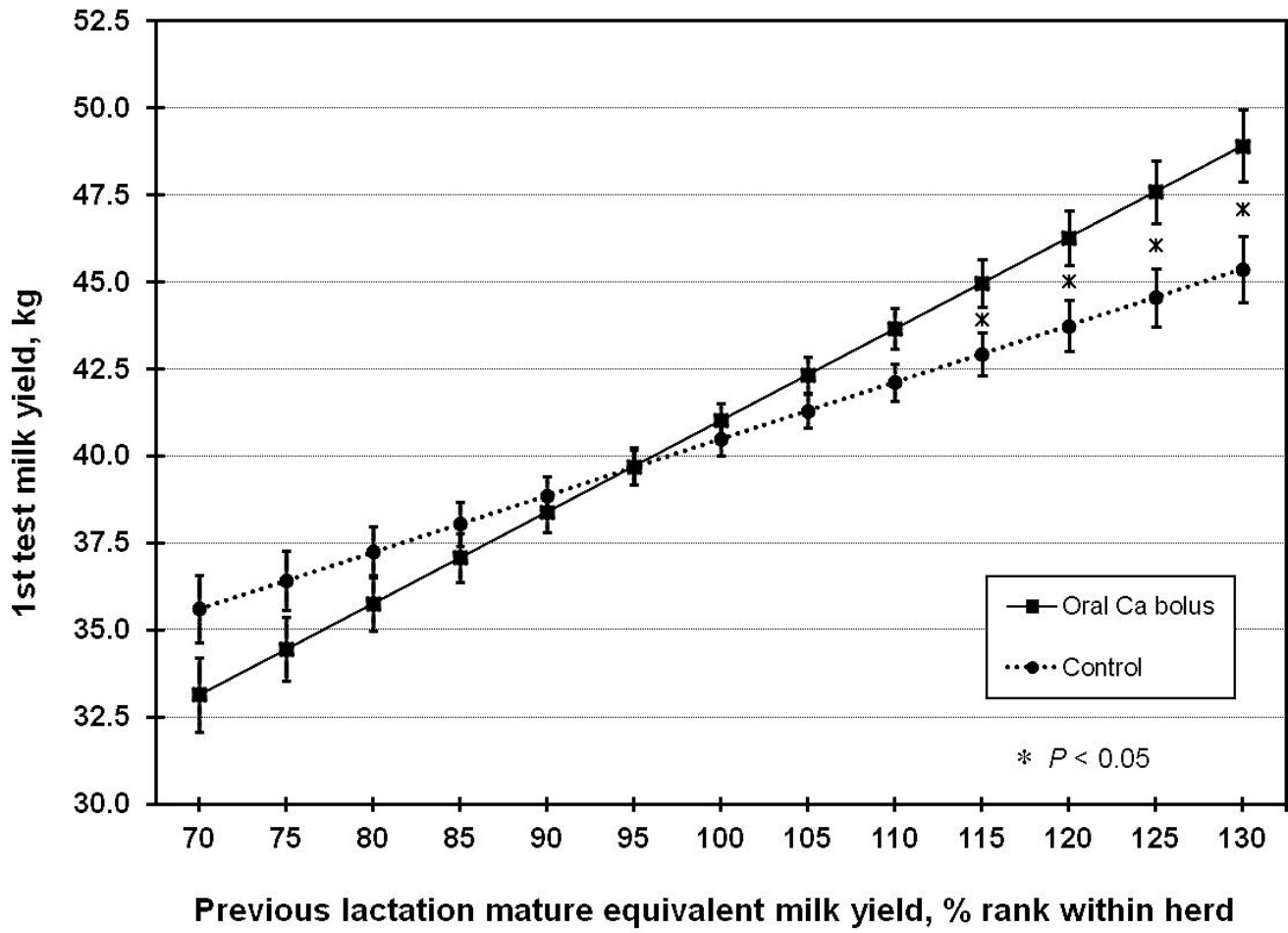
## ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS

