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Differential effects of a single dose of oral calcium based on postpartum plasma calcium concentration in Holstein cows

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ABSTRACT

Our objectives were to determine (1) the effect of a single dose of an oral Ca bolus within 24 h after parturition on plasma Ca concentration, (2) the response of primiparous (PP) and multiparous (MP) cows to this supplementation strategy, and (3) differential responses based on plasma Ca at enrollment. For objective 1, cows from 1 commercial dairy in New York State were enrolled within 19 h after parturition (mean ± standard deviation = 8.3 ± 5.3 h) and randomized within parity group (first, second, and ≥third) to control [CON (n = 25); no placebo] or a single dose bolus treatment [BOL (n = 25); 3 oral Ca boluses supplying 54 to 64 g of Ca]. Plasma Ca was measured repeatedly between 1 and 24 h following treatment. For objectives 2 and 3, cows on 6 commercial farms in New York State were assigned to treatment as for objective 1 (CON, n = 1,973; BOL, n = 1,976). Herd records for health, reproduction, and Dairy Herd Improvement Association test day milk production were collected. Mixed effect multivariable models were developed using repeated measures ANOVA, Poisson regression, or proportional hazard models. Objective 2 analyses considered treatment with periparturient risk factors, whereas objective 3 analyses also considered Ca status. No difference was observed for plasma Ca between 1 and 24 h after treatment. Primiparous cows assigned to BOL calving at >712 d old had decreased risk of one or more health disorders [≤30 d in milk; risk ratio (RR) = 0.65, 95% confidence interval (CI) = 0.51 to 0.84] and those with body condition score >3.5 responded to BOL with increased milk production (CON = 31.7 ± 1.1, BOL = 35.1 ± 1.1 kg/d), as did those with days carried calf >277 (CON = 31.9 ± 1.0, BOL = 34.7 ± 1.0 kg/d). Reduced risk of one or more health disorders was observed in parity ≥3 (RR = 0.85, 95% CI = 0.81 to 0.89) and MP cows with body condition score >3.5 (retained placenta; RR = 0.70, 95% CI = 0.58 to 0.84) or that were lame (displaced abomasum; RR = 0.49, 95% CI = 0.32 to 0.75). Differential responses for PP cows by Ca status were minimal. For MP cows with low plasma Ca, BOL decreased risk of additional Ca treatment (≤1.8 mmol/L; RR = 0.57, 95% CI = 0.40 to 0.80) as well as risk of one or more health disorders (≤2.15 mmol/L; RR = 0.90, 95% CI = 0.85 to 0.95). Supplementation with a single oral dose of Ca could be targeted to periparturient risk groups for improved health. Calcium status did not differentiate responses of PP cows, but MP cows with low Ca at parturition had improved health status when supplemented.
Key words: oral calcium bolus, subclinical hypocalcemia, transition cow

INTRODUCTION

Dairy cows experience many metabolic adaptations around the time of parturition (Bauman and Currie, 1980), one of which is meeting the increased demand for Ca that occurs at the onset of colostrum and milk production (Ramberg et al., 1970) compared with the relatively low Ca demand for fetal development in late gestation (House and Bell, 1993). Even with multiple physiological adaptations in place to maintain normocalcemia, many cows fail to meet this demand without compromising systemic Ca status. As a result, these cows can experience clinical hypocalcemia or, in the absence of clinical symptoms, have subclinical hypocalcemia (SCH). Recent research has demonstrated that cows with SCH suffer exacerbated negative energy balance and metabolic diseases (Chapinal et al., 2011; Chamberlin et al., 2013) as well as increased susceptibility to metritis in the early postpartum period (Martinez et al., 2012). Subclinical hypocalcemia has been associated with greater risk of early lactation culling (Roberts et al., 2012) and compromised reproductive performance (Chapinal et al., 2012; Martinez et al., 2012).

In an effort to mitigate some of the downstream consequences for cows that experience SCH, supplemental

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Ca can be provided by intravenous administration of readily available Ca or oral administration of Ca salts in the form of pastes or boluses. Whereas intravenous Ca is necessary for cows displaying clinical signs of hypocalcemia to prevent or treat recumbency (Oetzel, 2013), oral Ca supplements in the form of boluses may be more appropriate for suspected cases of SCH and have been demonstrated to provide a more moderate but sustained increase in blood Ca concentration (Blanc et al., 2014). Due to the lack of cow-side tests validated for measurement of blood Ca, selection of cows to receive supplemental Ca is often based primarily on parity, recognizing that older cows are more susceptible to SCH, with data suggesting that approximately 50% of older cows have subclinically low blood Ca in the day after parturition (Reinhardt et al., 2011; Caixeta et al., 2015). The variation in SCH incidence between herds has not been demonstrated and these estimates from previous work may not be reliable for application across populations. Recent research conducted with oral Ca boluses indicates that group level responses are minimal (Martinez et al., 2016b) or not evident (Oetzel and Miller, 2012). When specific risk groups are assessed, effects of Ca supplementation in the immediate postpartum period can be beneficial, such as in cows with high production potential and cows that are lame near parturition (Oetzel and Miller, 2012; Martinez et al., 2016b). However, detrimental responses have been observed in primiparous (PP) cows and multiparous (MP) cows that have lower productive potential or decreased risk of uterine disease (Martinez et al., 2016a,b). Accounting for blood Ca status of cows at parturition in conjunction with presence or absence of certain risk factors may further identify subgroups for more targeted supplementation.

Previous work investigating oral Ca bolus administration has been conducted using a multiple dosing strategy (Oetzel and Miller, 2012; Martinez et al., 2016a). This approach requires additional labor and cow handling. A single dose of oral Ca after parturition warrants investigation because this strategy would not require additional cow handling after cows are processed at the time of parturition and may result in more consistent field application. The efficacy of a single dose of oral Ca at parturition on health and performance outcomes has not been addressed previously. The primary objective of our study was to determine the effect of administering a single dose of an oral Ca bolus within 24 h after parturition in an all-inclusive approach on blood Ca concentration, health outcomes, and productive and reproductive performance with consideration of additional periparturient risk factors. Our secondary objective was to determine the differential response to bolus administration based on blood Ca status before treatment allocation. We hypothesized that a single dose of oral Ca would increase blood Ca concentration and that differential responses to oral Ca supplementation would be observed in health and performance outcomes because of periparturient risk factors and Ca status.

MATERIALS AND METHODS

Farms and Study Population

All animal use and procedures were approved by the Cornell University Institutional Animal Care and Use Committee (protocol 2014–0017). This study was conducted at 6 commercial dairy farms in New York State between February and December 2015. Criteria for farm enrollment included at least 500 milking cows, use of herd management software (DairyComp 305, Valley Agricultural Software, Tulare, CA), enrollment in monthly DHIA testing, no prophylactic use of supplemental Ca after calving or willingness to discontinue prophylactic use, and willingness of farm personnel to allocate animals to treatments and adhere to study protocols. These farms represent a convenience sample of eligible farms in close proximity to Lowville or Ithaca, New York. General herd information and details on transition cow management for each farm are outlined in Supplemental Tables S1 to S3 (https://doi.org/10.3168/jds.2017-13164).

Treatments, Animal Sampling, and Sample Analysis

Experiment 1. A subset of cows at one location (farm A) were enrolled to determine the effect of a single oral dose of Ca on blood Ca concentrations over the first 24 h after bolus administration. A total of 50 cows (10 PP and 40 MP) were enrolled within 24 h after parturition (mean $\pm$ SD = 8.3 $\pm$ 5.3 h) between June and July of 2015. A total of 32 d were needed to enroll and sample all cows. The enrollment period was defined as within the 24 h after parturition to allow for different enrollment strategies by farm in experiment 2. A sample size of 50 cows was determined to detect a difference in plasma Ca concentration of 0.12 mmol/L with 95% confidence and 80% power based on the variation in plasma Ca observed in the day following parturition in previous work conducted by our group (Leno et al., 2017). Cows enrolled in this study were a subset of cows enrolled in experiment 2. All cows that were not displaying clinical signs of hypocalcemia at the time of enrollment were eligible to be enrolled. Each farm was provided randomized enrollment sheets blocked by parity groups (first, second, and third or greater). The random number function of Microsoft

Excel (Microsoft Corporation, Redmond, WA) was used to allocate sequential pairs in enrollment sheets to 1 of 2 treatments. Cows were enrolled sequentially by calving date and time onto the next available row of the enrollment sheets for the appropriate parity group. Cows assigned to control (CON) received no intervention after parturition, and cows assigned to the bolus treatment (BOL) received a single dose of an oral Ca bolus (Quadrical, Bio-Vet Inc., Barneveld, WI), which delivered between 54 and 64 g of Ca per dose (3 boluses) in the form of Ca chloride, Ca sulfate, Ca propionate, and Ca lactate. Boluses also contained niacin and vitamin D_3. For the subset of cows included in experiment 1, cows calving over the night shift on farm A were enrolled by research personnel (authors I. L., R. N. and B. L.) the following morning, and therefore the subpopulation for experiment 1 represents the first 40 MP and 10 PP cows that calved overnight during this enrollment window on farm A. Treatment assignment adhered to the chronological order based on calving date and time for experiment 2 and cows were included in the study population for experiment 1 until 20 MP cows and 5 PP cows were enrolled on each treatment.

