Use of polyhalite mineral as an acidogenic product in diets of pre-partum and non-lactating dairy cows

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ABSTRACT

Polyhalite is a natural mineral that could be fed as an acidogenic product to induce a metabolic acidosis and prevent clinical hypocalcemia in dairy cows after calving. The overall objective of this study was to determine if the use of polyhalite mineral in the diets of pre-partum non-lactating dairy cows was effective as an acidogenic product. We measured the urine pH, dry matter intake, milk yield, and calcium and magnesium concentration of urine and serum in pre-partum and non-lactating dairy cows consuming diets containing a low dose of polyhalite (200 g/cow/day), a high dose of polyhalite (400 g/cow/day), calcium chloride (250 g/cow/day), or no acidogenic product. We hypothesized that including polyhalite mineral as an acidogenic product in the diets of pre-partum and non-lactating dairy cows will reduce urine pH and stimulate calcium metabolism mechanisms. We found that polyhalite effectively reduced urine pH and did not affect dry matter intake, and the stimulation of calcium metabolism was observed through an increase of calcium output in urine. In conclusion, feeding polyhalite mineral is an effective means for inducing metabolic acidosis without reducing dry matter intake. Based on these results, polyhalite should be fed at a dose of 400 g or more per cow per day to reduce urine pH.

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ABSTRACT

(General Audience)

Low blood calcium concentration, also known as hypocalcemia, is one of the common metabolic disorders that affect dairy cows transitioning from the pre-partum to post-partum period. Reducing the dietary cation anion difference (DCAD) in cows during the close-up period is known to effectively reduce the probability of cows developing hypocalcemia after calving. Polyhalite is a natural mineral that could be fed as an acidogenic product to induce a metabolic acidosis and prevent hypocalcemia in dairy cows after calving. The overall objective of this study was to determine if the use of polyhalite mineral in the diets of pre-partum and non-lactating dairy cows was effective as an acidogenic product. We evaluated the urine pH, dry matter intake, milk yield, and calcium and magnesium concentration of urine and serum in pre-partum and non-lactating dairy cows consuming diets containing a low dose of polyhalite (200 g/cow/day) , a high dose of polyhalite (400 g/cow/day), calcium chloride (250 g/cow/day), or no acidogenic product. We hypothesized that including polyhalite mineral as an acidogenic product in the diets of pre-partum and non-lactating dairy cows will reduce urine pH and stimulate calcium metabolism mechanisms. We found that polyhalite effectively reduced urine pH and did not affect dry matter intake, and the stimulation of calcium metabolism was observed through an increase of calcium output in urine. In conclusion, feeding polyhalite mineral is an effective means for inducing metabolic acidosis without reducing dry matter intake. Based on these results, polyhalite should be fed at a dose of 400 g or more per cow per day to reduce urine pH.

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LIST OF ABBREVIATIONS

Ca- Calcium

CHL- Positive control treatment (250 g/day of calcium chloride)

CON- Control treatment (no acidogenic product)

DCAD- Dietary cation-anion difference

DMI- Dry matter intake

ECD- Expected calving date

HIGH- High dose treatment (400 g/day of polyhalite)

K- Potassium

LOW- Low dose treatment (200 g/day of polyhalite)

Mg- Magnesium

PTH- Parathyroid hormone

CHAPTER 1

REVIEW OF LITERATURE

1.1 INTRODUCTION

Metabolic diseases in dairy cattle are most frequent during the transition period, which includes the last 3 wk of gestation through the first 3 wk of lactation (Mulligan and Doherty, 2008). Metabolic diseases following calving are prevalent due to the selection for high milk production and result in a negative impact on both profitability and animal welfare throughout the dairy industry. One of these diseases is periparturient hypocalcemia; a result of an imbalance of calcium **(Ca)** output and input. Hypocalcemia is characterized by a low blood Ca concentration and can have lasting effects throughout the duration of lactation, including a decrease in milk production and other metabolic diseases, such as retained placenta and metritis (Erb et al., 1985).

