The Epidemiology and treatment of Subclinical Hypocalcemia

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Introduction

The days following calving are a tumultuous time for a dairy cow as she adjusts to the metabolic and energetic demands that come along with colostrum and milk production. Of note is the appreciable increase in demand for Ca as the cow assumes lactation. Despite biological mechanisms in place to maintain Ca homeostasis, hypocalcemia befalls some cows in early lactation. Most dramatically this manifests as clinical hypocalcemia, however as our management of peripartal cows has improved, the incidence of clinical hypocalcemia has been reduced. Instead, nearly 50% of multiparous cows experience subclinical hypocalcemia; reductions in blood Ca below certain thresholds but display no physical signs of disease (Reinhardt et al., 2011). Early studies exploring subclinical hypocalcemia failed to reach a consensus as to what concentration of blood Ca was indicative of subclinical hypocalcemia and what day in milk it was best diagnosed. As such, the associations of subclinical hypocalcemia on health and production outcomes vary widely. Recent studies though have found that when the temporal patterns of blood Ca in early lactation are considered in the diagnosis of subclinical hypocalcemia, the outcomes associated with the disorder are more consistent. Parallel to the expansion in our understanding of subclinical hypocalcemia, has been the adoption of subclinical hypocalcemia mitigation and treatment strategies. While oral Ca supplementation, generally in the form of a bolus, is widely used in commercial settings, the impact that such boluses have on health, production, and reproductive parameters varies. This review aims to discuss both our current understanding of blood Ca dynamics in early lactation, how that impacts the diagnosis of subclinical hypocalcemia, and the available treatment strategies to minimize the associated negative outcomes of subclinical hypocalcemia.

Epidemiology of Subclinical Hypocalcemia

Hypocalcemia has long plagued the dairy industry as maintenance of the Ca pool during the first several days of lactation presents an appreciable challenge to the cow. As the cow assumes lactation, her demand for Ca more than doubles to support colostrum and milk production alone, and is bolstered by the essential role that Ca plays in many physiological and immunological pathways (Webb, 2003; Kimura et al., 2006; Goff et al., 2014). To meet the increased demand for Ca, several hormones and tissues work in tandem to restore and maintain normocalcemia in the hours and days following calving (Goff, 2000).

Unfortunately, some cows fail to regain normocalcemia and succumb to hypocalcemia, the reduction of blood Ca below physiologically normal concentrations. Most dramatically, this manifests as clinical hypocalcemia, arguably the most recognizable clinical diagnosis of dairy cows, as cows become recumbent soon after calving, often have cold ears, and lose the ability to raise their head or stand (McArt and Oetzel, 2023). Cases of clinical hypocalcemia, commonly referred to as milk fever, require immediate treatment with intravenous Ca to avoid death. Fortunately, over the last several decades, due to advancements in nutritional and management strategies of peripartal cows, the incidence of clinical hypocalcemia has substantially declined, affecting approximately 5% of multiparous dairy cows (Goff, 2008). However, up to 45% of multiparous dairy cows experience subclinical hypocalcemia (**SCH**) in the days following calving (Reinhardt et al., 2011).

Subclinical hypocalcemia is the occurrence of blood Ca concentrations below a given threshold at a specific time and is unaccompanied by physical signs of disease (McArt and Oetzel, 2023). Initial studies that aimed to identify SCH did so by characterizing blood Ca concentrations during the first several days of lactation and as serum Ca often reaches a nadir within the first 24 h of lactation, that was deemed the ideal time to diagnose SCH (Oetzel, 1988; Martinez et al., 2012). To improve the characterization of SCH and identify a more accurate diagnostic cutpoint of blood Ca, subsequent large field trials were conducted in which epidemiological methods were employed to explore the association of given Ca cutpoints with important outcomes such as milk production, disease events, and reproductive outcomes (Chapinal et al., 2011; Neves et al., 2018a; Venjakob et al., 2018).

