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Assessment of Noninferiority of Delayed Oral Calcium Supplementation on Blood Calcium and Magnesium Concentrations and Rumination Behavior in Dairy Cows

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Abstract: We investigated whether delaying oral calcium (Ca) bolus administration to the second day postpartum (DEL) was noninferior to bolus administration within 24 h of calving (CON) in its effects on plasma Ca concentrations during the first five days in milk (DIM). We also investigated the effects of DEL vs. CON strategies on magnesium (Mg) concentrations and daily rumination time (RT). Twenty-three multiparous (parity $\geq$ 3) dairy cows were randomly assigned to the CON (n = 11) or DEL (n = 12) treatment. Blood Ca and Mg were measured at 1–5 DIM and RT was monitored from −7 d to 7 d relative to calving. The noninferiority margin was a difference in Ca concentration of 0.15 mmol/L. Blood Ca and Mg concentrations and RT were analyzed by multivariable linear mixed models accounting for repeated measures. Blood Ca concentrations were 0.07 mmol/L (95% confidence interval: −0.30–0.17) less in DEL cows than CON cows, thus non-inferiority results were inconclusive. The Ca concentration increased across the first 5 DIM but did not differ between treatments while Mg concentrations decreased in both treatments (p < 0.001). There was no treatment difference in RT (CON: 436 ± 21, DEL: 485 ± 19 min/d). While noninferiority results were inconclusive, similar blood Ca dynamics between CON and DEL treatment strategies indicates that delayed Ca administration is a potential management option for commercial dairy farms; however, additional studies using large sample sizes are warranted to confirm these findings.

Keywords: calcium bolus; magnesium; multiparous; rumination behavior; subclinical hypocalcemia

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1. Introduction

Hypocalcemia is associated with the occurrence of other metabolic diseases in the periparturient period and can also lead to decreased production and overall performance [1]. Hypocalcemia is traditionally classified as clinical or subclinical hypocalcemia (SCH). Both are defined by low blood calcium (Ca) concentration, with clinical hypocalcemia differentiating from SCH based on the presence of clinical signs (e.g., recumbency, lethargy, hypothermia, incoordination, decubitus, muscle weakness).

Although well-designed control programs using acidogenic diets prepartum can reduce clinical hypocalcemia incidences to <1% [1], 25% of primiparous and >50% of multiparous cows have SCH in the first 48 h postpartum [2–4]. Considering the economic impact of SCH ($246/case) [5] and lack of effective on-farm diagnostic tools for SCH, supplementing dairy cows immediately postpartum with oral Ca boluses is a widely adopted management strategy in the US dairy industry. Administration of oral Ca supplements to multiparous cows increases blood Ca concentration in the first few hours following treatment [6–8]. The effect of postpartum oral Ca administration on health and performance, however, is inconsistent and affected by previous lactation milk yield, previous lactation length, and locomotion score at calving [7,9,10].

Recent research brings into question the necessity of Ca administration to all cows postpartum. Nearly half of postpartum cows have low blood Ca concentrations in the first 24 h postpartum only, after which concentrations increase without supplementation [11,12]. Notably, cows with persistent SCH (low blood Ca from 1–2 DIM) or delayed SCH (low blood Ca after 3 DIM) had impaired health and performance compared to cows with transient SCH (low blood Ca 24 h postpartum only) or normocalcemic cows [11,12]. Considering these results, an alternative management strategy to the current conventional approach of herdwide Ca supplementation at calving would be to delay postpartum Ca supplementation by at least one day. This strategy would provide time for cows capable of successfully adapting to the demands of their new physiological state to do so, while still allowing for identification and supplementation of cows that fail to successfully adapt.

To determine if this new strategy is a viable alternative, a noninferiority study can be used. Noninferiority studies seek to prove that a new intervention (e.g., delaying Ca supplementation) is not unacceptably worse than the current intervention (e.g., Ca supplementation at calving), based on a pre-designated noninferiority margin [13]. Thus, the main study objective was to determine whether delaying postpartum administration of oral Ca boluses by one day was noninferior to Ca administration on the day of calving in its effect on Ca concentration dynamics in multiparous cows during the first 5 DIM. Secondary objectives were to describe blood Mg concentration dynamics during the first 5 DIM and rumination behavior from 7 d before to 7 d after calving. We hypothesized that delaying Ca administration by 24 h would not be worse than the conventional method of administration on the day of calving, within a noninferiority margin of −0.15 mmol/L, in its effects on Ca concentrations over the first 5 DIM.