There are multiple approaches to prevent hypocalcemia in dairy cattle. Prevention of hypocalcemia is essentially stimulation of parathyroid hormone **(PTH)** synthesis and release from parathyroid glands to aid in Ca metabolism. Stimulation of PTH can be accomplished through the inclusion of a negative dietary cation-anion difference **(DCAD)** in the 3 wk before calving, which has possible ramifications, such as depressed dry matter intake **(DMI)**. A negative DCAD can be achieved through the addition of an acidogenic product to the close-up ration. Another approach to stimulate PTH synthesis and release is including low concentrations of dietary Ca in the 3 wk before calving, which can be somewhat difficult to accomplish on high forage diets.

1.2 PERIPARTURIENT HYPOCALCEMIA

Hypocalcemia, or milk fever, is a metabolic disease typically prevalent around parturition and beginning lactation (Hibbs, 1950). This disease is characterized by low concentrations of Ca in the blood and can be further classified into one of two categories: clinical hypocalcemia and subclinical hypocalcemia. Subclinical hypocalcemia is defined as blood Ca concentrations of 5 to 8 mg/dL with no physical signs of hypocalcemia (Goff, 2008; Reinhardt et al, 2011). Clinical hypocalcemia is prevalent with blood Ca concentrations < 5 mg/dL and clinical signs present, such as cold ears and recumbency or a wobbly gait due to the role of Ca in skeletal muscle tone and smooth muscle function (NRC, 2001; Goff and Kosewski, 2018; McArt et al., 2018). Additional clinical symptoms can include bloating, constipation, loss of anal reflex, and gastrointestinal atony as hypocalcemia can impair muscle function (Oetzel, 1988). Incidence rates of clinical hypocalcemia are 5 to 6% of all dairy cows, while subclinical hypocalcemia affects approximately 50% of multiparous cows (Reinhardt et al., 2011; Goff et al., 2014).

Hypocalcemia occurs when intestinal Ca absorption, bone Ca resorption, or renal reabsorption are not sufficiently increased to keep up with the loss of Ca through colostrum and milk production (Horst et al., 1997). This delay in Ca absorption and resorption is due to selection for high milk production. The amount of Ca secreted in the volume of milk to sustain the growth of the calf once was equal to the rate of placental Ca transport during late gestation. However, selection for high milk production has increased the volume of milk produced to nourish at least 10 calves (Ramberg et al., 1984).

In addition to high milk production, parity is another factor that can increase a dairy cow's susceptibility to hypocalcemia. Dairy cows with a lower parity are known to have greater bone Ca resorption rates compared to those of greater parity (Neves et al., 2017). Calcium

metabolism slows with age due to multiple factors, such as a decline in intestinal Ca absorption and kidney function (Veldurthy et al., 2016). Neves et al. (2017) found that cows in their third or greater parities were 70% more likely to be diagnosed with subclinical hypocalcemia than cows in their second parity.

Long-term effects of both clinical and subclinical hypocalcemia include a decrease in milk production and increased risk of other disorders, such as retained placenta, metritis, and displaced abomasum (Erb et al., 1985; Massey et al., 1993; Rajala-Schultz et al., 1999). A study evaluating the association between subclinical hypocalcemia and various metabolic diseases found an increased risk of subclinically hypocalcemic cows to be diagnosed with metritis, retained placenta, and displaced abomasum with odds ratios of 4.25, 3.43, and 3.71, respectively (Rodríguez et al., 2017). Massey et al. (1993) found that, in a study involving 510 Holstein cows, both clinically and subclinically hypocalcemic cows were 4.8 times more likely to develop a left displaced abomasum than normocalcemic cows. Liang et al. (2017) found that the average cost per case of clinical hypocalcemia is $246 ± 52 with factors such as culling, death, labor and treatment costs, and milk production decrease included in the model. Therefore, both clinical and subclinical hypocalcemia are a major concern for the dairy industry, negatively affecting both profitability and animal welfare.

1.3 CALCIUM METABOLISM

Calcium is a macro-mineral that plays a major role in physiological functions such as bone mineralization, cell signaling, and muscle contraction (Wilkens et al., 2020). Calcium is distributed throughout the adult cow body with approximately 10 kg total, 98% of which is included in bones and the remainder in extracellular fluids (Martin-Tereso and Martens, 2014).