With these field trials, the black box of SCH was opened; no consensus was made as to what concentration of serum total Ca (**tCa**), or day in milk to diagnose, was most accurately associated with negative health and production outcomes. Diagnostic cutpoints of tCa ranged from 1.8 to 2.2 mmol/L and the day in which blood was analyzed varied from 0 to 7 days in milk (Couto Serrenho et al., 2021). When Chamberlin et al. (2013) classified multiparous Holstein cows as SCH when blood ionized Ca was <1.0 mmol/L (approximately 2.0 mmol/L tCa) within the first 24 h of calving, they saw no difference in the incidence of clinical mastitis, ketosis, metritis, displaced abomasum, or reproductive outcomes between SCH cows and those that remained normocalcemic. Additionally, there was no difference in milk production for the first 35 DIM between SCH and normocalcemic cows. When Neves et al. (2018) explored the association of tCa concentrations of multiparous Holstein cows within 12 h of calving, they found that cows with tCa ≤1.85 mmol/L were at increased risk for developing a displaced abomasum compared to those with tCa above the cutpoint. Interestingly, they also found that multiparous cows with tCa ≤1.95 mmol/L produced more milk than cows with tCa above the given cutpoint. Furthermore, in a large field trial conducted on 55 herds across North America in which SCH was diagnosed as tCa <2.1 mmol/L at any point during the first week of lactation, they found that SCH cows were at increased odds of developing a displaced abomasum and produced less milk than normocalcemic cows.

In studies where the temporal patterns of blood Ca during the first serval days of lactation were considered in the diagnosis of SCH, a clearer picture of SCH emerged, and the associated negative impacts of SCH were more consistent. When only 1 DIM tCa concentrations were considered, Neves et al. (2018b) observed no association between SCH diagnosis and risk of metritis, however they found that if parity 2 cows had tCa ≤1.97 mmol/L at 2 DIM and parity 3 cows had tCa ≤2.2 mmol/L at 4 DIM, they were at increased risk for developing metritis, displaced abomasum, or both compared to cows that remained above the stated cutpoints. Furthermore, they observed that reduced blood tCa at 1 DIM was associated with greater milk production while reduced blood tCa at 4 DIM was associated with decreased milk production. Caixeta et al. (2017) also found that cows with tCa ≤2.15 mmol/L for the first 3 DIM took significantly longer to return to cyclicity and had decreased odds of becoming pregnant to first service compared to cows that only had 1 sample ≤2.15 mmol/L or that remained above the cutpoint during the first 3 DIM.

These studies indicated that the absolute nadir of tCa in early lactation may not be indicative of associated negative outcomes, but rather the timing and persistency of SCH should be considered. McArt and Neves (2020) further investigated the dynamics of tCa during the first 4 DIM by classifying multiparous Holstein cows (n = 263) into 1 of 4 SCH groups based on tCa at 1 and 4 DIM. The tCa cutpoints were identified through receiver operator characteristic curves established by Neves et al. (2018b) and were ≤1.77 mmol/L and ≤2.20 mmol/L for 1 and 4 DIM, respectively. If cows had tCa above both cutpoints at 1 and 4 DIM, they were deemed normocalcemic (**NC**; n = 109); cows with tCa ≤1.77 mmol/L at 1 DIM but >2.20 mmol/L at 4 DIM were classified as transient SCH (**tSCH**; n = 50), those that were below the cutpoints at both 1 and 4 DIM were classified as persistent SCH (**pSCH**; n = 34), and finally those with tCa >1.77 mmol/L at 1 DIM but ≤2.20 mmol/L at 4 DIM were deem delayed SCH (**dSCH**; n = 70). The dynamics of tCa during the early lactation period are outlined in Figure 1. When the risk of adverse events, defined as hyperketonemia, metritis, displaced abomasum, herd removal, or a combination thereof during the first 60 DIM, was explored in these cows, the authors found that pSCH and dSCH cows were nearly twice as likely to experience an adverse event compared to NC cows. Furthermore, tSCH cows produced significantly more milk throughout the first 10 wk of lactation compared to NC cows.