Blood samples were collected just before treatment allocation (time 0) and at 1, 2, 4, 8, 12, and 24 h after treatment administration. Cows were restrained in headlocks or stalls only for blood sample collection and were otherwise loose in pens with access to feed during the sampling period. Samples were collected from the coccygeal vessels using 20-G evacuated needles and 10-mL lithium heparin evacuated tubes (Greiner Bio-One, Monroe, NC). Plasma was separated by centrifugation at 2,000 × *g* for 10 min in a portable centrifuge at room temperature, harvested, snap frozen in liquid nitrogen, and stored at −20°C. Within 14 h after sample collection, samples were transported to the laboratory for storage at −80°C. Plasma samples were analyzed for total Ca concentration using the colorimetric orthocresolphtalein method (Burtis and Bruns, 2015) at the University of Illinois Veterinary Diagnostic Laboratory (Urbana, IL) using an Olympus AU680 automated chemistry analyzer (Beckman Coulter Inc., Brea, CA) with reagents from Beckman Coulter. The inter- and intraassay coefficients of variation were less than 1%.

Experiment 2. This experiment was conducted at all 6 farms to determine the effect of a single oral dose of Ca on health and performance outcomes with consideration of differential responses to treatment based on periparturient risk factors and subsequently responses to this supplementation strategy based on plasma Ca status before bolus administration. Eligibility criteria, enrollment, and treatments were according to the aforementioned scheme. Cows were enrolled within 24 h after parturition and treatments were administered by trained farm personnel. On farms A and D, certain personnel were responsible for enrollment and therefore enrollment did not necessarily occur at first handling after parturition depending on the shift during which the cow calved. On all other farms, enrollment was conducted by the person who first handled the cow after parturition. The number of personnel responsible for enrollment were 8 on farm A, 3 on farm B, 8 on farm C, 3 on farm D, 5 on farm E, and 3 on farm F. Protocol compliance was monitored daily (farms A and E), twice weekly (farms B, D, and F), or every other week (farm C) at which point enrollment sheets were checked for completeness and any inconsistencies. The blocking of cows within parity groups (first, second, and third or greater) was employed to allow for more even distribution of treated and control groups across parities. A sample size of 500 per group for PP cows was planned to detect a difference between 8.0 and 3.5% for retained placenta (RP), 12.0 and 6.5% for metritis, 15.0 and 9.0% for mastitis, 38 and 47% for pregnancy to first service, and a 1.5 kg/d response in milk yield with 95% confidence and power ≥80%. For MP cows, a sample size of 1,500 per group was planned to detect a difference between 8.0 and 5.0% for RP, 12.0 and 8.0% for metritis, 5.0 and 2.5% for displaced abomasum (DA), 15.0 and 11.0% for mastitis, 35 and 41% for pregnancy to first service, and a 1 kg/d response in milk yield with 95% confidence and power ≥90%. The variation in early lactation milk yield (SD = 6 kg/d) was determined from previous work by our group (Leno et al., 2017).

Cows enrolled in experiment 2 had a blood sample collected before treatment administration using a 20-G needle and 6-mL polypropylene syringe containing no anticoagulant, which was immediately dispensed into a 4-mL evacuated tube containing lithium heparin (Greiner Bio-One, Monroe, NC). Sample collection was conducted by trained farm personnel (the same personnel responsible for enrollment). Blood samples were stored at 4°C and shipped to a central location twice per week at which point they were centrifuged at 1,000 × *g* for 10 min at 22°C and plasma was stored at −20°C until analysis. Total Ca concentration was determined by the methods described previously for experiment 1. Previous research has demonstrated no measureable deviation in total Ca for this period of storage when human whole blood is refrigerated (Heins et al., 1995).

Samples of the TMR fed to the close-up dry cow pens and pens housing cows in early lactation were collected once per week at each farm for the duration of cow enrollment. Within farms, the rations fed during the close-up period and the fresh period were the same for all parity groups despite separate housing for parity groups on 3 farms. Samples were collected immediately

after feed delivery on farms A and C, within 1 h on farm E, within 4 h on farms B and D, and within 6 h on farm F. Wet TMR samples were frozen at −20°C, dried at 55°C for 48 h in a forced-air oven, and ground to 2 mm in a Wiley mill. All samples from individual weeks were combined and a composite sample was sent to a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD) for analysis of chemical composition according to wet chemistry methods as described by McCarthy et al. (2015). Average ingredient composition and chemical analysis of diets are presented in Supplemental Table S4 (https://doi.org/10.3168/jds.2017-13164).

Health, Milk Production, and Reproduction Data Collection

Cows were assigned a BCS and locomotion score between 0 and 10 DIM. Body condition scores were assigned within 3 categories [≤2.5 (thin), 2.75 to 3.5 (normal), ≥3.75 (overconditioned)] based on a 5-point scale in quarter-point increments (Edmonson et al., 1989). Locomotion scores were assigned into 2 categories (not lame, lame). On all farms, locomotion was assessed while cows were walking in pens (either to the lactating pen from maternity or within the lactating pen). Cows with normal gait or an abnormal gate without a favored limb were categorized as not lame, while cows favoring a limb, as evidenced by limping or a hunched back, were categorized as lame. At farms B, C, D, and F, BCS and locomotion scores were assigned by the same trained farm personnel that enrolled cows (described above). At farms A and E, scores were assigned by one trained researcher. Because BCS and locomotion scoring were often conducted at or near the time of enrollment, the personnel conducting the assessments were not blinded to treatment. The aforementioned categories for BCS and locomotion scores were adopted to reduce the potential variation in scoring between observers.

Farms were provided case definitions for identification of clinical health disorders that were identified and recorded by farm personnel in DairyComp 305. Clinical hypocalcemia was defined as signs of weakness or recumbency with cold extremities causing the inability to rise within 72 h after parturition and no evidence of physical injury. Retained placenta was diagnosed if the placenta was not expelled within 24 h after parturition. Metritis was defined as foul-smelling, watery uterine discharge that was red to brown in color within 14 d of parturition. Mastitis was defined as abnormal milk without consideration of SCC and cows with one or more recorded cases of mastitis within the first 30 DIM were coded as having mastitis. Displaced abomasum was defined as a high-pitched ping upon simultaneous percussion and auscultation on the right or left side of the cow along the line from the elbow to the hip which extends forward of the 10th rib if on the right side. Farm recorded occurrence of health disorders and culling (sold or died) within 30 DIM were collected from DairyComp 305 along with monthly test day milk production for the first 4 DHIA tests (milk yield was not corrected for component production), success or failure of first service insemination, and days to pregnancy by 150 DIM. For test day milk production, tests occurring less than 7 DIM were deleted due to variability in reporting, and the following test was considered the first test day. For cows that aborted and were then rebred, pregnancy date was considered to be the date of the breeding for which the first pregnancy was diagnosed. Reproductive data from farm E were removed due to an extensive superovulation program at that farm.

Statistical Analysis

Experiment 1. Statistical analyses were conducted using SAS (version 9.4, SAS Institute Inc., Cary, NC). A mixed effect repeated measures ANOVA model was conducted using the MIXED procedure. The repeated effect was hour with the effect of cow within treatment included as the subject of repeated measures. There was no difference between CON and BOL for plasma Ca concentration before treatment assignment when analyzed by univariate ANOVA in the GLM procedure, and plasma Ca concentration before treatment assignment was included as a covariate in the model. Other fixed effects included treatment, hour, parity group (first, second, and third or greater), and the interaction between treatment and hour. The Kenward-Roger method was used for estimating denominator degrees of freedom. Five covariance structures were tested (compound symmetry, heterogeneous compound symmetry, Toeplitz, antedependence 1, unstructured), and the option resulting in the lowest Akaike's information criterion was selected. Model residuals were inspected for normality and homogeneity of variance.

Experiment 2. All milk production, reproduction, health disorder, and culling outcomes were analyzed separately for PP and MP cows. Descriptive data were generated using the FREQ and UNIVARIATE procedures. The difference between treatment groups for PP and MP cows for the occurrence of twinning, stillbirth, lameness, and the distribution of BCS category and calving season were subject to a chi-squared test with the FREQ procedure. The difference in days carried calf (DCC), previous lactation days open, age at first calving (AFC), dry period length, previous lactation 305-d mature equivalent milk production, and DIM at first test day between treatment groups was tested us-

ing univariate ANOVA with the GLM procedure. Variables that were not normally distributed (previous days open, AFC, and dry period length) were transformed for analysis. Two separate multivariable models were developed for each outcome to address the 2 objectives of experiment 2. First, to address the efficacy of an all-inclusive approach to treatment with consideration of periparturient risk factors, multivariable models were developed without considering plasma Ca measured in samples collected before treatment assignment. In the second model for each outcome, Ca status before treatment assignment was also considered, and therefore only the cows with Ca concentration determined on a plasma sample collected before enrollment were included in the analysis. The percentage of cows in each treatment group included in this analysis is outlined in Table 4. Because BCS and locomotion scores were not available for all cows (percentage with BCS and locomotion scores presented in Table 4), multivariable modeling within each objective was initially conducted for just the subset of cows with complete data. If BCS or locomotion score did not remain in the final model, the process was repeated with the complete study population and BCS and locomotion scores were no longer considered as predictors.