Approximately 50% of Ca in blood is protein bound, with the majority of this portion consisting of albumin. The remaining 50% of Ca in blood is either complexed to soluble anions such as citrate and sulfate (3-7%) or ionized depending on pH conditions (42-48%) (Goff, 2014). Calcium concentration in blood in an adult dairy cow is normally maintained between 8.5 and 10 mg/dL (Goff, 2014). Any small deviation from this typical Ca concentration range can alter the movement of Ca throughout the body, leading to an array of problems including loss of skeletal and smooth muscle tone. Therefore, Ca must be tightly regulated in the extracellular fluids to maintain these physiological functions.

Typically, Ca intake for a non-lactating dairy cow includes at least 15.4 mg/kg BW of absorbable Ca and 0.44% DM of dietary Ca (Visek et al., 1953; Hansard et al., 1957; NRC, 2001). Dietary Ca is the amount of Ca ingested, while absorbable Ca increases the bioavailability of Ca. Forage Ca absorption rates vary based on forage type. For example, the Ca absorption rate for alfalfa opposed to an alfalfa-corn silage mixture is 25% and 42%, respectively (NRC, 2001). Therefore, the average efficiency of Ca absorption from forages is approximately 30% (NRC, 2001). Non-forage feedstuffs have a greater Ca absorption efficiency of 60% (NRC, 2001).

The tight regulation of Ca in the body is accomplished by vitamin D and PTH maintaining the balance of intestinal absorption, bone resorption, and renal reabsorption (McCarthy and Kumar, 1999). Intestinal absorption of dietary Ca can be accomplished by either a transcellular or a paracellular mechanism. Transcellular Ca absorption is dependent on the hormonal form of vitamin D, 1,25-$(OH)_2D$, also known as calcitriol (McCarthy and Kumar, 1999). Once calcitriol is formed by the hydroxylation of vitamin D in the kidney, it is secreted to interact with the vitamin D receptor from macrophages to eventually initiate the active transport of Ca across epithelial cells. Active transport of Ca is achieved by calcitriol stimulating 3

vitamin-D dependent proteins: TRPV-6, calbindin-9KD, and a plasma membrane Ca-ATPase pump (Goff, 2014). Due to the dependency of calcitriol on stimulating these proteins, it takes about 48 h for the transcellular transport of Ca to cause a significant increase in blood Ca (Goff et al., 1986).

The second intestinal Ca absorption mechanism, the paracellular or passive mechanism, is the movement of Ca between epithelial cells rather than across epithelial cells. This means that this mechanism is independent of vitamin D, and Ca can directly flow across tight junctions into the blood (Christakos, 2012). However, paracellular absorption can be somewhat influenced by calcitriol due to calcitriol's ability to change the structure of the tight junctions by activation of protein kinase C. This increases tight junction permeability, increasing the flow of Ca (Blaine et al., 2015). Contribution of transcellular and paracellular Ca absorption in dairy cows is unknown, but it is thought that greater dietary Ca increases the chance of paracellular absorption being used as the predominant mechanism (Goff, 2014).

The PTH is essential for Ca bone resorption and renal reabsorption. After intestinal absorption, if blood Ca concentrations continue to decrease after calving, the next mechanism used to maintain Ca homeostasis is bone resorption. The average dairy cow loses approximately 20 to 30 g of Ca per day when producing colostrum and during early lactation (Goff, 2014). Therefore, the cow must utilize her largest amounts of stored Ca, which is in the bone. This is called lactational osteoporosis, and typically results in a 9 to 13% loss of skeletal Ca during the first month of lactation (Ellenburger et al., 1932).

When dietary Ca absorption fails to maintain normocalcemia, bone Ca can be mobilized with the help of osteoclasts (secretory cells) and osteoblasts (PTH target cells), both of which are found in bone tissue (Erben, 2001; Brown et al., 2013; Goff, 2014). About 99% of Ca in bone is

stored in bone crystals, known as hydroxyapatite. Osteoclasts return Ca to the blood by secreting enzymes and acid to mobilize the Ca in hydroxyapatite. The PTH receptors on osteoblasts signal the secretion of kβ ligand and macrophage colony-stimulating factor to alert osteoclasts to begin secretion of enzymes and acid (Hoorn and Zietse, 2013). There is also a small but readily available amount of Ca in bone fluid that can be mobilized. This solution surrounds osteocytes, cells derived from osteoblasts located within calcified bone matrix, that quickly respond to the PTH to pump Ca from the bone fluid into the extracellular fluid to increase Ca levels in the blood (Teti and Zallone, 2009; Brown et al., 2013).