2. Materials and Methods

All study procedures followed the University of Minnesota Dairy Cattle Teaching and Research Center standard operating procedure (2002-37896A) approved by the University of Minnesota Institutional Animal Care and Use Committee. The study was conducted from September 2020 to May 2021. A total of 23 multiparous (parity ≥ 3) Holsteins cows at the dairy were enrolled 21 d prior to their expected calving date. Cows were housed in tie-stalls and fed ad libitum to stimulate greater postpartum intake and help reduce postpartum energy deficits [14]. The total mixed ration (TMR, Table 1) was formulated following NRC recommendations. Prepartum TMR contained anionic salts and was formulated to have a negative DCAD of −280 mEq/kg of DM. Feed was delivered once daily and cows were milked twice daily.

At enrollment, cows were randomly assigned to one of two treatments. Cows in the conventional treatment group (CON; n = 11) received oral Ca boluses within the first 24 h postpartum (i.e., day of calving). Cows in the delayed treatment group (DEL; n = 12) receive oral Ca boluses between 24 and 48 h postpartum (i.e., day after calving). Cows in each treatment group received a single administration of two Ca boluses (Rumilife CAL24, Genex Cooperative Inc., Shawano, WI, USA), each containing 100 g Ca (400 g of bolus weight/dose), following manufacturer recommendations. Bolus administration was carried out by the research team.

Prepartum blood samples were collected one week prior to the expected calving dates. Postpartum blood samples were collected within 24 h of calving for both treatment groups and collected daily thereafter around 2 h after the morning milking (07:30–08:30 h) through 5 DIM. Bolus administration occurred immediately after the first postpartum blood collection for CON cows and after blood sampling at 2DIM for DEL cows. Blood samples were collected from a coccygeal vessel via evacuated tubes with lithium heparin and centrifuged within 2 h of collection at 1200× g for 15 min. Plasma was harvested and stored at −20 °C until analyzed. Total Ca and Mg were measured in duplicate with a small-scale chemistry analyzer (CataChemWell-T, Catachem Inc, Oxford, CT, USA) using the Arsenazo III (assay range: 0.09–4.3 mmol/L) and Xylidyl blue (assay range: 0.35–3.5

mmol/L) methods, respectively. Intra- and inter-assay coefficients of variation, respectively, were 8.3 and 11.2% for Ca and 6.8 and 11.7% for Mg.

Table 1. Ingredient and nutrient content of diets fed pre- and postpartum.

Components	Prepartum Diet	Postpartum Diet
Ingredient, % of DM		
Corn gluten	4.38	6.18
Corn silage	44.38	35.90
Grass hay	29.68	-
MegAnion premix [a]	21.56	-
Alfalfa hay	-	10.91
Lactating cow protein mix [b]	-	14.54
QLF commercial dairy mix [c]	-	5.00
Cottonseed, Fuzzy	-	5.46
Corn extra fine rolled	-	21.37
Energy Booster 100 [d]	-	0.64
Nutrient content, DM basis		
DM, %	50.88	57.05
CP, %	15.44	16.30
Crude fat, %	2.77	4.51
ADF, %	28.73	16.55
NDF, %	35.25	26.00
Starch, %	15.00	28.17
NE_L, Mcal/kg	1.52	1.67
Ca, %	0.76	0.88
P, %	0.40	0.39
Mg, %	0.49	0.37
K, %	1.11	1.43
Na, %	0.09	0.50
S, %	0.34	0.28
Cl ion, %	1.39	0.58
DCAD [e], mEq/100 g	−28.03	23.79