**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).

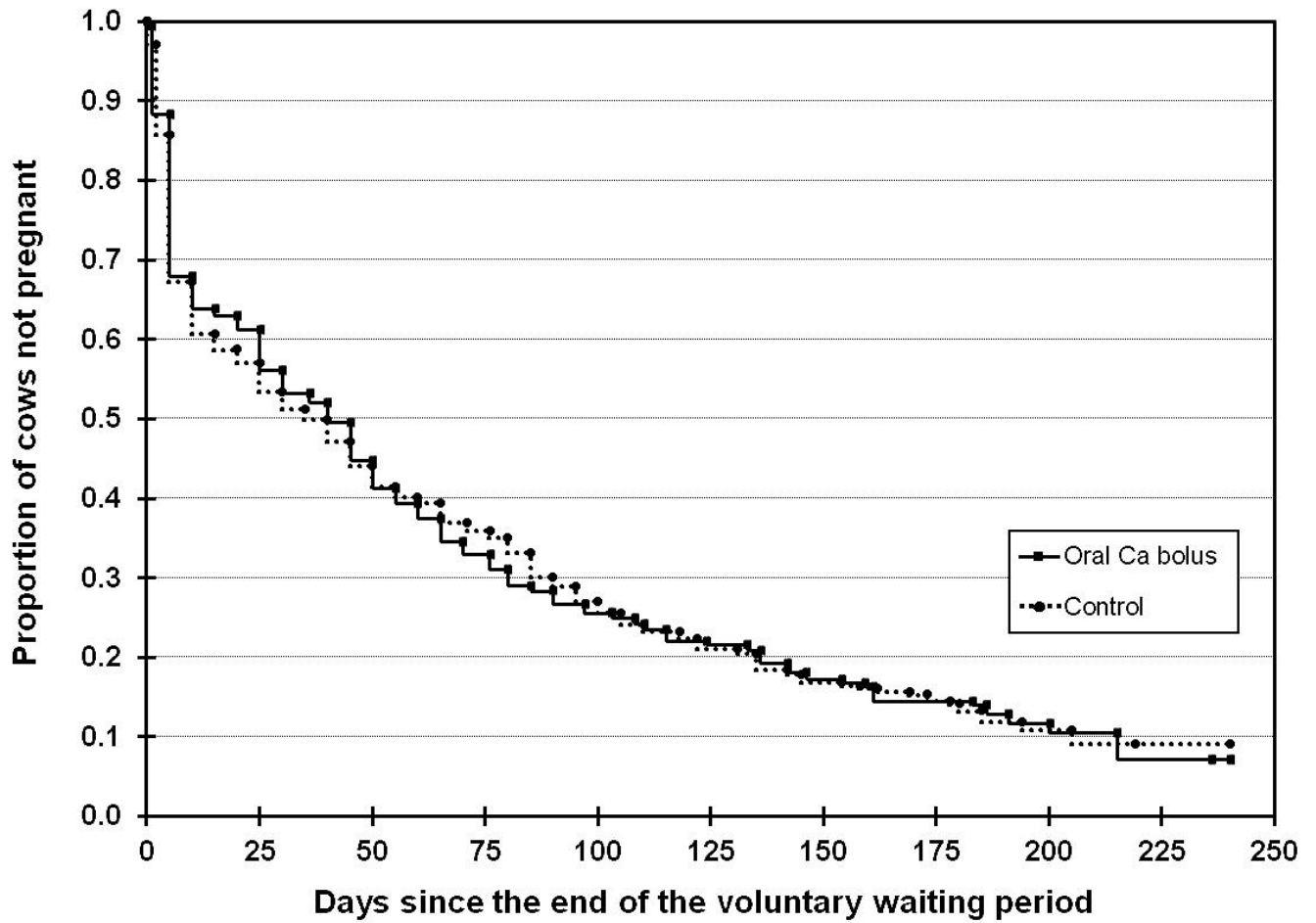
ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS



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

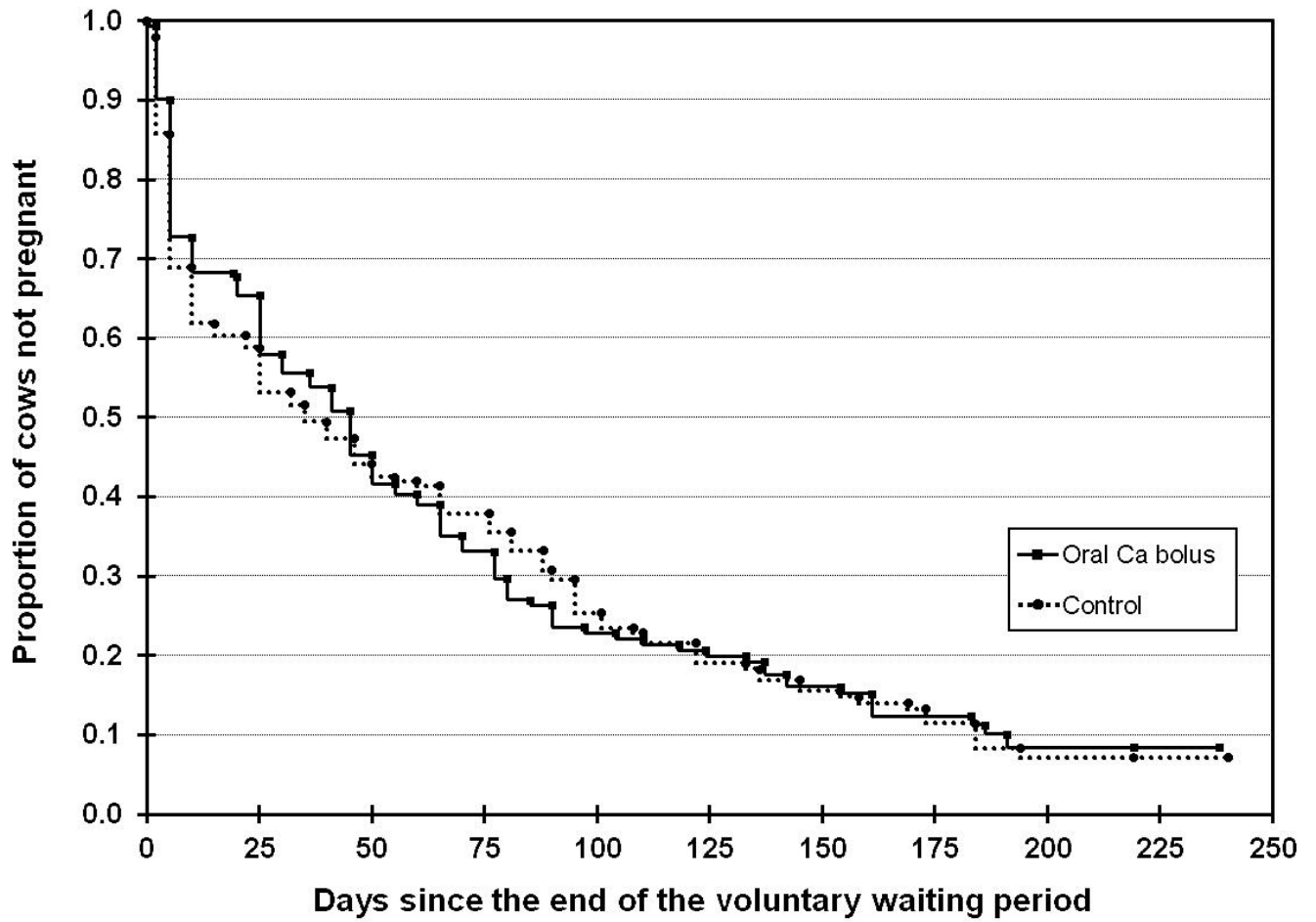


ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS



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

ORAL CALCIUM BOLUSES FOR PARTURIENT DAIRY COWS



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