Renal reabsorption is another mechanism for Ca homeostasis if blood Ca concentration slightly decreases. Typically, urine Ca excretion is about 0.5 g Ca/day when cows are fed diets with a positive DCAD (Goff, 2014). When blood Ca concentration is stable, decreasing urine Ca excretion through renal Ca reabsorption is sufficient enough to maintain normocalcemia. Approximately 80% of Ca reabsorption occurs via paracellular pathways of the proximal convoluted tubules of the nephrons (Blaine et al., 2015).

The PTH needs to bind its receptors to stabilize the Ca balance through these mechanisms. Unfortunately, there are instances, such as metabolic alkalosis and hypomagnesemia, that delay PTH response to hypocalcemia. During metabolic alkalosis, the PTH receptor is altered, preventing the activation of osteoclasts to begin Ca bone resorption (Goff, 2008). Lack of PTH receptor responsiveness also can decrease renal Ca reabsorption. Metabolic alkalosis can also cause the kidneys to fail to convert vitamin D to calcitriol. Metabolic alkalosis is mainly caused by a diet with a positive DCAD (i.e., diet with more cation than anion equivalents). One preventative measure to decrease the risk of metabolic alkalosis and maintain Ca homeostasis is to reduce the DCAD in the close-up period before calving.

1.4 PREVENTION OF HYPOCALCEMIA THROUGH NUTRITION

1.4.1 Reduction of DCAD

Dietary cation-anion difference, also known as DCAD, is the difference of the major cations [sodium (Na) and potassium (K)] and anions [chloride (Cl) and sulfur (S)] in the diet expressed in mEq/kg DM. There have been multiple equations created to calculate DCAD, but the most common equation is [DCAD = (Na + K) – (Cl + S)], which has been used in fundamental studies such as Ender et al. (1971) and Block (1984). Ender et al. (1971) used the equation [DCAD = (Na + K) – (Cl + S)] to formulate diets with the objective of being hypocalcemia-inducing (3,010 mEq/kg DM) or hypocalcemia preventing (-255 mEq/kg DM). Ender et al. (1971) also incorporated varying levels of dietary Ca to further investigate Ca-balance. Dietary Ca treatments consisted of groups 1 and 2 having a DCAD of 3,010 mEq/kg DM and groups 3 and 4 having a DCAD of -255 mEq/kg DM. Groups 1 and 3 included calcium carbonate as a supplement (140 and 145 g dietary Ca, respectively) and groups 2 and 4 did not include a calcium supplement (34 and 39 g dietary Ca, respectively). Ender et al. (1971) found that there were no incidences of hypocalcemia in groups 3 and 4, both of which were formulated for a negative DCAD. Hypocalcemia incidences for groups 1 (positive DCAD, calcium carbonate) and 2 (positive DCAD, no calcium carbonate) were 4 out of 10 and 4 out of 6, respectively. The average Ca balance values were negative for groups 1 and 2 during the first 4 d postpartum. Groups 3 and 4 had average Ca balance values that were slightly positive and negative, respectively, during the first 4 d postpartum. Ender et al. (1971) concluded that these results supported theories that negative Ca-balance values post-partum could be an indicator of

hypocalcemia and that trends of negative Ca-balance values are related to diet composition. Lean et al. (2006) conducted a meta-analysis evaluating the various DCAD equations found in literature and concluded that the equation [DCAD = (Na + K) – (Cl + S)] provides the most accurate model for explanation of hypocalcemia risk through DCAD. A typical negative DCAD diet is about -50 mEq/kg DM to -150 mEq/kg DM for close-up prepartum cows (NRC, 2001). Prepartum diets high in cations tend to induce milk fever, while an anion-dominant prepartum diet will induce a metabolic acidosis (Goff et al., 1991). This acidic state aids in the functionality of the mechanisms used for Ca metabolism, such as dietary Ca intestinal absorption and bone Ca resorption.