Potential predictor variables considered in all models included calving season (winter = December, January, February; spring = March, April, May; summer = June, July, August; fall = September, October, November), DCC, locomotion score, and BCS. Very few cows were enrolled in the winter category (n = 10) and those cows were recategorized as fall calving. Primiparous cow enrollment began later and therefore only 6 PP cows were categorized as spring calving and were recategorized to summer. Because few cows had BCS $\leq$2.5, this category was combined with the normal BCS category (>2.5 to $\leq$3.5). The BCS categories were then overconditioned (BCS >3.5) versus normal to thin (BCS $\leq$3.5). Models developed for analysis of PP cow outcomes also included AFC. Models developed for analysis of MP cow outcomes also included parity group (second, and third or greater) and dry period length. Days carried calf, dry period length, and AFC were considered as continuous predictors. The effects of stillbirth and twin calving were not considered because of the small number of animals in those groups (Table 3). For all models, the effect of farm was included as a random effect (repeated measures and proportional hazards models) or cows were clustered within farm and an exchangeable correlation matrix was used (Poisson regression models). The multivariable modeling approach involved initially offering all potential variables, as well as the treatment variable, to the model. Manual backward stepwise elimination was conducted to reach the base model by removing main effects with $P > 0.10$; however, the effect of treatment and parity group (for MP cows) was forced into all models regardless of P-value. Subsequently, interactions between treatment and additional variables that had biologically plausible relationships were offered to the base model one at a time. All possible interactions were considered, regardless of the significance of the main effect, considering that differential responses to treatment within stratified groups could mask the effects observed when the variable was only offered to the model as a main effect. All interactions resulting in $P < 0.10$ when considered individually were then added to the final multivariable model. At that point, any variables that were no longer important ($P > 0.10$) were removed. Type III sums of squares P-values for model effects were used throughout the modeling process so that the order of variable inclusion did not affect interpretation. If interactions between treatment and continuous predictors were retained, the continuous predictor was categorized into quartiles and the categorized variable was forced into the final model in place of the continuous variable for ease of interpretation. Estimates of treatment effects at each quartile were observed to determine any groups that responded similarly to simplify categories. The categorized variables were then considered in the model for the same outcome when analyzing the differential effects of bolus administration based on Ca status. For interaction terms in the final model, the LSMEANS statement with the PDIFF option was used to generate pairwise comparisons between CON and BOL within each level of the confounding variable. For all results, significance was declared at $P \leq 0.05$ and trends discussed at $0.05 < P \leq 0.10$. Importance of multiple contrasts from interaction terms was adjusted using a Bonferroni correction for the number of levels of the interacting variable.

Milk production across the first 4 DHIA test days after parturition was analyzed by repeated measures ANOVA using the MIXED procedure. Based on previous work demonstrating that production responses to oral Ca bolus supplementation were dependent on previous lactation 305-d mature equivalent milk production (Oetzel and Miller, 2012; Martinez et al., 2016b), this variable was dichotomized based on the DairyComp 305 reported relative value of the previous lactation 305-d mature equivalent milk production (RANK305ME) and considered in multivariable models for test day milk production of MP cows. Cows with relative value >105% were categorized as high RANK305ME and cows with relative value $\leq$105% were categorized as low RANK305ME. The random ef-

fect of farm and the repeated measure of test number with cow within treatment as the subject of repeated measures were included. The Kenward-Roger method was used for estimation of denominator degrees. In addition to treatment and parity group (for MP cows), the effect of DHIA test number and the interaction between treatment and DHIA test number were forced into the model. Three covariance structures were considered: heterogeneous autoregressive 1, Toeplitz, and unstructured. The initial model containing all potential main effects was analyzed with each structure and the option with the lowest Akaike's information criterion was selected for further modeling. After interaction consideration and variable selection, the final model was analyzed with each of the 3 structures again to ensure that the structure chosen remained the best option.

Reproductive outcomes assessed included pregnancy risk to first service and days to conception within 150 DIM. Pregnancy risk to first service was analyzed using the same methods as described for health events. Additional variables considered included breeding code at first service (timed AI or heat breeding) and previous lactation days open (continuous) for MP cows. Days to pregnancy from the end of the voluntary waiting period (VWP) to 150 DIM was analyzed with a Cox proportional hazard regression model using the PHREG procedure of SAS. Voluntary waiting period was accounted for by specifying different entry times into the analysis and therefore results represent the hazard for pregnancy between the end of the VWP and 150 DIM. Cows that were removed from the herd, classified as "do not breed," or bred before the end of the VWP were removed from the analysis. Censoring was used to account for cows that were removed from the herd or classified as "do not breed" between the end of the VWP and before conceiving, or were not pregnant by 150 DIM. Variables offered to the model were the same as for the analysis of pregnancy to first service with the exception of breeding code at first service. Sensitivity analysis was conducted with final models to ensure that censoring was noninformative. Kaplan-Meier analysis was used to generate survival curves by treatment (stratified by the level of interacting variables if applicable based on the final multivariable model) to determine median days from the end of the VWP to conception.

Health event outcomes that were analyzed included additional supplemental Ca (administration of oral or intravenous Ca $\leq$3 DIM), RP, metritis, DA, mastitis, and culling within 30 DIM as well as the occurrence of one or more health outcome (RP, metritis, DA, or mastitis). Cows that received additional Ca supplementation remained in all further analyses for health, milk production, and reproduction. For PP cows, only 0.3% of cows were given additional supplemental Ca. For MP cows, 4.5% of CON cows and 3.9% of BOL cows were administered additional supplemental Ca. To be included in the analysis as eligible to have the above health disorders, cows had to either be diagnosed with the disorder or have survived within the herd until at least 2 DIM for RP, 10 DIM for metritis, and 30 DIM for DA, mastitis, and one or more health disorders. The distribution between treatments of cows excluded from each health event analysis due to DIM at culling was tested using the chi-squared test of the FREQ procedure to ensure that results were not biased by this screening. Analysis of these outcomes was conducted using a mixed effect Poisson regression model with a log link function with the GENMOD procedure to enable reporting of risk ratios.

To address the differential responses of PP and MP cows to treatment based on plasma Ca status at enrollment, a second analysis was conducted for all outcomes that included a variable representing Ca status before treatment administration. Blood Ca measured at this time was dichotomized according to thresholds in 0.05 mmol/L intervals ranging from 1.6 to 2.3 mmol/L. To ensure enough statistical power to assess the interaction between treatment and Ca status, only the thresholds resulting in at least 10% of cows within each level of the contingency table between treatment and the dichotomized Ca variables were considered. This resulted in a range of thresholds for PP cows of 2.15 to 2.30 mmol/L and for MP cows of 1.80 to 2.15 mmol/L. Models were developed according to the method described previously with the addition of one of the dichotomized Ca status variables as an additional predictor. This Ca status variable and its interaction with treatment were forced into all models. For MP models, parity was included as a main effect but was not considered as an interaction with treatment. Although a strong univariate association was present between Ca status and parity ($P < 0.0001$ for all thresholds), there were still many observations in the discordant cells of the contingency table, and parity remained an important predictor in many of the models despite forcing Ca status into the models as a main effect. The main effect of parity remained in the models to account for outcome risk due to parity that was not described by the Ca status variable alone. Because the effect of interest was the treatment by Ca status interaction, the treatment by parity interaction was not tested to avoid collinearity and because it had been investigated as part of objective 1. A separate model for each outcome was developed with each Ca status threshold. The final reported model was decided

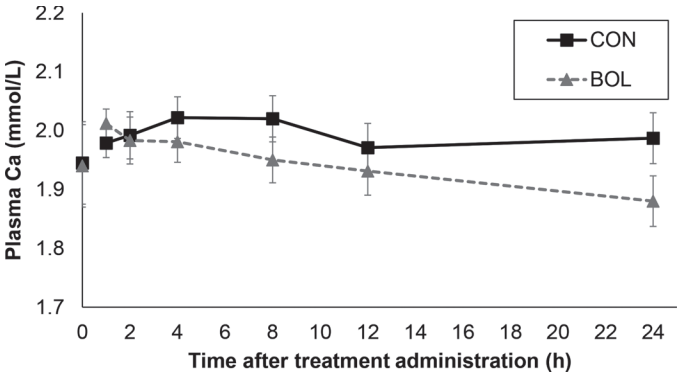

Figure 1. Plasma Ca concentration (mmol/L) by hour after treatment administration for a subset of cows (n = 25 per treatment) enrolled on farm A. Cows were randomly assigned to treatments within parity groups (first, second, and third or greater lactation) within 19 h after parturition (CON = no intervention; BOL = a single dose of an oral Ca bolus providing 54 to 64 g of Ca in the form of 3 boluses). A tendency was observed for an effect of hour ($P = 0.07$), but no overall effect of treatment ($P = 0.36$) or interaction between treatment and hour ($P = 0.14$) was observed. Error bars represent the SEM.

by the treatment by Ca status interaction with lowest probability of type I error.

RESULTS

Experiment 1

The distribution of treatment groups and baseline Ca status for cows enrolled in experiment 1 are presented in Table 1. The results for plasma Ca concentration during the 24 h following treatment administration are presented in Figure 1. No effect was observed of treatment on average plasma Ca concentration over the observation period (CON = 2.00 and BOL = 1.96 mmol/L, $P = 0.36$), and no interaction was observed between treatment and time. There tended to be an effect of hour such that plasma Ca concentration decreased over time ($P = 0.07$).

Experiment 2

Of the 4,438 cows that calved during the enrollment period, 3,949 were enrolled in experiment 2 and remained in the data set. Reasons for exclusion from the study population are outlined in Table 2. The final number of cows in each group was 1,973 for CON and 1,976 for BOL. Descriptive data for the final study population by treatment and farm, as well as the average enrollment time by treatment and farm, are presented in Table 3. The balance of treatments are presented separately for PP and MP cows in Table 4. The balance of treatments was analyzed for the subset of cows with BCS and locomotion scores, as well as the subset with plasma Ca concentration measured before treatment administration, and the treatment balance of subpopulations was not markedly different. Prevalence of lameness tended to differ by treatment in the PP cow population ($P = 0.06$); however, since lameness was considered in all statistical models, results were controlled to reduce this potential bias.