[a] Bio-Sel Dry Cow 1000-Rum (Vita Plus Corporation, Madison, WI, USA), 2.4%; vitamin E-20,000, 0.5%; di-calcium phosphate 21% phosphorus, 0.7%; blood meal, 2.5%; calcium carbonate, 2.9%; canola meal, 13%; ReaShure Choline (Balchem Coorporation, New Hapton, NY, USA), 1.9%; fat, 0.75%; magnesium oxide 54%, 1%; soy hulls, 42%; soybean meal 47% protein, 13%; Yeast Original XPC (Diamond V, Cedar Rapids, IA, USA), 0.4%; MegAnion (Origination O2D, Maplewood, MN, USA), 18.75%; Smartamine (Adisseo USA. Inc, Alpharetta, GA, USA), 0.25%. [b] Soybean meal 47% protein, 28.25%; canola meal, 30.00%; Amino Plus (AG Processing, Inc., Omaha, NB, USA), 8.50%; blood meal, 6.50%; calcium carbonate, 6.75%; sodium bicarbonate, 6.00%; WR Elite Dairy Micro (Vita Plus Corporation, Madison, WI, USA), 3.50%; potassium carbonate, 2.00%; UltraMet (Vita Plus Corporation, Madison, WI, USA), 2.75%; sodium chloride, 2.70%; urea 46% N, 2.10%; Alimet (Novus International, Inc., Saint Charles, MO, USA), 0.90%; Rumensin 90 (Elanco Animal Health, Greenfield, IN, USA), 0.05%. [c] Molasses-based liquid supplement of soluble sugars and protein developed from cane molasses, condensed whey, urea, and sulfuric acid. The liquid supplement DM contained total sugars as invert 58.6%, CP 11.8 % with 38% NPN, fat 0.5%, Ca 1.16%, P 0.42%, K 4.82%, Mg 0.47%, S 0.95%, Na 0.47%, Cl 2.86% (Quality Liquid Feeds, Dodgeville, WI, USA). [d] Hydrolyzed animal and vegetable fat (Milk Specialties Global, Eden Prairie, MN). [e] Calculated using the equation [(mEq of Na + mEq of K) − (mEq of Cl + mEq of S)].

Rumination behavior was monitored from −7 d to 7 d relative to calving and recorded continuously using the CowManager ear-tag accelerometer (Agis Automatisering BV, Harmelen, The Netherlands). Rumination data was extracted as the total min/h spent ruminating and reported as total daily rumination time (RT, min/d). The 24 h time interval used to calculate total daily RT was based on time of calving.

Milk yield (kg/d) during the first 7 DIM was compared between CON and DEL groups to assess whether delaying the administration of Ca boluses affects cow performance in early lactation. Dry matter intake (kg/d) 7 days prior to and 7 days after calving was also recorded and compared between groups.

The study was designed as a randomized noninferiority intervention trial to determine if the DEL management strategy was at least as effective as the CON strategy, within a pre-specified margin of noninferiority, in its impact on blood Ca dynamics in the first

5 DIM. The predetermined noninferiority margin for Ca concentration was −0.15 mmol/L, representing the lower boundary of a 2-sided 95% confidence interval (CI) of the difference of DEL relative to CON. This margin reflects a biologically important difference that would, on average, be great enough to impact hypocalcemic diagnosis [6]; i.e., if DEL was inferior, cows would also be hypocalcemic. Based on sample size calculations for a noninferiority trial, 18 cows ($n = 9$ per group) were required to be 80% sure that the lower bound of a one-sided 95% CI was greater than the −0.15 mmol/L noninferiority margin. The number of cows was increased by 25% to account for potential exclusions due to factors unrelated to our experiment.

Statistical Analysis

Total Ca and Mg plasma concentrations during the first 5 DIM and total daily RT from −7 to +7 days relative to the time of bolus administration were evaluated. The noninferiority for calcium blood concentration of DEL to CON was inferred if the lower bounds of a 2-sided 95% confidence interval (CI) of the difference between DEL and CON was greater than the noninferiority margin of −0.15 mmol/L. Primary (Ca) and secondary (Mg and RT) outcomes were assessed using a multivariable linear mixed model with repeated measures using an autoregressive covariance structure. The model consisted of the fixed effects of treatment (CON, DEL), DIM, and the interaction between treatment and DIM. Cow nested within treatment was considered as a random effect. Parity and postpartum concentrations of Ca and Mg at 1 DIM were examined as possible covariates. The Bonferroni correction method was used to account for multiple comparisons within the model when needed. Differences in milk-yield (kg/d), DMI (kg/d) prepartum, and DMI during the first week postpartum were assessed using a simple linear regression. Results are reported as least square means (95% CI) and treatments are considered to differ when $p < 0.05$.