Metabolic acidosis can be monitored by measuring urine pH. A reduction in urine pH is typically seen within 36 h of the cow ingesting a ration with a negative DCAD (Goff and Horst, 1998). Typically, urine pH values will decrease from a pH of about 8.0 to below 6.0 when metabolic acidosis is induced. Zimpel et al. (2018) analyzed close-up prepartum diets with a DCAD of 196 mEq/kg DM, 194 mEq/kg DM, 192 mEq/kg DM, -114 mEq/kg DM, and -113 mEq/kg DM. The urine pH values for these diets were 8.14, 7.86, 7.92, 5.66, and 5.54, respectively. The urine pH values of the cows fed the two negative DCAD diets were significantly more acidic than the remaining three diets with a positive DCAD. Goff et al. (2004) found that the addition of at least 1.5 Eq of either chloride of sulfate as the anionic source significantly decreased urine pH compared to water as the control. When comparing a new acidogenic product, MegAnion, with a positive control, SoyChlor, it was found that both treatments resulted in a similar urine pH decrease from 8.15 to 6.12. Both treatments were formulated to have a similar DCAD; with MegAnion and SoyChlor treatments having a DCAD of -200 mEq/kg DM and -228 mEq/kg DM, respectively (Caixeta et al., 2020). The negative

DCAD inducing a metabolic acidosis was accurately reflected in the drop in urine pH values for both treatments. Ryan et al. (2020) found a large difference in urine pH values when comparing a positive DCAD diet (60 mEq/kg DM) with two diets of low and high dietary Ca (0.4% and 2% DM Ca, respectively) having a negative DCAD (-240 mEq/kg DM). The three diets resulted in urine pH values of 8.11, 5.79, and 5.71, respectively.

Despite potential benefits of DCAD reduction on periparturient Ca metabolism, low DCAD diets often result in depressed DMI. Although there is normally a decrease in DMI as calving approaches regardless of DCAD reduction, the use of a negative DCAD can further depress DMI (Bertics et al., 1992; Robinson and Garrett, 1999). Horst et al. (1994) explained that prepartum diets with a DCAD < -300 mEq/kg DM tend to reduce DMI. A recent study by Caixeta et al. (2020) compared a diet with a DCAD of -200 mEq/kg DM with a further reduced diet with a DCAD of -228 mEq/kg DM. The further reduced diet resulted in a DMI less than that of the other diet, with values of 11.0 and 12.2 kg/day, respectively. Ryan et al. (2020) interestingly found a greater DMI (18.4 and 18.8 kg/d) with a DCAD of -240 mEq/kg DM for diets containing 0.4% and 2.0% dietary Ca, respectively, than a diet composed of 0.4% dietary Ca and a DCAD of 60 mEq/kg DM. Zimpel et al. (2018) hypothesized that this decrease in DMI is a result of metabolic acidosis. Zimpel et al. (2018) found that cows fed rations with a DCAD averaging 193 mEq/kg DM had an increased DMI of about 0.6 kg/d more than cows fed rations with Biochlor and a DCAD averaging -113.5 mEq/kg DM. Although, one of the treatments with a positive DCAD also included Biochlor. Therefore, it is possible that depressed DMI with acidogenic diets is due to the acid-base status of the cow, rather than the acidogenic product.

Charbonneau et al. (2006) conducted a meta-analysis with the objective of evaluating the impact of DCAD reduction on DMI. Twenty-two studies with 75 different treatment groups were

included with a DCADs range of -284.2 mEq/kg to 612.7 mEq/kg. Using the DCAD equation DCAD = [(Na + K)- (Cl + 0.6 S)], they found that, although most individual studies reported no reduction in DMI with a negative DCAD, there was a reduction in DMI of 1.3 kg DM/d (11.3%) when DCAD was reduced from 300 mEq/kg to 0 mEq/kg. Therefore, the benefits of preventing milk fever through reduced DCAD must be weighed against the issues that might arise with depressed DMI near calving, such as greater prepartum body condition score loss and decreased colostrum synthesis (Lopera et al., 2018).

1.4.2 Acidogenic sources

Multiple acidogenic sources have been formulated into rations to achieve a metabolic acidosis in close-up dairy cows. When comparing chloride and sulfate as an anionic source, Goff et al. (2004) found that urine pH with the inclusion of chloride was reduced significantly lower than with the inclusion of the same dose of sulfate. This could mean that there is a difference in their acidogenic properties, which might be a lack of absorption or excess in excretion rate for sulfate. To evaluate alternative acidogenic products, Goff and Horst (1998) tested hydrochloric acid as an acidogenic source. They found that the addition of hydrochloric acid significantly reduced the incidence of milk fever (11%) compared to the control diet (63%). The hydrochloric acid treatment also showed a higher DMI compared to the control treatment.