Health and Production Responses of PP Cows to Oral Ca Administration. For PP cows, an interaction between treatment and BCS category ($P = 0.008$) and an interaction between treatment and DCC group ($P = 0.05$) were observed for milk yield over the first 4 DHIA test days; the treatment contrasts for milk yield by level of BCS and DCC group are presented in Table 5. Other variables retained in the model for test day milk production included AFC and treatment by DHIA test (not significant). Reproductive outcomes and incidence of health disorders and culling by treatment for PP cows are presented in Table 6 along with the associated contrasts comparing BOL to CON (by level of periparturient risk factors when applicable). No effect of treatment was observed for pregnancy to first service ($P = 0.72$) and hazard of pregnancy between the VWP and 150 DIM ($P = 0.63$). No other variables were retained in the models for pregnancy to first ser-

Table 1. The distribution of treatment groups for cows enrolled in experiment 1

	Treatment[1]		
Variable	CON	BOL	*P*-value
Total (no.)	25	25	
Parity (no.)			0.95
First	5	5	
Second	7	8	
≥Third	13	12	
Time of enrollment[2] (h)	7.4 ± 5.7	9.3 ± 4.9	0.32
Baseline plasma Ca[2] (mmol/L)	1.95 ± 0.35	1.94 ± 0.31	0.42
Baseline plasma Ca ≤2.15 mmol/L (%)	68.0	72.0	0.76

[1]CON = no intervention; BOL = a single dose of an oral Ca bolus providing 54 to 64 g of Ca in the form of 3 boluses within 24 h after parturition.

[2]Mean and SD.

Table 2. Total number of eligible cows calving during the enrollment period for experiment 2, reasons for exclusion from the study, and the final study population

Exclusion reason	N
Calving not recorded by farm personnel	172
Farm personnel identified abortion or days carried calf <260	118
Enrolled before calving or >24 h after calving	83
Missing enrollment information	41
Clinical hypocalcemia at parturition	20
Dry period <30 d	18
Extreme calving difficulty (cesarean section/prolapse)	7
Inappropriate treatment allocation or administration	7
Sold as replacements	7
Died the day of calving	5
Other[1]	11
Total eligible	4,438
Final study population	3,949
Final study population with pretreatment plasma Ca data	3,341

[1]Includes conformational or behavioral issues prohibiting treatment administration, mastitis in the dry period, and calving off of the farm premises.

vice and days to pregnancy. Incidence of additional Ca supplementation (0.3% overall) and DA (0.6% overall) in PP cows were too low for statistical analysis. An interaction between treatment and AFC group was observed for risk of RP ($P = 0.04$) while controlling for calving season and DCC. No effects of treatment on risk of metritis in PP cows ($P = 0.68$) were detected while controlling for BCS category. An interaction between treatment and AFC group was also observed for risk of mastitis ($P = 0.04$). Overall, an interaction tended to be present between treatment and AFC group for risk of having one or more health disorder ($P = 0.08$) while controlling for DCC. There tended to be increased risk for early removal from the herd for PP cows receiving BOL ($P = 0.09$) while controlling for DCC.

Health and Production Responses of MP Cows to Oral Ca Administration. For milk yield across the first 4 DHIA test days for MP cows, an interaction tended to be present between treatment and DCC group ($P = 0.06$) while controlling for parity, locomotion score, BCS, RANK305ME, calving season, and treatment by DHIA test number. Results of the treatment contrasts for milk yield by DCC group are presented in Table 5. Reproductive outcomes and incidence of health disorders and culling by treatment for MP cows are presented in Table 6 along with the associated contrasts comparing BOL with CON (by level of periparturient risk factors when applicable). No association was observed between treatment and risk of pregnancy at first service ($P = 0.76$) controlling for parity and previous lactation days open. An interaction between treatment and dry period length group was observed for days to pregnancy from the end of the VWP ($P = 0.0006$) while controlling for parity, calving season, and previous lactation days open. No association between treatment and risk of additional Ca supplementation ($P = 0.16$) was detected while controlling for parity and dry period length. An interaction between treatment and BCS for risk of RP ($P = 0.04$) was detected and an interaction tended to be present between treatment and parity ($P = 0.10$) while controlling for DCC and locomotion score. There tended to be an interaction between treatment and locomotion score ($P = 0.06$) for risk of metritis while controlling for parity and BCS. There tended to be an interaction between treatment and locomotion score for risk of DA ($P = 0.09$) while controlling for parity. An interaction between treatment and parity for risk of one or more health disorder ($P = 0.05$) was detected while controlling for DCC, locomotion score, and BCS. Mastitis was unaffected by treatment ($P = 0.49$) in a model containing parity. No association was observed between treatment and risk of early removal from the herd ($P = 0.28$) while controlling for parity, locomotion score, and dry period length.

Differential Responses of PP Cows to Oral Ca Based on Plasma Ca Status at Enrollment. Differential responses to treatment based on Ca status for test day milk production of PP cows are presented in Table 5. Milk production tended to be higher for PP cows with high plasma Ca that received BOL ($P = 0.03$). Other variables retained in the model for test day milk production included AFC, treatment by DHIA test number (not significant), and treatment by DCC group. The differential responses of PP cows to treatment based on Ca status for reproductive outcomes, health disorders, and culling are presented in Table 7 with the contrasts comparing BOL with CON within each level of Ca status. The Ca status threshold resulting in the smallest probability of type I error for the interaction between Ca status and treatment in all models was 2.20 mmol/L, with the exception of metritis and days to pregnancy for which a threshold of 2.15 mmol/L was used. No effect was observed of treatment in either Ca status group for pregnancy to first service ($P \geq 0.30$) or days to pregnancy from the end of the VWP ($P \geq 0.43$). Risk of RP was decreased for PP cows assigned to BOL with high plasma Ca ($P = 0.01$) while controlling for DCC and treatment by AFC group. There were no effects of treatment for PP cows in either Ca status group for risk of metritis ($P \geq 0.12$). Primiparous cows in the higher Ca status group had increased risk of mastitis when administered a bolus ($P < 0.0001$), and other predictors in the model included an interaction between treatment and AFC as a continuous variable ($P = 0.04$). Risk of one or more health disorder tended to increase for PP cows

Table 3. Descriptive statistics of the study population and health outcomes by farm for experiment 2

	Farm											
	A		B		C		D		E		F	
Variable	CON[1]	BOL[1]	CON	BOL	CON	BOL	CON	BOL	CON	BOL	CON	BOL
Total (no.)	297	298	240	238	449	448	249	264	276	267	462	461
Parity (no.)												
First	87	83	67	66	127	129	63	67	73	73	75	77
Second	83	89	73	71	130	134	87	89	81	75	143	142
≥Third	127	126	100	101	192	185	99	108	122	119	244	242
Twin calving (%)	5.8	6.4	1.7	4.6	5.1	6.7	4.4	2.7	2.5	2.2	6.7	4.8
Stillbirth (%)	7.1	5.4	2.9	4.2	4.2	3.6	4.8	3.8	5.1	4.9	6.9	4.8
BCS[2] (%)												
≤2.5	3.0	3.8	6.6	6.9	3.2	4.3	4.3	6.9	0.8	2.5	2.8	2.2
2.75 to 3.5	82.1	81.4	81.8	83.3	79.4	77.9	87.6	80.1	68.5	67.9	70.4	76.0
≥3.75	14.9	14.8	11.7	9.7	17.4	17.8	8.1	13.0	30.6	29.6	26.9	21.8
Lame[2] (%)	29.1	28.3	24.1	20.8	41.0	43.1	18.8	25.6	36.3	38.1	12.1	8.6
Ca treatment risk[3] (%)	3.4	1.7	1.7	0.8	3.1	2.2	2.0	0.8	7.2	9.4	4.8	3.0
RP risk[4] (%)	3.0	4.0	3.4	4.2	11.8	9.2	0.0	0.4	8.0	9.1	11.3	10.1
DA risk[5] (%)	3.1	2.8	0.9	0.9	2.8	3.1	1.3	0.0	4.4	1.2	2.7	3.0
Metritis risk[6] (%)	16.7	14.6	3.0	6.5	9.7	9.9	1.6	0.8	8.1	8.9	14.2	15.1
Mastitis risk[7] (%)	2.4	1.7	7.4	7.4	5.6	6.1	5.4	6.7	1.9	1.9	5.1	3.9
Health disorder risk[8] (%)	22.5	20.0	14.7	18.7	22.8	23.6	7.9	7.9	18.5	17.3	24.3	22.3
Early removal risk[9] (%)	3.4	3.7	5.8	5.9	4.9	5.8	4.4	4.9	3.3	3.4	3.0	5.0
Pregnancy risk at first service[10] (%)	38.3	40.9	32.7	35.5	42.3	42.4	34.4	34.9	—	—	42.1	38.0
Enrollment time, median (h)	4.6	5.0	2.0	1.5	1.5	1.9	10.3	10.0	0.7	0.7	1.3	1.3
Enrollment time, IQR[11] (h)	1.2–12.1	1.3–11.6	0.8–5.2	0.5–5.0	0.8–5.2	1.0–5.7	4.0–15.5	4.5–16.5	0.3–1.0	0.3–1.3	0.5–4.0	0.5–4.0

[1]CON = no intervention; BOL = a single dose of an oral Ca bolus providing 54 to 64 g of Ca in the form of 3 boluses within 24 h after parturition.

[2]BCS and locomotion scoring conducted by farm personnel (farms B, C, D, and F) or researchers (farms A and E) between 0 and 10 DIM.

[3]Ca treatment risk = number of cows treated with supplemental Ca (intravenous or oral) ≤3 DIM/number of fresh cows.