3. Results

Five of the 23 cows enrolled were excluded from the analysis due to a calving communication error (one CON), instrument malfunction (one CON), dystocia (two DEL) and injury (one DEL). Parity of the remaining 18 cows (nine per group) were evenly distributed between CON and DEL and other descriptive characteristics were similar between groups (Table 2). Bolus administration occurred within 24 h of calving for all CON cows and six DEL cows were administered Ca boluses between 24 and 48 h postpartum. Due to the timing of treatment administration of boluses for all cows (8:00 AM), 3 DEL cows were administered boluses 21–24 h postpartum.

Blood plasma Ca concentration at the day of calving was 2.36 ± 0.58 mmol/L in the CON and 2.00 ± 0.27 mmol/L in the DEL group, but did not differ, and increased similarly from 2 to 5 DIM (Figure 1a). The number of cows that had Ca < 2.0 mmol/L from 1 to 5 DIM were 2, 3, 1, 0 and 0 for CON and 5, 4, 2, 0 and 1 for DEL, respectively. Two CON cows and 4 DEL cows had Ca concentrations < 2.0 mmol/L for two or more days between 1 to 3 DIM. Only one cow in each group had low Ca concentration at 2 DIM but not at 1 DIM. No cows with Ca < 2.0 mmol/L displayed any clinical signs of hypocalcemia and thus met the criteria for SCH [1,2].

The average blood plasma Ca concentrations did not differ ($p = 0.52$) between CON (2.46 ± 0.08 mmol/L; 95% CI: 2.29–2.63) and DEL (2.38 ± 0.08 mmol/L; 95% CI: 2.22–2.55). There was no interaction between treatment and DIM ($p = 0.48$). However, DIM affected ($p < 0.001$) Ca concentrations as concentrations increased over the course of the first 5 DIM for both CON and DEL treatment groups. Blood Ca concentrations were 0.07 (95% CI: −0.30 to 0.17) mmol/L lower for DEL cows than for CON.

Table 2. Descriptive statistics of the study population for parity, time between calving and calcium bolus administration (Time-to-treat), measures of prepartum blood calcium (Ca) and magnesium (Mg), taken 1 week prior to expected calving date, and total daily rumination time (RT). Parity is presented as the number of cows/group/parity and all other outcomes are presented as the mean ± SD and range per treatment group.

Outcome Measure	CON [a]	DEL [b]	*p*-Value
Parity			0.52
3	5	5	
4	4	4	
Time-to-treat, h	9.3 ± 9.0	26.1 ± 4.0	<0.001
Range	1.0 to 24.0	21.0 to 31.0	
Prepartum Ca, mmol/L	2.47 ± 0.52	2.00 ± 0.27	0.09
Range	1.46 to 3.22	1.58 to 2.35	
Prepartum Mg, mmol/L	0.96 ± 0.25	1.07 ± 0.16	0.38
Range	0.62 to 1.26	0.88 to 1.40	
Prepartum RT [c], min/d	416 ± 24	435 ± 16	0.85
Range	277 to 604	114 to 707	

[a] CON = cows in the control group received their first postpartum oral calcium supplementation within the first 24 h after calving. [b] DEL = cows in the delayed group received their first postpartum oral calcium supplementation between 24 and 48 h postpartum. [c] Prepartum RT = total duration of time spent ruminating daily (min/d), averaged across the 7 days prior to the time of bolus administration.