Acidogenic salts acidify the cow's urine, which decreases the efficiency of renal Ca reabsorption, resulting in increased Ca output through urine (Goff and Koszewski, 2018). In cows that have a urine pH < 6.0, urine Ca output typically increases from 4 g/day to 7 g/day with the addition of acidogenic salts (Schonewille et al., 1999; Goff et al., 2014). When comparing a diet including an acidogenic product, Biochlor, and positive DCAD (192 mEq/kg DM) with a

diet including Biochlor and negative DCAD (-114 mEq/kg DM), Zimpel et al. (2018) found that urine Ca output increased from 0.66 g/day to 7.53 g/day. Schonewille et al. (1999) compared diets with a positive DCAD (332 mEq/kg DM) and a negative DCAD (-230 mEq/kg DM) and found that urinary Ca concentrations were about six times greater in cows fed the diet with a negative DCAD compared to cows fed the diet with a positive DCAD. Vagnoni and Oetzel (1998) also found an increase in Ca urine output from diets containing a DCAD of -51 mEq/kg DM, -40 mEq/kg DM, and -63 mEq/kg DM compared to a diet with a DCAD of 203 mEq/kg DM. The urinary Ca output values were 0.9 g/day, 6.7 g/day. 7.9 g/day, and 6.9 g/day, respectively.

1.4.3 Low dietary calcium

Low concentrations of dietary Ca have shown to prevent hypocalcemia. A Ca deficient diet puts cows into a negative Ca balance, which activates bone resorption and renal reabsorption by PTH and Calcitriol (NRC 2001; Goff and Koszewski, 2018). A low dietary Ca concentration is about 0.5% Ca (Goff and Koszewski, 2018). This method aids the cow in achieving calcium homeostasis after calving, which acts in a similar fashion to the dietary addition of an acidogenic product. Boda and Cole (1954) were the first to administer this method, using diets high in phosphorus **(P)** and low in Ca. In this study including 16 cows, they found no incidences of hypocalcemia or negative effects from the Ca deficient diet. Goings et al. (1974) achieved similar results with hypocalcemia seen in 0 of the 12 cows fed the low Ca high P diet (52 g Ca/day and 35 g P/day) and 5 out of 13 cows fed the control diet (41 g Ca/day and 34 g P/day). However, this hypocalcemia prevention method is extremely difficult to execute in the United States due to forages not having a low Ca content, and cows tend to increase their DMI with low

Ca diets to ensure that their Ca requirements are met (Goff and Koszewski, 2018). Goff and Horst (1997) compared 0.5% dietary Ca with 1.5% dietary Ca in various amounts of dietary K. They found no difference in the incidence of hypocalcemia between diets, with 12 of 31 cows and 11 of 32 cows having hypocalcemia after being fed 0.5% dietary Ca and 1.5% dietary Ca, respectively.

In a recent study, Amundson et al. (2018) compared low (0.45%), medium (1.13%), and high (2.02%) concentrations of dietary Ca with negative DCADs of -167 mEq/kg DM, -207 mEq/kg DM, and -172 mEq/kg DM, respectively. All cows were nonlactating and nonpregnant, and hypocalcemia was induced intravenously with 5% EGTA solution randomly every other day from day 22-24. Blood Ca concentrations declined similarly among treatments after the EGTA solution was administered, although, cows fed the high dietary Ca had significantly greater blood Ca concentrations compared to cows fed the low and medium dietary Ca. In order to ease the difficulty of formulating a Ca-deficient diet, compounds can be added to the diet to bind to Ca, reducing Ca absorption, resulting in a pseudo Ca-deficient diet effect.