To further explore the etiology of SCH and the dynamics of tCa during early lactation, Seely et al. (2021) again classified multiparous Holstein cows (n = 78) as NC, tSCH, pSCH, or dSCH based on tCa at 1 and 4 DIM (1 DIM cutpoint: tCa ≤1.95 mmol/L and 4 DIM cutpoint: tCa ≤2.20 mmol/L) and measured daily dry matter intake (**DMI**) from 14 days prepartum to 21 days postpartum. During the prepartum period, DMI was similar between SCH groups ($P = 0.6$), however following parturition DMI was significantly different between SCH groups ($P < 0.001$) with NC and tSCH cows consuming more feed than their pSCH and dSCH counterparts (NC = 20.9 ± 1.0 kg/d, tSCH = 21.2 ± 1.0 kg/d, pSCH = 17.5 ± 1.2 kg/d, and dSCH = 18.6 ± 1.0 kg/d). Of note, was the unique pattern of DMI during the first 4 DIM between SCH groups that closely mirrored the temporal pattern of blood tCa occurring simultaneously, further highlighting the unique nature of tCa concentrations during the early lactation period.

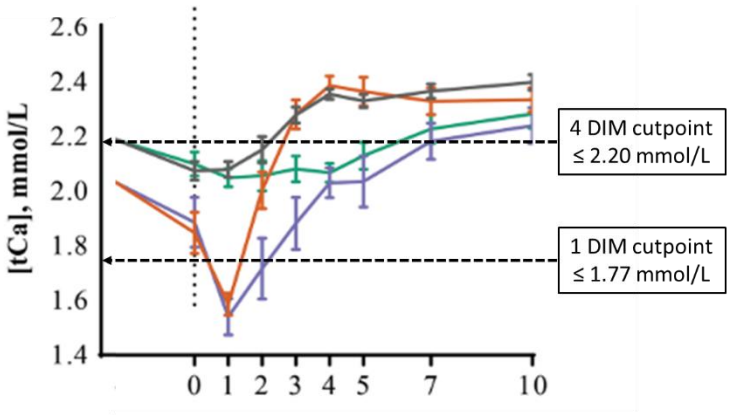

Figure 1. Dynamics of tCa for multiparous Holstien cows (n = 263). Cows were classified as follows: normocalcemic (NC, gray line; [Ca] >1.77 at 1 DIM and 2.20 mmol/L at 4 DIM); transient subclinical hypocalcemia (tSCH, orange line; [Ca] ≤1.77 at 1 DIM and >2.20 mmol/L at 4 DIM); persistent subclinical hypocalcemia (pSCH, purple line; [Ca] ≤1.77 at 1 DIM and ≤2.20 mmol/L at 4 DIM); or delayed subclinical hypocalcemia (dSCH, green line [Ca] >1.77 at 1 DIM and ≤2.20 mmol/L at 4 DIM). Adapted from McArt and Neves (2020).

The culmination of findings of McArt and Neves (2020) and Seely et al. (2021) as well as earlier work by Caixeta et al. (2017) and Neves et al. (2018b) considering the dynamic patterns of tCa during early lactation in the diagnosis of SCH demonstrate homogeneous results. Together, these works suggest that a transient drop in blood Ca at 1 DIM may be necessary as the cow prepares for the demands of lactation, while low blood Ca at 3 and 4 DIM is representative of a larger metabolic disruption, putting the cow at increased risk for additional disease development and decreased reproductive success and production. Considering the consistent negative production and health outcomes associated with low tCa at 4 DIM, we postulate that these cows are experiencing a state of dyscalcemia where the demands of early lactation have outpaced biological mechanisms in place to restore and maintain tCa concentrations.

To explore the associated impact of dyscalcemia on reproductive outcomes, Seely and McArt (2023) classified multiparous Holstein cows (n = 697) as dyscalcemic if 4 DIM tCa ≤2.20 mmol/L (n = 182) or normocalcemic if 4 DIM tCa >2.20 mmol/L (n = 515). They found that the odds of becoming pregnant to first service was significantly reduced in dyscalcemic cows compared to normocalcemic cows (odds ratio = 0.75; P = 0.01). While 27.4% of normocalcemic cows became pregnant to first service, 18.1% of dyscalcemic cows became pregnant to first service. Similarly, the median time to pregnancy was longer for dyscalcemic cows (119 ± 16 d) compared to normocalcemic cows (103 ± 11d; P = 0.1). The hazard of pregnancy by 150 DIM was also reduced in dyscalcemic cows (incidence = 65.4%; hazard ratio = 0.82; P = 0.06) compared to normocalcemic cows (incidence = 70.7%).