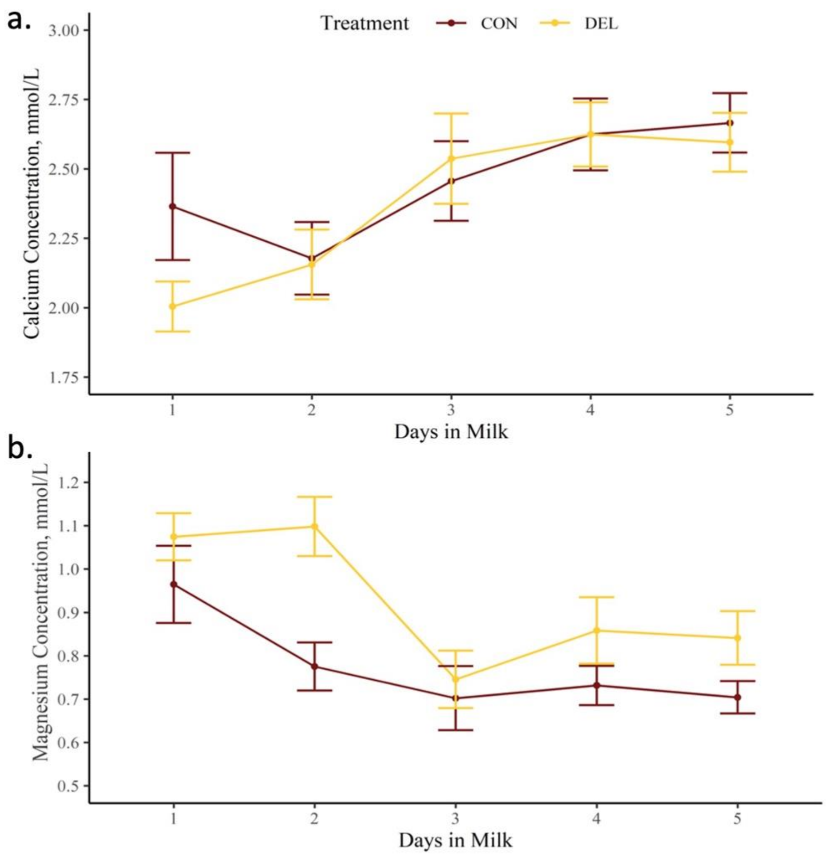

Figure 1. Blood plasma calcium (**a**) and magnesium (**b**) concentrations in multiparous cows administered oral Ca boluses. Two Ca boluses were administered simultaneously within 24 h (CON) or between 24 and 48 h (DEL) of calving. Least square means (±SEM) from 1 to 5 days in milk (DIM) are reported for 9 CON and 9 DEL cows. Blood plasma Ca concentration did not differ between treatments (p = 0.52), increased with DIM (p < 0.001) and was not affected by the interaction of treatment by DIM (p = 0.48). Blood plasma Mg concentration was greater in DEL cows (p = 0.04), decreased with DIM (p < 0.001), and was altered by the interaction of treatment * DIM (p = 0.02).

Blood plasma Mg concentrations were lower ($p = 0.04$) in CON (0.78 ± 0.04 mmol/L; 95% CI: 0.68–0.87) than in DEL (0.92 ± 0.04 mmol/L; 95% CI: 0.83–1.02) cows across the first 5 DIM and decreased ($p < 0.001$) as DIM increased (Figure 1b). There was an interaction of treatment by DIM ($p = 0.02$) as Mg concentrations decreased after Ca supplementation which, by design, occurred at 1 DIM for CON and 2 DIM for DEL cows.

Total daily RT did not differ ($p = 0.07$) between CON (418 ± 21 min/d; 95% CI: 373–464) and DEL (475 ± 18 min/d; 95% CI: 435–514) and there was no interaction of treatment and DIM ($p = 0.65$). Daily RT was altered by DIM ($p < 0.001$) as pre-partum RT was relatively static for both treatment groups, decreased at calving, returned to prepartum levels by 5 DIM and continued to increase through 7 DIM (Figure 2). There was an interaction between treatment and DIM ($p < 0.01$) for RT, primarily attributed to the decrease, followed by increase, in RT that occurs relative to calving.