[4]RP risk = number of cows diagnosed with retained placenta (RP)/number of fresh cows.

[5]DA risk = number of cows diagnosed with displaced abomasum (DA) ≤30 DIM/number of fresh cows (excluding those that were not diagnosed with DA and were culled ≤30 DIM).

[6]Metritis risk = number of cows diagnosed with metritis/number of fresh cows (excluding those that were not diagnosed with metritis and were culled ≤10 DIM).

[7]Mastitis risk = number of cows diagnosed with mastitis/number of fresh cows (excluding those that were not diagnosed with mastitis and were culled ≤30 DIM).

[8]Health disorder risk = number of cows diagnosed with RP, metritis, DA, or mastitis/number of fresh cows (excluding those that were not diagnosed with disorders and were culled ≤30 DIM).

[9]Early removal risk = number of cows that died or were sold ≤30 DIM/number of fresh cows.

[10]Pregnancy risk at first service = number of cows that conceived to the first breeding after parturition/number of cows that were bred at least once after parturition. Reproductive data for farm E was removed from analysis due to an extensive superovulation program.

[11]IQR = interquartile range.

Table 4. The distribution of cow characteristics for treatment groups by parity for experiment 2

	Primiparous			Multiparous		
Variable	CON[1]	BOL[1]	*P*-value	CON	BOL	*P*-value
Cows (n)	492	495		1,481	1,481	
Parity (%)						0.91
Second	—	—		40.3	40.5	
≥Third	—	—		59.7	59.5	
Twin calving (%)	1.4	1.0	0.55	5.8	6.1	0.76
Stillbirth (%)	9.6	7.9	0.35	3.9	3.2	0.32
Scored for locomotion and BCS (%)	76.6	78.0		72.5	73.5	
BCS[2] (%)			0.89			0.33
≤2.5	3.9	4.5		2.9	4.0	
2.75 to 3.5	78.5	78.6		77.2	76.9	
≥3.75	17.6	16.9		20.0	19.2	
Lame[2] (%)	7.2	11.1	0.06	36.4	36.2	0.91
Cows with blood Ca (%)	74.6	73.5		88.0	87.0	
Plasma Ca ≤2.15 mmol/L (%)	23.4	20.6	0.35	76.3	74.6	0.30
Calving season (%)			0.97			0.92
Spring	—	—		27.0	26.9	
Summer	54.1	53.9		38.0	38.7	
Fall	45.9	46.1		35.0	34.4	
Days carried calf[3] (d)	275 ± 5	275 ± 5	0.96	277 ± 5	277 ± 5	0.32
Previous lactation days open[4] (d)	—	—	—	90 (71–139)	90 (71–139)	0.94
Dry period length[4] (d)	—	—	—	52 (47–59)	52 (47–60)	0.37
Age at calving[4] (d)	672 (649–714)	675 (649–711)	0.84	—	—	—
Previous lactation 305ME[3,5] (kg)	—	—	—	13,485 ± 2,024	13,468 ± 2,044	0.81
First DHIA test day[3] (DIM)	22.5 ± 9.1	22.7 ± 9.0	0.68	22.2 ± 9.1	22.3 ± 9.2	0.72

[1]CON = no intervention; BOL = a single dose of an oral Ca bolus providing 54 to 64 g of Ca in the form of 3 boluses within 24 h after parturition.

[2]BCS and locomotion scoring conducted by farm personnel (farms B, C, D, and F) or researchers (farms A and E) between 0 and 10 DIM.

[3]Mean and SD.

[4]Median and interquartile range.

[5]305-d mature equivalent milk production.

Table 5. Least squares means and SEM for DHIA test-day milk production over the first 4 tests following parturition for primiparous (PP) and multiparous (MP) cows receiving a single oral dose of Ca within 24 h following parturition (BOL) and those receiving no intervention (CON) by level of periparturient risk factors and by Ca status group

		Treatment		Sample size (n)		
Outcome	Level of interaction[1]	CON	BOL	CON	BOL	*P*-value[2]
Efficacy of Ca bolus administration[1]						
PP milk yield (kg/d)	BCS ≤3.5	33.0 ± 0.8	33.0 ± 0.8	322	326	0.96
	BCS >3.5	31.7 ± 1.1	35.1 ± 1.1	55	60	0.003
	DCC ≤277[3]	32.7 ± 0.9	33.7 ± 0.9	267	272	0.12
	DCC >277	31.9 ± 1.0	34.7 ± 1.0	110	114	0.0009
MP milk yield (kg/d)	DCC ≤277	47.0 ± 1.2	46.8 ± 1.2	444	454	0.64
	DCC >277	46.8 ± 1.2	47.7 ± 1.2	629	635	0.03
Differential responses to Ca bolus administration due to Ca status[1]						
PP milk yield (kg/d)	>2.2 mmol/L	32.1 ± 1.0	33.3 ± 1.0	236	236	0.03
	≤2.2 mmol/L	34.2 ± 1.1	33.4 ± 1.1	131	128	0.32
MP milk yield (kg/d)	>2.15 mmol/L	46.2 ± 1.2	45.8 ± 1.2	224	240	0.55
	≤2.15 mmol/L	47.1 ± 1.1	47.8 ± 1.1	734	721	0.06

[1]For determining the efficacy of Ca bolus administration, periparturient risk factors resulting in interactions with treatment with $P \leq 0.10$ were retained in the model and the resulting contrasts are presented. For determining differential responses to Ca bolus administration due to Ca status, a separate model was developed that considered Ca status in addition to other periparturient risk factors and the interaction between treatment and Ca status was forced into the model using several thresholds. The threshold resulting in the smallest probability of type I error for the interaction between treatment and Ca status is presented with the resulting contrasts.

[2]*P*-values are the result of contrasts between CON and BOL at each level of the interacting variable. The threshold for significance was adjusted using a Bonferroni correction ($P \leq 0.025$).

[3]DCC = days carried calf.

Table 6. Contrasts comparing cows receiving a single oral dose of Ca within 24 h following parturition (BOL) to those receiving no intervention (CON) for risk of pregnancy to first AI service postpartum, hazard of conception between the end of the voluntary waiting period (VWP) and 150 DIM, and risk of health disorders

Outcome	Level of interaction[1]	Treatment		RR[2]	95% CI	*P*-value[3]
		CON	BOL			
Primiparous						
Pregnancy risk at first service[4] (%)	—	43.7 (171/391)	44.6 (176/395)	1.02	0.92 to 1.14	0.72
Median time to conception[5] (d)	—	29 (26–35)	29 (26–37)	1.04[6]	0.89 to 1.22	0.63
RP risk[7] (%)	AFC Q1 to Q3[8]	5.2 (19/364)	4.8 (18/374)	0.98	0.70 to 1.37	0.91
	AFC Q4[8]	5.6 (7/126)	2.5 (3/119)	0.42	0.18 to 0.98	0.04
Metritis risk[9] (%)	—	11.0 (41/373)	11.0 (42/383)	1.03	0.89 to 1.19	0.68
Mastitis risk[10] (%)	AFC Q1 and Q2[8]	1.6 (4/248)	4.6 (11/238)	3.10	1.47 to 6.55	0.003
	AFC Q3 and Q4[8]	3.8 (9/238)	1.6 (4/245)	0.45	0.24 to 0.86	0.02
Health disorder risk[11] (%)	AFC Q1[8]	21.6 (27/125)	28.5 (35/123)	1.46	0.81 to 2.64	0.20
	AFC Q2 and Q3[8]	14.8 (35/237)	16.3 (40/246)	1.09	0.61 to 1.96	0.77
	AFC Q4[8]	16.0 (20/125)	10.3 (12/117)	0.62	0.43 to 0.88	0.008
Early removal risk[12] (%)	—	1.4 (7/492)	2.6 (13/495)	1.91	1.12 to 3.25	0.09
Multiparous						
Pregnancy risk at first service[4] (%)	—	37.5 (432/1,151)	37.0 (431/1,166)	0.99	0.91 to 1.07	0.76
Median time to conception[5] (d)	Dry ≤52 d	41 (30–46)	50 (46–57)	0.81[6]	0.71 to 0.93	0.002
	Dry >52 d	48 (42–54)	39 (32–46)	1.14[6]	0.99 to 1.32	0.07
Ca treatment risk[13] (%)	—	4.9 (73/1481)	3.8 (57/1481)	0.78	0.53 to 1.15	0.16
RP risk[7] (%)	Parity = 2	5.0 (21/423)	6.7 (29/431)	1.11	0.72 to 1.73	0.64
	Parity ≥3	9.9 (64/647)	7.4 (48/651)	0.66	0.52 to 0.83	0.0003
	BCS ≤3.5	7.5 (65/865)	7.6 (67/885)	1.05	0.76 to 1.46	0.17
	BCS >3.5	9.8 (20/205)	5.1 (10/197)	0.70	0.58 to 0.84	0.0002
Metritis risk[9] (%)	Not lame	6.4 (43/673)	8.4 (57/679)	1.31	0.99 to 1.74	0.06
	Lame	11.1 (42/380)	7.4 (28/381)	0.66	0.39 to 1.11	0.12
DA risk[14] (%)	Not lame	2.0 (13/665)	2.3 (15/668)	1.15	0.56 to 2.35	0.70
	Lame	6.3 (23/366)	3.3 (12/368)	0.52	0.32 to 0.83	0.01
Mastitis risk[10] (%)	—	5.3 (76/1422)	5.1 (72/1410)	0.95	0.84 to 1.08	0.49
Health disorder risk[11] (%)	Parity = 2	12.2 (51/419)	15.9 (68/427)	1.29	1.04 to 1.60	0.02
	Parity ≥3	25.8 (161/623)	20.6 (129/625)	0.80	0.70 to 0.90	0.0005
Early removal risk[12] (%)	—	4.4 (47/1,073)	5.2 (57/1,089)	1.18	0.89 to 1.57	0.28

[1]Periparturient risk factors resulting in interactions with treatment with $P \leq 0.10$.