Beyond the established negative associations that dyscalcemia has on health and production, low blood Ca concentrations at large can negatively impact rumen function and rumination time. The strength and speed of rumen contractions rely on cytosolic Ca (Webb, 2003). In cases of clinical hypocalcemia, it is not uncommon for rumen contractions to cease (Jørgensen et al., 1998), and (Goff et al., 2020) reported a positive association between rumination time and blood Ca during the first 2 DIM. In an effort to explore the association between dyscalcemia and rumination time during the periparturient period and perhaps identify an alternative to blood sampling for the diagnosis of dyscalcemia, Seely and McArt (2023) recorded rumination and activity time for multiparous Holstein cows (n = 182). Cows were classified as dyscalcemic if 4 DIM tCa ≤2.20 mmol/L (n= 57) or normocalcemic if 4 DIM tCa >2.20 mmol/L (n = 125) and rumination and activity time was recorded for the 14 d before and after calving. While there was no difference in rumination or activity time prepartum between dyscalcemic and eucalcemic cows (both $P > 0.3$), postpartum rumination and activity times were both significantly reduced in dyscalcemic cows compared to normocalcemic cows (both $P < 0.01$). Dyscalcemic cows ruminated an average of 480.5 ± 15 min/d while normocalcemic cows ruminated an average of 512.3 ± 10.5 min/d during the first 14 DIM. Dyscalcemic cows were also less active than normocalcemic cows (407.8 ± 15.5 arbitrary units/d versus 436.1 ± 11.0 arbitrary units/d). Predictive models utilizing rumination and activity variables recorded during the first 4 DIM also showed promise in correctly identifying dyscalcemic cows (best performing model: sensitivity = 38.6%, specificity = 94.4%, accuracy = 77.0%).

Recent works exploring the associations of dyscalcemia challenge the traditional framework of SCH diagnosis and Figure 2 summarizes the outcomes associated with the temporal patterns of blood Ca during early lactation. What remains to be elucidated though is the driving force that allows some cows to regain normocalcemia after a transient drop in blood Ca at 1 DIM, while reductions in blood Ca persist or do not drop until 4 DIM in others. Furthermore, despite our improved understanding of blood Ca dynamics following parturition, hypocalcemia still befalls many dairy cows in early lactation and for that we turn to a variety of treatment and prevention strategies.

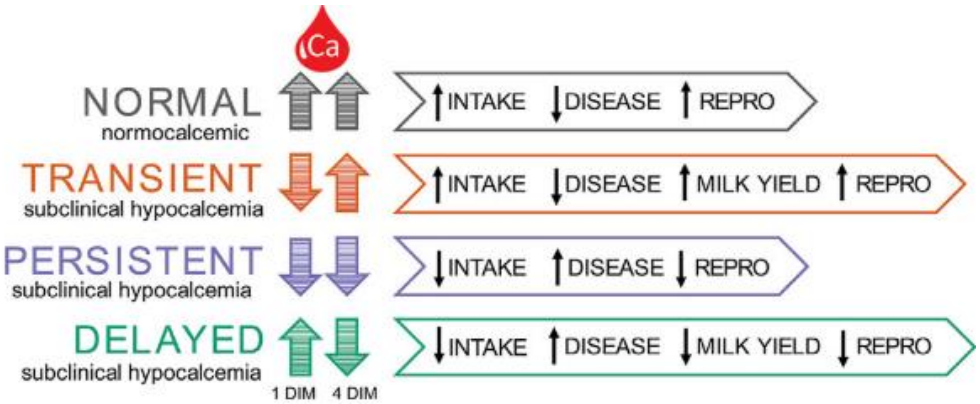

Figure 2. Calcium dynamic groups based on blood total calcium concentrations of multiparous cows at 1 and 4 DIM and their association with dry matter intake, subsequent disease incidence, early lactation milk yield, and reproductive success.