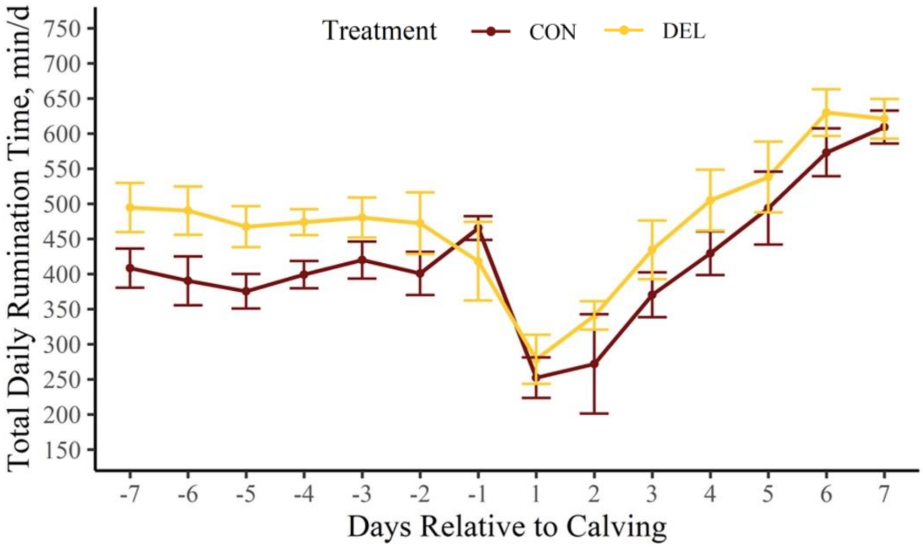

Figure 2. Total daily rumination time (RT, min/d) in multiparous cows administered oral Ca boluses. Two Ca boluses were administered simultaneously within 24 h (CON) or between 24 and 48 h (DEL) of calving. Least square means (±SEM) from −7 to +7 days in milk (DIM) are reported for 6 CON and 8 DEL cows. The first hour of day 1 coincides with the time of reported calving. Effect of DIM on total Daily RT differed among days ($p < 0.001$) but was not affected by treatment ($p = 0.07$) or the interaction of treatment by day ($p = 0.65$).

Milk yield during the first 7 DIM did not differ between CON (29.0 kg/d.; 95% CI: 26.0–31.9) and DEL (31.9 kg/d.; 95% CI: 29.0–34.7) groups ($p = 0.36$). Dry matter intake during the week prior to calving also did not differ between CON (11.3 kg/d.; 95% CI: 6.2–16.3) and DEL (12.4 kg/d.; 95% CI: 7.0–17.8) groups ($p = 0.74$). During the first week postpartum, both groups had an increase in DMI when compared to the week before parturition but no differences when comparing groups (CON: 15.9 kg/d., 95% CI: 11.9–19.8 vs. DEL: 20.0 kg/d., 95% CI: 15.8–24.1; $p = 0.15$)

4. Discussion

The primary study objective was to determine if delaying Ca oral bolus supplementation to the second day after calving was noninferior to the current industry standard of Ca administration on the day of calving on blood Ca concentrations in the first 5 DIM, within a −0.15 mmol/L noninferiority margin. The results regarding the comparative efficacy of the two strategies were inconclusive. Results of a noninferiority trial are deemed 'inconclusive' when the lower and upper bounds surpass the noninferiority margin and a mean difference equal to 0, respectively. However, lack of noninferiority is not inherently synonymous in noninferiority studies with the new intervention strategy being inferior to the existing one.

Postpartum blanket treatment of multiparous cows with Ca bolus supplementation is a widely used SCH treatment and prevention strategy [15]. However, an economic analysis determined that, amongst all supplementation strategies investigated, Ca supplementation for high producing multiparous cows and lame cows, instead of blanket treatment, results in the greatest net impact [16]. Recent findings show Ca homeostasis does not follow the same pattern in all animals, with the majority of cows having normal blood Ca levels within 2–3 days of calving, independently of Ca supplementation [11,12]. Furthermore, the use of negative DCAD diets in the prepartum period results in metabolic acidosis at calving [17–19] and Ca bolus administration can induce uncompensated acidosis [1]. The rationale for delaying oral Ca dosing chosen for our study was to avoid inducing uncompensated acidosis and leverage the dairy cow's physiological adaptations to sustain postpartum health without compromising the health of maladapted cows that require Ca supplementation after calving.

The blood Ca concentration profiles through the first 5 DIM in our study agrees with those in previous reports for multiparous cows administered oral Ca boluses [20,21]. Looking at Ca dynamics across a longer postpartum period is important, as SCH that persists across the first three days postpartum or for which onset is delayed to after 3 DIM results in decreased health and performance by comparison to SCH that occurs only within 24 h of calving [11,12]. As expected, blood Ca concentrations increased over the first 5 DIM in both treatment groups. Even with more cows classified with SCH in the first 2 DIM amongst DEL cows (5/9 DEL vs. 2/9 CON cows), Ca concentrations were able to match those of CON cows within the first 5 DIM. These results suggest that an alternative management program for Ca supplementation in which bolus administration is delayed could allow farmers to provide targeted treatment to those cows unable to meet the Ca demands of their new physiological state while forgoing treatment of those that can satisfy Ca demands without supplementation.