Thilsing et al. (2007) and Kerwin et al. (2019) have tested synthetic zeolite A as a means to create a low-absorbable-Ca diet for hypocalcemia prevention. Thilsing et al. (2007) included four treatments with either 39 g/day or 100 g/day of dietary Ca, and/or 36 g/day or 136 g/day of dietary P. All cows were supplemented with 600 g/day of zeolite A during the last 2 weeks of gestation. Incidence of hypocalcemia was seen in 3 of 5 cows given low concentrations of dietary Ca and P, 2 of 6 cows given high dietary P and low dietary Ca, 0 of 4 cows given high dietary Ca and low dietary P, and 1 of 5 cows given high dietary Ca and P. This study concluded that the hypocalcemia preventing capability of zeolite A is also due to its ability to bind to P to reduce P availability. Kerwin et al. (2019) compared diets with (DCAD=268 mEq/kg DM) or

without (DCAD=110.3 mEq/kg DM) the addition of zeolite A. Thirteen of 26 cows fed zeolite A and 18 of 29 cows not fed zeolite A were categorized as having subclinical hypocalcemia. Chronic subclinical hypocalcemia, defined as subclinical hypocalcemia present for more than 3 weeks, was observed in 34% of cows not fed zeolite A. There were no cases of chronic subclinical hypocalcemia observed in cows fed zeolite A. Therefore, zeolite A has an effect on reducing Ca absorption in the weeks following calving but lacks effectiveness for reducing Ca absorption for hypocalcemia prevention immediately after calving.

Amundson et al. (2018) conducted a recent study that hypothesized the addition of acidogenic salts to the prepartum diet would increase blood total Ca **(tCa)** and ionized Ca **(iCa)** concentrations during times of Ca stress due to the increased stimulation of PTH for Ca metabolism mechanisms (Block, 1984; Goff, 2008). Ionized Ca is a more accurate measure of Ca in the blood compared to tCa, as iCa only measures Ca accessible for absorption, while tCa includes protein-bound and non-protein bound Ca. Amundson et al. (2018) found significantly different iCa concentrations among treatments of low dietary Ca (1.185 mM), medium dietary Ca (1.208 mM), and high dietary Ca (1.228 mM). Block (1984) found that plasma Ca concentrations were greater in cows fed an anionic diet compared to cows fed a cationic diet. Block (1984) also found that plasma Ca concentrations in cows fed a cationic diet had a significantly larger drop around parturition and at calving than cows fed an anionic diet. Goff et al. (1991) found similar results when comparing an anionic diet with a cationic diet, with the cationic diet having a significantly lower plasma Ca concentration on day of calving than the anionic diet (6.58 mg/dL and 7.63 mg/dL, respectively).

1.5 SUMMARY

Hypocalcemia is a metabolic disease that greatly affects the dairy industry. Hypocalcemia is defined as low blood concentration and subcategorized as subclinical (< 8 mg/dL) or clinical (< 5 mg/dL) (NRC, 2001; Goff, 2008). Hypocalcemia occurs when Ca metabolism mechanisms cannot keep up with Ca output through colostrum production (Horst et al., 1997). Intestinal Ca absorption and bone Ca resorption can increase by feeding a diet with a negative DCAD through the use of acidogenic products to stimulate the PTH. An important factor to consider when using acidogenic products is possible decrease in DMI, either due to the acid-base status of the cow or the palatability of the acidogenic product itself. Calcium-deficient diets are another method of increasing Ca metabolism, although, this method is much more difficult to use. In order to test the effectiveness and safety of a new acidogenic product, these aspects must be taken into consideration.