[2]Contrasts generated from multivariable Poisson regression models. Control was the reference group for all contrasts.

[3]The threshold for significance is adjusted using a Bonferroni correction (primiparous health disorder risk $P \leq 0.016$, all other comparisons $P \leq 0.025$).

[4]Pregnancy risk at first service = number of cows that conceived to the first breeding after parturition/number of cows that were bred at least once after parturition.

[5]Median days to conception from the end of the farm's VWP, expressed as median (95% CI).

[6]Hazard ratio. Contrasts generated from proportional hazards models.

[7]RP risk = number of cows diagnosed with retained placenta (RP)/number of fresh cows.

[8]Age at first calving (AFC) quartiles (Q) were as follows: Q1 ≤ 649 d, Q2 = 650 to 673 d, Q3 = 674 to 712 d, Q4 > 712 d.

[9]Metritis risk = number of cows diagnosed with metritis/number of fresh cows (excluding those that were not diagnosed with metritis and were culled ≤10 DIM).

[10]Mastitis risk = number of cows diagnosed with mastitis/number of fresh cows (excluding those that were not diagnosed with mastitis and were culled ≤30 DIM).

[11]Health disorder risk = number of cows diagnosed with RP, metritis, DA, or mastitis/number of fresh cows (excluding those that were not diagnosed with disorders and were culled ≤30 DIM).

[12]Early removal risk = number of cows that died or were sold ≤30 DIM/number of fresh cows.

[13]Ca treatment risk = number of cows treated with supplemental Ca (injection or oral) ≤3 DIM/number of fresh cows.

[14]DA risk = number of cows diagnosed with displaced abomasum (DA) ≤30 DIM/number of fresh cows (excluding those that were not diagnosed with DA and were culled ≤30 DIM).

administered boluses in the higher Ca status group ($P = 0.03$), and no other predictors were included in the model. This analysis was not conducted for risk of early removal from the herd because too few observations were available to estimate the treatment by Ca status interaction.

Differential Responses of MP Cows to Oral Ca Based on Plasma Ca Status at Enrollment. Differential responses to treatment based on Ca status for test day milk production of MP cows are presented in Table 5. Milk production over the first 4 DHIA test days did not differ for MP cows at either level of Ca

status ($P \geq 0.06$). Other variables retained in the model include parity, locomotion score, BCS, RANK305ME, calving season, treatment by DHIA test number (not significant), and treatment by DCC group ($P = 0.06$). The differential responses to treatment of MP cows based on Ca status for reproductive outcomes, health disorders, and culling are presented in Table 8 with the contrasts comparing BOL with CON for each level of Ca status. For MP cows, the plasma Ca threshold used in the final model varied by outcome and ranged from 1.80 to 2.15 mmol/L. Risk of pregnancy to first service was increased for cows receiving BOL with higher plasma Ca ($P = 0.001$); other variables retained in the model included parity and previous lactation days open. Days to pregnancy from the end of the VWP was not affected by treatment in either Ca status group ($P \geq 0.06$) while controlling for parity, previous lactation days open, calving season, and treatment by dry period length. Treatment by dry period length was significant when dichotomized (as in the model for this outcome without inclusion of pretreatment Ca status) but was retained as a continuous variable in this model to allow for interpretation of the interaction of interest at an average dry period length. Risk of additional Ca treatment was reduced for cows with low plasma Ca that received BOL ($P = 0.001$) while controlling for parity and dry period length. Risk of RP was reduced for cows in both high ($P = 0.001$) and low ($P = 0.0007$) Ca status groups while controlling for parity, DCC, and treatment by BCS. Bolus administration reduced metritis risk for cows with low plasma Ca ($P = 0.0001$) and increased risk for cows with high plasma Ca ($P = 0.02$) and other predictors retained in the model included parity, locomotion score, and BCS. Risk of DA was reduced for cows with low plasma Ca that received BOL ($P = 0.01$) in a model containing parity and dry

Table 7. Contrasts comparing primiparous cows receiving a single oral dose of Ca within 24 h following parturition (BOL) with those receiving no intervention (CON) by blood Ca status before treatment for risk of pregnancy to first AI service postpartum, hazard of conception between the end of the voluntary waiting period (VWP) and 150 DIM, and risk of health disorders

Outcome	SCH group[1] (mmol/L)	Treatment		RR[2]	95% CI	*P*-value[3]
		CON	BOL			
Pregnancy risk at first service[4] (%)	>2.2	41.4 (72/174)	46.3 (81/175)	1.12	0.90 to 1.39	0.30
	≤2.2	44.6 (45/101)	41.9 (44/105)	0.95	0.74 to 1.21	0.66
Median time to conception[5] (d)	>2.15	28 (24–35)	26 (24–31)	1.00[6]	0.81 to 1.24	1.00
	≤2.15	31 (21–49)	47 (24–66)	0.85[6]	0.55 to 1.29	0.43
RP risk[7] (%)	>2.2	5.9 (14/236)	3.8 (9/236)	0.52	0.31 to 0.87	0.01
	≤2.2	4.6 (6/130)	7.1 (9/127)	1.36	0.75 to 2.46	0.32
Metritis risk[8] (%)	>2.15	10.4 (29/278)	11.1 (32/289)	1.03	0.92 to 1.16	0.56
	≤2.15	9.3 (8/86)	2.7 (2/74)	0.45	0.17 to 1.24	0.12
Mastitis risk[9] (%)	>2.2	0.9 (2/234)	4.3 (10/232)	4.20	2.27 to 7.74	<0.0001
	≤2.2	4.7 (6/128)	3.2 (4/124)	0.80	0.38 to 1.69	0.56
Health disorder risk[10] (%)	>2.15	13.7 (38/277)	17.8 (51/286)	1.29	1.02 to 1.62	0.03
	≤2.15	14.1 (12/85)	9.7 (7/72)	0.83	0.49 to 1.41	0.49

[1]The blood Ca threshold used to categorize subclinical hypocalcemia (SCH) for each outcome was determined by the treatment by SCH interaction with the lowest probability of type I error for models derived along a range of Ca thresholds.

[2]Contrasts generated from multivariable Poisson regression models. Control was the reference group for all contrasts.

[3]The threshold for significance is adjusted using a Bonferroni correction and is $P \leq 0.025$ for all comparisons.

[4]Pregnancy risk at first service = number of cows that conceived to the first breeding after parturition/number of cows that were bred at least once after parturition.

[5]Median days to conception from the end of the farm's VWP, expressed as median (95% CI).

[6]Hazard ratio. Contrasts generated from proportional hazards models.

[7]RP risk = number of cows diagnosed with retained placenta (RP)/number of fresh cows.

[8]Metritis risk = number of cows diagnosed with metritis/number of fresh cows (excluding those that were not diagnosed with metritis and were culled ≤10 DIM).

[9]Mastitis risk = number of cows diagnosed with mastitis/number of fresh cows (excluding those that were not diagnosed with mastitis and were culled ≤30 DIM).

[10]Health disorder risk = number of cows diagnosed with RP, metritis, displaced abomasum, or mastitis/number of fresh cows (excluding those that were not diagnosed with disorders and were culled ≤30 DIM).

Table 8. Contrasts comparing multiparous cows receiving a single oral dose of Ca within 24 h following parturition (BOL) with those receiving no intervention (CON) by blood Ca status before treatment for risk of pregnancy to first AI service postpartum, hazard of conception between the end of the voluntary waiting period (VWP) and 150 DIM, and risk of health disorders

Outcome	SCH group[1] (mmol/L)	Treatment		RR[2]	95% CI	*P*-value[3]
		CON	BOL			
Pregnancy risk at first service[4] (%)	>2.1	37.5 (126/336)	43.6 (154/353)	1.16	1.06 to 1.28	0.001
	≤2.1	37.1 (247/666)	34.6 (226/653)	0.94	0.87 to 1.01	0.11
Median time to conception[5] (d)	>1.95	43 (33–47)	34 (29–34)	1.02[6]	0.90 to 1.16	0.18
	≤1.95	48 (42–54)	58 (49–65)	0.90[6]	0.75 to 1.07	0.06
Ca treatment risk[7] (%)	>1.8	2.5 (25/1005)	3.0 (30/990)	1.26	0.72 to 2.19	0.42
	≤1.8	13.4 (40/299)	8.0 (24/299)	0.57	0.40 to 0.80	0.001
RP risk[8] (%)	>1.9	8.4 (50/599)	8.2 (50/609)	0.81	0.71 to 0.92	0.001
	≤1.9	7.6 (27/357)	5.2 (18/345)	0.57	0.41 to 0.79	0.0007
Metritis risk[9] (%)	>2.15	8.1 (18/222)	11.8 (28/237)	1.56	1.07 to 2.26	0.02
	≤2.15	7.8 (56/720)	6.3 (44/697)	0.80	0.71 to 0.90	0.0001
DA risk[10] (%)	>2.15	1.4 (4/297)	1.9 (6/317)	1.46	0.56 to 3.81	0.44
	≤2.15	4.3 (41/951)	2.4 (22/904)	0.53	0.33 to 0.86	0.01
Mastitis risk[11] (%)	>1.95	5.2 (38/731)	3.7 (27/726)	0.71	0.45 to 1.10	0.13
	≤1.95	5.5 (29/523)	7.0 (35/500)	1.25	0.95 to 1.66	0.11
Health disorder risk[12] (%)	>2.15	19.7 (43/218)	23.4 (55/235)	1.24	0.91 to 1.68	0.17
	≤2.15	21.0 (150/714)	17.0 (118/693)	0.80	0.72 to 0.89	<0.0001
Early removal risk[13] (%)	>1.8	4.0 (29/724)	5.7 (41/718)	1.14	0.71 to 1.81	0.59
	≤1.8	5.6 (13/234)	4.9 (12/243)	0.71	0.36 to 1.43	0.34

[1]The blood Ca threshold used to categorize subclinical hypocalcemia (SCH) for each outcome was determined by the treatment by SCH interaction with the lowest probability of type I error for models derived along a range of Ca thresholds.