Treatment of Subclinical Hypocalcemia

Management strategies aimed at supporting blood Ca concentrations during the early lactation period are commonplace in high producing dairy herds in North America. Supplemental Ca can be provided in several forms; intravenous or subcutaneous infusion of readily available Ca, or oral administration of Ca salts in the form of liquid, paste, or bolus. Intravenous infusion of Ca elicits a rapid and robust increase in blood Ca, however within hours of infusion, blood Ca falls and remains low for the hours and days to follow (Braun, 2009; Blanc et al., 2014). While intravenous Ca infusion is necessary for the treatment of clinical milk fever, it is not appropriate for cows with SCH as the dramatic increase and subsequent decrease in blood Ca may further inhibit the return to normocalcemia by impeding Ca homeostatic mechanisms. Oral Ca supplementation may be more appropriate for the treatment and prevention of SCH as it yields a moderate, but sustained increase in blood Ca (Domino et al., 2017).

Due to the relative ease of administration, oral Ca boluses have become a common prophylactic strategy to mitigate SCH. Several Ca boluses are commercially available and are generally administered in 2 doses, the first at calving and the second 12 to 24 h later. Despite their widespread use in commercial settings, there are a limited number of scientific reports that explore the effects of oral Ca boluses on health and production outcomes, and those that have report varied results. When explored as a prophylactic treatment, administered at the herd level to all early lactation cows, oral Ca boluses have minimal effects on milk production, health outcomes, and reproductive measures (Oetzel and Miller, 2012; Valldecabres et al., 2023). The neutral effect that Ca boluses have on health and production outcomes at the population level indicates that while some cows may benefit from supplementation, others realize no gains or may even be detrimental to their success.

At the population level, there is little evidence for a difference in milk production or reproductive measures between cows given oral Ca boluses at and around calving and those that are not supplemented (Domino et al., 2017; Valldecabres et al., 2023). However, subpopulations of cows appear to respond differently to oral Ca supplementation as Oetzel and Miller (2012) reported significantly increased milk production in cows with above-average production in the previous lactation that were supplemented with an oral Ca bolus at 0 and 24 h of lactation compared to their high producing herdmates who were not supplemented. In the same study, cows with below average production produced less milk when given an oral Ca bolus after calving compared to non-supplemented, low producing cows. Other reports also suggest that lame cows or those with high (≥3.5) body condition score at calving are at reduced risk for experiencing a negative health event in early lactation when they are supplemented with oral Ca at or around calving (Oetzel and Miller, 2012; Leno et al., 2018). Interestingly though, Leno et al. (2018) observed an increased risk for risk for negative health events in parity 2 cows that were given an oral Ca bolus at calving compared to parity 2 cows that were not supplemented. Similarly, Martinez et al. (2016a;b) reported reduced reproductive success and increased risk of disease in primiparous cows given oral Ca boluses in early lactation.

Oral Ca boluses elicit a slow and sustained increase in blood Ca over the course of 8 to 24 h following administration and there is little impact of supplementation on blood Ca concentration 48 h post administration (Martinez et al., 2016a; Domino et al., 2017; Frost et al., 2022). The differential effects that oral Ca bolus supplementation at 0 and 1 DIM has on health and production outcomes may be due to the varying nature of Ca dynamics in the days following parturition. The traditional bolus supplementation strategy was implored to target the nadir in blood Ca that often occurs within 24 h of parturition, however as our understanding of early lactation blood Ca dynamics has grown and the phenomenon of dyscalcemia has emerged, more targeted strategies to support blood Ca and minimize the associated negative impacts of dyscalcemia are required.

Seely et al. (2022) sought to explore the impact of oral Ca bolus timing on milk production and health events and blood Ca dynamics in the early lactation period in an effort to target cows with dyscalcemia. They enrolled multiparous Holstein cows (n = 998) at the time of calving and cows were randomly assigned to 1 of 3 treatments: 1) control; no supplemental Ca at or around the time of parturition (**CON**; n = 343), 2) conventional bolus; conventional administration of an oral Ca bolus at the time of calving and again 24 h later (**BOL-C**; n = 330), or 3) delayed bolus; delayed administration of an oral Ca bolus at 48 and 72 h post-calving (**BOL-D**; n = 325).