Sample size calculations used a noninferiority margin of −0.15 mmol/L—a biologically significant difference in the context of exploring our two SCH intervention strategies. The lack of noninferiority could be the result of an insufficiently powered analysis, suggesting a larger sample size may be required to more conclusively evaluate the noninferiority of the DEL treatment. Moreover, SCH cows have a greater increase in blood Ca concentrations following postpartum oral Ca supplementations than normocalcemic cows [8]. As such, our findings need to be carefully interpreted and future studies with larger study populations able to differentiate between normocalcemic and SCH cows are warranted to establish the effects of delaying oral Ca supplementation according to blood Ca concentration at the time of treatment. Use of an equivalence trial as opposed to looking solely at noninferiority would further elucidate the comparative effects of delaying Ca bolus administration versus the current standard management practices.

Postpartum plasma Mg concentrations followed the same pattern published by others, with Mg concentrations found to decrease following Ca supplementation [8,20]. The maintenance of blood Mg concentration is not regulated by hormones and instead depends on the influx of Mg via gastrointestinal absorption [22], which can be significantly reduced when higher concentrations of Ca are present in the gastrointestinal tract [23,24]. Increased concentration of Ca in the rumen following boluses administration may have affected Mg absorption, leading to the decreased Mg concentration observed in our study. Nonetheless, although Mg concentration decreased within 24 h following bolus administration and remained stable and less than prepartum concentrations in both groups from 3 to 5 DIM, concentrations stayed within a normal range (0.70–1.23 mmol/L) in both groups during the study.

Rumination behavior was not affected by Ca supplementation strategies in our study. Our results substantiate previous reports that oral Ca supplementation had no effect on dairy cow rumination behavior during the first week postpartum [25]. Indeed, our results are more concurrent with calving-related changes in rumination, as RT decreases in the two days around parturition [26]. As such, the large decreases and subsequent increase in RT for DEL and CON cows from −1 to 1 day relative to calving, respectively, are likely not attributable to the treatments administered. It is important to note, however, that there was a larger proportion of DEL cows that had SCH at 1 DIM (5/9 DEL cows vs. 2/9 CON cows) and at least one third of cows in both groups had SCH at 2 DIM. It is possible that rumination behavior around calving may differ for cows that develop SCH and those that do not, which could not be tested in this study, warranting further investigation into potential relationships between RT and postpartum Ca concentrations.

No difference was observed in the first week postpartum for milk-yield or for DMI. Even though these measurements are generally quite variable during this short interval, the values reported here are consistent with what authors have reported previously for multiparous cows in this facility [15].

5. Conclusions

The noninferiority results were inconclusive for blood Ca concentrations in multiparous cows (parity ≥ 3) when administration of oral Ca boluses was delayed to the second day of lactation compared to cows that received Ca boluses on the day of calving. Blood Ca concentrations were similar from d 2 to 5 postpartum, suggesting both strategies have similar impacts on Ca dynamics. Blood Mg concentrations differed by treatment and time, but did not deviate from biologically normal ranges during the study. Total RT similarly was not impacted by management strategy. These findings suggest that delaying bolus administration to the day after calving can be done without detriment to blood Ca dynamics. These results also contribute to our knowledge regarding the optimization of fresh cow management, presenting an alternative management strategy for subclinical hypocalcemia that provides an opportunity for cows to naturally adapt to their new lactation stage while still allowing for timely intervention for cows that require Ca supplementation. Further research with a larger study population is needed to investigate the comparative effects of these two management strategies on health and performance outcomes associated with SCH.

Author Contributions: C.C.F. was involved in data acquisition, data management, formal analysis and interpretation, and original draft preparation; E.S. was involved in the formal analysis and interpretation, and in manuscript review and editing; M.R. and J.V.L.S. were involved in acquisition and manuscript review and editing; B.A.C. was involved in manuscript review and editing; L.S.C. was involved in the study conceptualization and design, manuscript review and editing, funding acquisition and supervision. All authors have read and agreed to the published version of the manuscript.

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