[2]Contrasts generated from multivariable Poisson regression models. Control was the reference group for all contrasts.

[3]The threshold for significance is adjusted by a Bonferroni correction and is $P \leq 0.025$ for all comparisons.

[4]Pregnancy risk at first service = number of cows that conceived to the first breeding after parturition/number of cows that were bred at least once after parturition.

[5]Median days to conception from the end of the farm's VWP, expressed as median (95% CI).

[6]Hazard ratio. Contrasts generated from proportional hazards models.

[7]Ca treatment risk = number of cows treated with supplemental Ca (injection or oral) ≤3 DIM/number of fresh cows.

[8]RP risk = number of cows diagnosed with retained placenta (RP)/number of fresh cows.

[9]Metritis risk = number of cows diagnosed with metritis/number of fresh cows (excluding those that were not diagnosed with metritis and were culled ≤10 DIM).

[10]DA risk = number of cows diagnosed with displaced abomasum (DA) ≤30 DIM/number of fresh cows (excluding those that were not diagnosed with DA and were culled ≤30 DIM).

[11]Mastitis risk = number of cows diagnosed with mastitis/number of fresh cows (excluding those that were not diagnosed with mastitis and were culled ≤30 DIM).

[12]Health disorder risk = number of cows diagnosed with RP, metritis, DA, or mastitis/number of fresh cows (excluding those that were not diagnosed with disorders and were culled ≤30 DIM).

[13]Early removal risk = number of cows that died or were sold ≤30 DIM/number of fresh cows.

period length as predictors. Mastitis risk did not differ by treatment for cows in either Ca status group ($P > 0.11$) while controlling for parity. Risk of one or more health disorder was decreased for cows with low plasma Ca assigned to BOL ($P < 0.0001$) while controlling for parity and locomotion score. Risk of culling was not affected by treatment in either Ca status group ($P \geq 0.34$) while controlling for parity, locomotion score, dry period length, and treatment by BCS.

DISCUSSION

Experiment 1

Our primary hypothesis of experiment 1 was that a single dose of an oral Ca bolus within 24 h after parturition would result in increased plasma Ca concentration. Recent studies that have assessed oral Ca bolus supplementation have implemented multiple dosing

strategies. Although this strategy has proven effective for increasing blood Ca concentration (Martinez et al., 2016a), the additional doses requires either more time away from the pen or more time restrained in headlocks within the days immediately following parturition. The additional handling time required to administer doses that occur after cows are handled at parturition are an added management complication and may interfere with cow behavior. Results of experiment 1 demonstrated that administering a single dose of an oral Ca bolus did not increase blood Ca concentration between 1 and 24 h following administration. The amount of Ca supplied by this single dose of oral Ca may have been insufficient to result in an increase in systemic blood Ca concentration. Alternatively, the resulting increase in blood Ca from this supplementation strategy may have been too transient to be detected with the sampling scheme used due to the composition and form of the product. Martinez et al. (2016a) demonstrated that supplying either 43 or 86 g of Ca in the form of an oral bolus resulted in transiently increased blood Ca concentration with increases lasting for 2 to 4 h. Although the amount of Ca supplied in our study was intermediate to those 2 treatments, the source used in the trial by Martinez et al. (2016a) was different from our study in both the Ca salts incorporated in the source as well as the presence of a fat coating on the bolus, which may alter the dissolution time of the bolus in the rumen. Previous work using oral Ca drenches has demonstrated that different Ca salts (i.e., Ca chloride vs. Ca propionate) result in a different magnitude and duration of increased blood Ca (Goff and Horst, 1994). The product used in our study contained a different combination of Ca salts that may have had different dissolution times or bioavailability. Martinez et al. (2016a) demonstrated that the most pronounced response in blood Ca was observed at the first blood sample collected after bolus administration at 0.5 h, indicating that release and absorption of Ca occurs rapidly after bolus administration. With the first sample collected 1 h after bolus administration in the current study, a possible short spike in plasma Ca would not be detected. Blanc et al. (2014) demonstrated that administration of intravenous Ca resulted in a transient spike in blood Ca concentration and a subsequent decrease in blood Ca below that of the control group at 20 h after treatment administration, which is a similar pattern (albeit to a smaller magnitude) to the numerical differences observed in our study. Blanc et al. (2014) had another treatment group utilizing the same bolus product as that administered by Martinez et al. (2016a) and observed numerically higher blood Ca for 24 h following treatment initiation.

A limitation to experiment 1 was the range in timing of enrollment of cows relative to parturition (mean $\pm$ SD = 8.3 $\pm$ 5.3 h), which may have increased the variation in blood Ca changes over the 24-h observation period. A further limitation to experiment 1 is that cows enrolled were not identified as hypocalcemic before enrollment. In a study investigating the administration of a subcutaneous dose of Ca, the magnitude of the blood Ca response at 24 h after treatment administration was found to be dependent on pretreatment blood Ca concentration and cows with lower pretreatment blood Ca were found to have more pronounced responses to treatment (Miltenburg et al., 2016). Cows with compromised Ca status may have been more likely to retain Ca administered in the bolus treatment in our study, and therefore if the entire study population were hypocalcemic at enrollment, a difference in blood Ca concentrations between groups may have been observed.

Experiment 2

Health and Production Responses of PP and MP Cows to Oral Ca Administration. Previous work conducted on the use of oral Ca boluses for the mitigation of downstream consequences associated with SCH has demonstrated that responses at the group level are minimal, but specific risk groups can be identified that have positive responses to bolus administration (Oetzel and Miller, 2012; Martinez et al., 2016a,b). Further, the negative responses to bolus administration observed in some groups of cows, identified in a study in which boluses were administered in doses above the label indication (Martinez et al., 2016a,b), necessitates the determination of specific target groups to optimize the profitability of supplementation. Administering a single dose of oral Ca may be a less disruptive approach, both physiologically and from a management and cow behavior perspective, resulting in fewer negative outcomes associated with the treatment. Our primary hypothesis for this portion of the study was that responses to a single dose of oral Ca at parturition would be dependent on periparturient risk factors. Indeed, the majority of responses that were observed were the result of treatment interactions. Cows that received additional supplemental Ca remained in the analysis for all outcomes because the need to administer additional Ca would be indicative of displaying symptoms of a more severe hypocalcemia. The subsequent health of that cow would therefore be important information to capture in regards to the efficacy, or lack thereof, of administering oral Ca.

Positive responses to bolus administration for PP cows were limited to cows calving with higher AFC and to those that were overconditioned at parturition. Positive responses to supplementation based on these risk factors for PP cows have not been previously identified.

Higher BCS around parturition has been associated with greater risk of metabolic disease and compromised performance (Ospina et al., 2010; McArt et al., 2012). Supplementing Ca to overconditioned cows could alleviate some of the underlying causes of increased risk for metabolic disease in these animals by supporting gut motility, which can be compromised when blood Ca declines (Daniel, 1983; Martinez et al., 2014) secondary to poor intake in overconditioned cows. The improved gut motility could be supportive of more rapid increases in intake postpartum and some reduction in the incidence or severity of metabolic disease, and higher DMI would be supportive of greater milk yield in those cows. In this study population, increasing AFC was positively associated with milk yield over the first 4 DHIA test days. Two previous studies have identified that MP cows with greater production potential have positive responses to Ca supplementation (Oetzel and Miller, 2012; Martinez et al., 2016b). Primiparous cows calving with higher AFC in our study might have had increased demand for Ca, precipitating greater responses to supplementation. Increasing age has also been associated with decreased intestinal absorption efficiency of Ca (Hansard et al., 1954) and lower indication of bone metabolism at parturition (Taylor et al., 2008), which might contribute to a greater challenge for older PP cows at parturition. The additional Ca supplied to those animals might have been used to support metabolic and immune health, contributing to decreased risk of disorders in those cows.

Previous work conducted in PP cows has demonstrated marked detrimental effects of bolus administration including increased risk of metritis and compromised reproductive performance (Martinez et al., 2016a,b). In that study, the amount of Ca supplemented was much greater than in the current study, and in one group, the cessation of bolus administration in PP cows was followed by an increase in SCH prevalence compared with PP cows that were not supplemented. This suggests that the administration of this quantity of oral Ca may have impaired Ca homeostatic mechanisms, exacerbating the challenge of adapting to the increased demands of early lactation. The dose of Ca administered in the current study might have been sufficient to aid in the recovery of blood Ca in certain risk groups without impairing homeostatic mechanisms in those cows that were not at increased risk of hypocalcemia, providing support for a single dose approach to Ca supplementation in PP cows. Overall, PP cows that received supplemental Ca in the current study did have increased risk of early removal from the herd and PP cows with lower AFC had greater risk of mastitis when administered boluses. Taken together, the data suggest that a blanket treatment approach for PP cows would not be beneficial but that targeting treatment with a single dose approach to the risk groups identified could result in positive responses.