As with previous reports exploring the effects of oral Ca boluses, Seely et al. (2022) reported differential results. At the study population level, there was no evidence for a difference in milk production for the first 10 wk of lactation between treatments (P = 0.2), however a parity × treatment effect was evident (P = 0.002). There was no evidence for a difference in milk production between treatment groups for parity 2 cows, however parity 3 cows that received oral Ca at 2 and 3 DIM produced significantly more milk than BOL-C treated cows and CON cows (BOL-D: 52.0 ± 1.3 kg/d, BOL-C: 47.9 ± 1.6 kg/d, CON: 49.8 ± 1.5 kg/d; P = 0.003). Interestingly, this improvement in milk production by BOL-D treatment did not appear in parity ≥4 cows and milk production was similar between treatments. Furthermore, the incidence of adverse events (metritis, displaced abomasum, herd removal, or a combination thereof within the first 30 DIM) was similar between treatment groups (BOL-D: 11.5%, BOL-C: 8.0%, CON: 10.8%; P = 0.4). Similarly, while controlling for parity group, there was no evidence for a difference in serum tCa or incidence of dyscalcemia (defined as 4 DIM serum tCa ≤2.20 mmol/L) between treatment groups (BOL-D: dyscalcemia incidence 32%, [tCa] 2.09 ± 0.02 mmol/L, BOL-C: dyscalcemia incidence 28%, [tCa] 2.11 ± 0.02 mmol/L, CON: dyscalcemia incidence 26%, [tCa] 2.11 ± 0.02 mmol/L).

The results of the Seely et al. (2022) study are not unlike previous reports exploring the impact of oral Ca bolus supplementation on health and production outcomes, further supporting the idea that postpartum oral Ca supplementation differentially impacts cohorts of cows. In culmination with findings reported by Martinez et al. (2016a) and Leno et al. (2018) it is likely that parity ≤2 cows do not require Ca supplementation after calving as they are able to efficiently navigate the Ca challenge of

early lactation through homeostatic pathways as milk production is not as great as advanced parity cows. Alternatively, despite minimal impact of blood Ca, delaying oral Ca supplementation to 2 and 3 DIM in parity 3 cows appeared to positively impact milk production and may offer a promising strategy to mitigate dyscalcemia. While parity ≥4 cows saw no benefit from delayed or conventional bolus supplementation, older cows are at increased risk for SCH and more appropriate Ca supplementation strategies are needed for older cows. Perhaps advanced parity cows require larger doses of supplementation Ca or for a longer duration? Regardless, future work should be dedicated to optimizing the prevention and treatment of SCH and dyscalcemia in advanced parity cows with focus given to Ca dynamics in early lactation.

Conclusions

Hypocalcemia has been an ever-present threat to dairy cows. Over the years we have reduced the incidence of clinical milk fever and instead have focused on the subclinical manifestation of the disorder. With that, other challenges have arisen such as when to diagnose and what threshold of blood Ca is associated with negative health and production outcomes. With an improved understanding of the dynamics of blood Ca in early lactation, we have come to realize that the timing and persistency of reduced blood Ca may be more indicative of associated negative outcomes than the absolute nadir of blood Ca. Through a series of large field trials, it has become apparent that reductions in blood Ca at 4 DIM are consistently associated with decreased milk production, decreased intake, increased risk for negative health events, and reduced reproductive success and has since been coined dyscalcemia. Regardless of how and when subclinical hypocalcemia or dyscalcemia is diagnosed, treatment and prevention strategies aimed at supporting blood Ca in early lactation are widely employed across the dairy industry. Traditionally administered at 0 and 1 DIM, oral Ca boluses are commonplace in the commercial setting however have varied impacts on production and health outcomes. Recent works have explored the effects of delaying oral Ca bolus supplementation to 2 and 3 DIM, to reduce the associated impacts of dyscalcemia and resulted in improved milk production in parity 3 cows. Future work is needed to better understand the etiology of dyscalcemia and what allows certain cows to regain normocalcemia after a transient drop in blood Ca at 1 DIM. Knowing this may inform us of more optimized treatment and prevention strategies that will minimize the negative impact that dyscalcemia has on health and production and improve the productive potential of the dairy industry.

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