Responses of MP cows to treatment for milk production and reproduction were minimal. Milk production tended to be higher for MP cows receiving boluses with DCC greater than 277. Interestingly, a similar response was observed in PP cows with DCC greater than 277. For MP cows, this difference was small (0.9 kg/d more for cows assigned to BOL across the first 4 test days), whereas the response was larger for PP cows (2.8 kg/d for cows assigned to BOL). The reason for this response is unclear; however, DCC may be an indirect measure of the time spent in the close-up pen consuming a preventative negative DCAD ration. In this study, all herds were feeding prepartum rations with a negative DCAD, with the exception of one farm (Supplemental Table S4; https://doi.org/10.3168/jds.2017-13164). Those cows may have been more prepared to respond to supplemental Ca postpartum. Multiparous cows that received BOL with shorter dry periods had decreased hazard of pregnancy from the end of the VWP to 150 DIM. Cows with a shorter dry period may represent a group of cows at lower risk of health and reproductive issues. Other researchers have also identified that subsets of MP cows thought to be at lower risk of periparturient health problems responded negatively to bolus administration (Martinez et al., 2016a). Taken with the increased risk of health disorders in MP cows entering their second parity, our data suggest that a blanket approach to treatment may not be appropriate even for MP cows.

Beneficial health responses to Ca supplementation in MP cows were observed in cows entering parity 3 or greater, overconditioned cows, and lame cows. As discussed previously, age is a risk factor for hypocalcemia and compromised Ca metabolism in the periparturient period. Decreased risk of RP and overall health disorder risk in older cows supplemented with oral Ca may be a reflection of supplying additional Ca to support immune function in those cows. Functional capacity of innate immune cells is an important component of expelling the placenta after parturition (Kimura et al., 2002) and clearing the uterus of bacterial contamination introduced during parturition. Previous work has demonstrated that cows with hypocalcemia have compromised immune cell function (Kimura et al., 2006; Martinez et al., 2014) and greater risk of uterine disease (Martinez et al., 2012; Wilhelm et al., 2017), and this may have been alleviated to some extent in cows that received boluses in the current study. Cows that are lame have previously been identified to respond to oral Ca supplementation with decreased occurrence of health disorders (Oetzel and Miller, 2012). Dry mat-

ter intake is likely further compromised in lame cows in the periparturient period, which will impair the effectiveness of preventative strategies implemented in prepartum rations and result in reduced Ca intake around parturition. Although similar health responses to Ca bolus administration in certain risk groups have been identified in previous work (Oetzel and Miller, 2012; Martinez et al., 2016a,b), our study is the first to demonstrate positive responses to a single-dose administration of oral Ca boluses. These results suggest that the additional labor required for multiple doses could be spared and benefits could still be realized by targeting a single dose of oral Ca to cows that are most likely to respond. It should be noted that previous work that compared different dosing strategies administered Ca boluses at doses higher than label indication (Oetzel and Miller, 2012; Martinez et al., 2016a,b), and therefore more work is needed comparing the merit of a single dose versus more moderate administration of multiple doses to determine the optimal strategy.

Differential Responses of PP and MP Cows to Oral Ca Based on Plasma Ca Status at Enrollment. The industry has shown increased interest in application of blood Ca monitoring of cows in the immediate postpartum period. To date, this is only practically useful for herd-level monitoring because of the lack of cow-side tests available for blood Ca determination, which would be necessary for making individual cow treatment decisions. As these technologies advance, the opportunity to use this information for identification of cows to supplement should be investigated to aid in targeting our treatment strategies. In the current study, we aimed to determine thresholds for blood Ca measured within the day after parturition that differentiated responses to supplemental Ca. Other studies have investigated thresholds for blood Ca that were predictive of subsequent disease (Chapinal et al., 2011, 2012; Martinez et al., 2012); however, these thresholds were developed based on blood Ca measurements determined within the first 3 DIM or over the first week of lactation. The relationship between blood Ca concentration measured in the day following parturition and subsequent health and performance is complicated by the Ca utilization that is required to mount an immune response (Waldron et al., 2003; Martinez et al., 2014) as well as the Ca that is required to be excreted in milk (Kume and Tanabe, 1993). Consequently, low blood Ca in the day after parturition, within a reasonable range, could be reflective of a strong immune response at parturition or be necessary for appropriate adaptation to the lactational Ca demand. For these reasons, the interaction between Ca status and oral Ca supplementation was not limited to recent thresholds used for identification of SCH. Utilizing a threshold of $\leq$2.15 mmol/L for pretreatment plasma Ca measurements, 74.6 and 76.3% of CON and BOL cows, respectively, were categorized as having SCH. This prevalence of SCH is similar to that observed in studies that used similar thresholds and sampling time points (Caixeta et al., 2015) and in one case also measured blood Ca in cows that were being fed a negative DCAD prepartum as a preventative strategy (Leno et al., 2017).

Minimal differential responses of PP cows to Ca supplementation were observed based on Ca status, indicating that utilization of periparturient risk factors for targeting treatment is more valuable than measurement of blood Ca concentration within the day following parturition. For MP cows, those with low plasma Ca ($\leq$1.8 mmol/L) that received boluses had decreased risk of receiving additional supplemental Ca, suggesting that those cows were less likely to display signs of clinical hypocalcemia. Although the results of experiment 1 did not show differences in plasma Ca concentration, this finding suggests that for cows with low plasma Ca at enrollment, there was likely enough of an increase in blood Ca concentration to prevent muscular and nervous signs that would be detected by farm personnel to identify clinical hypocalcemia (Oetzel, 2013). Further, MP cows with low plasma Ca ($\leq$2.15 mmol/L) were less likely to have a DA when given boluses. Subclinical reductions in blood Ca concentration have been demonstrated to reduce gut motility (Daniel, 1983; Martinez et al., 2014) and supplementation of Ca could have alleviated this to some degree, which would support greater intake in the immediate postpartum period and potentially aid in prevention of DA. Risk of RP was reduced for MP cows receiving a bolus that were above and below the blood Ca threshold, and risk of metritis was reduced for MP cows with low plasma Ca. The associations between hypocalcemia and poorer immune function discussed previously may have been alleviated by bolus supplementation in those cows. Overall, risk of having one or more health disorder in early lactation was reduced for cows that received boluses that also had low plasma Ca. The thresholds that differentiated responses to Ca supplementation ranged from 1.80 to 2.15 mmol/L. The majority of plasma Ca thresholds that differentiated responses to bolus administration in our study were close to those identified by Martinez et al. (2012) and Wilhelm et al. (2017) as thresholds for SCH identification based on the sensitivity and specificity for identifying cows that would subsequently be diagnosed with metritis.

Responses of both PP and MP cows that were in the higher Ca status groups were inconsistent. In some cases, cows receiving boluses in this group responded

positively with decreased risk of RP in all parties, increased milk yield in PP cows, and decreased risk of mastitis and increased pregnancy risk at first service in MP cows. In other cases, cows in the higher Ca status group responded negatively to bolus administration, such as with greater risk of mastitis in PP cows and greater risk of metritis in MP cows. Martinez et al. (2016a) also observed that administering Ca boluses increased risk of metritis in MP cows that had normal calving (no dystocia, twins, stillbirth, laceration, or RP). Although it is possible with the large sample size that some of these findings are the result of type I error, these results do suggest that responses to bolus supplementation in cows with higher plasma Ca are inconsistent. This is in contrast to responses observed in cows with low plasma Ca, which were positive when identified.

Although the results of experiment 1 do not show an increase in blood Ca concentration after the administration of a single dose of the oral Ca bolus, the results of experiment 2 demonstrate improvements in health outcomes that are indicative of some effect of the treatment. One possible explanation is that a response in blood Ca does occur, but we were unable to detect this difference in experiment 1 due to reasons discussed previously. It does appear that if any response in blood Ca occurs, it is a small increase, which brings into question how this change can affect subsequent health so profoundly. It may be that Ca provided from the bolus is rapidly being used to support immune cells, and thus not measured in the blood, subsequently increasing efficiency of the inflammatory response after parturition. Additionally, the small increase in blood Ca could be affecting the cow through modulation of homeostatic mechanisms that trigger better adaptation of cows through the transition period. The reasons for these discrepancies cannot be elucidated from our study, but the findings warrant further investigation into underlying mechanisms.

A limitation of our study was the variability in herd reporting of disease incidence. Although all herds were provided standardized case definitions, very low disease occurrence in one herd suggested some inconsistency in reporting (Table 3). Randomization within farm ensured that risk of disease diagnosis was equal across treatments and inclusion of farm as a random effect accounted for the differences in likelihood of disease diagnoses between farms. Another limitation of the study is the lack of blinding of farm personnel to treatments. However, treatments were administered sequentially by calving time, and therefore randomization eliminated treatment administration bias and cows were not differentiated by treatment beyond the time of handling at parturition.

CONCLUSIONS

Administration of a single dose of oral Ca at parturition supplying 54 to 64 g of Ca did not result in an observable increased plasma Ca concentration between 1 and 24 h postpartum; however, responses observed for health and performance outcomes suggest that cows in certain risk categories and MP cows with low plasma Ca at enrollment still responded positively to bolus administration. Supplementation of PP cows with higher AFC or higher BCS at parturition positively affected health status and early lactation performance, respectively. In MP cows, supplementation of cows with higher parity, higher BCS, and lameness also resulted in improved health status. Differential responses to treatment based on blood Ca concentration demonstrated that blood Ca was less reliable than other periparturient risk factors for identification of PP cows with potential to respond positively to Ca supplementation. For MP cows, those with low plasma Ca responded with decreased health disorders, but cows with higher plasma Ca had varied responses.

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