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CHAPTER 2

USE OF POLYHALITE MINERAL AS AN ACIDOGENIC PRODUCT IN DIETS OF PRE-PARTUM AND NON-LACTATING DAIRY COWS

2.1 ABSTRACT

Polyhalite ($K2SO_4 \cdot 2CaSO_4 \cdot MgSO_4 \cdot 2H_2O$) is a natural mineral with acidogenic properties. Dietary inclusion of polyhalite in pre-partum diets might induce metabolic acidosis and prevent peri-parturient hypocalcemia in dairy cows. We hypothesized that including polyhalite as an acidogenic product in the diets of pre-partum and non-lactating dairy cows would reduce urine pH by inducing metabolic acidosis and stimulate mineral metabolism The objectives of this study were to determine if pre-partum feeding of polyhalite: negatively affects DMI during the pre-partum period; alters Ca and Mg concentrations in serum and urine; and reduces urine pH of pre-partum non-lactating dairy cows. Forty non-lactating and pregnant Holstein cows entering their second or greater lactation were randomly assigned 1 of 4 experimental diets. Experimental diets included: 48% corn silage, 25% grass hay, and 27% of one of four pelleted concentrates (DM basis). Pelleted concentrates contained: no acidogenic product (**CON**), 200 g/day of polyhalite (**LOW**), 400 g/day of polyhalite (**HIGH**), or 250 g/day of calcium chloride (**CHL**). At 35 d before expected calving date (ECD), cows were transferred from pasture to a bedded pack barn. While in the bedded pack barn, cows were fed once daily (1200 h) using a Calan gate feeding system (American Calan Inc., Northwood, NH). Individual DMI was measured daily. Before feeding on d −21, −14, −7, 0, 7 and 28 relative to ECD, serum was collected by coccygeal vessel venipuncture and urine samples were collected after vulva stimulation in the morning before feeding. Calcium and Mg concentrations in serum and urine

were determined after being diluted with a lanthanum stock solution followed by analysis at the Virginia Tech Soil Testing Laboratory. Calcium and Mg concentrations in urine were normalized after determining creatinine concentrations by colorimetric assay. Urine pH was measured immediately after collection using a portable pH meter. Post-partum milk yield was recorded automatically after each milking. Data were analyzed with PROC MIXED procedure of SAS. The statistical model included the fixed effects of diet, day, and diet by day and the random effects of block and block by diet. All variables were tested using the autoregressive covariance structure for repeated measures (cow = subject). Dry matter intake did not differ among diets in the pre-partum or post-partum periods ($P > 0.11$ and $P > 0.68$, respectively) but decreased towards calving ($P < 0.01$). Dry matter intake towards calving decreased similarly for all diets ($P > 0.68$). Dry matter intake following calving increased similarly for all diets ($P > 0.51$). Serum Ca concentration did not differ among diets ($P > 0.83$) but decreased towards calving ($P <0.01$). Serum Mg concentration differed among diets and by day (both effects $P <0.01$), but no interaction between diet and day was found ($P > 0.75$). Urine Ca concentration differed among diets ($P > 0.01$) but did not differ in urine Mg concentration ($P > 0.46$).Urine pH pre-partum differed among diets ($P < 0.01$). Cows consuming CONTROL and LOW diets had the highest urine pH (7.98 and 7.88, respectively), and cows consuming HIGH and CHLOR diets had the lowest urine pH (6.62 and 6.18, respectively). Milk yield increased similarly for all diets ($P > 0.06$). In conclusion, feeding polyhalite is an effective means for inducing metabolic acidosis without reducing DMI. Based on these results, polyhalite should be fed at a dose of 400 g or more per cow per day to decrease urine pH and stimulate Ca metabolism mechanisms to aid in the prevention of clinical hypocalcemia after calving.

2.2 INTRODUCTION

Periparturient clinical hypocalcemia, also known as milk fever, is a metabolic disease affecting fresh cows, and is a major concern for the dairy industry. Hypocalcemia is defined as a low concentration of Ca in blood. Normocalcemia in dairy cows is maintained typically at 8 mg/dL or greater (Reinhardt et al., 2011). In post-partum cows, decreased blood Ca concentration is a consequence of increased Ca requirements at the onset of lactation. Blood Ca concentration decreases when Ca is not mobilized from tissue reserves fast enough to keep up with Ca withdrawal through colostrum and milk (Hibbs, 1950). In dairy cows, clinical hypocalcemia manifests once blood Ca is <5 mg/dL (NRC, 2001) and signs such as wobbly gait, recumbency, or cold ears (Goff et al., 2018) are present. After calving, about 50% of cows in second lactation or greater may have blood Ca concentrations < 8 mg/dL with no physical signs of disease; this is known as subclinical hypocalcemia (Goff, 2008). Implications of both clinical and subclinical hypocalcemia can include a decrease in milk production (Block 1984; Chapinal et al., 2012) and an increased risk of other periparturient disorders such as ketosis, displaced abomasum, and metritis (Rodríguez et al., 2017).

Feeding acidogenic diets to non-lactating pregnant cows has reduced the incidence of both clinical and subclinical hypocalcemia after parturition (Ender et al., 1971; Block, 1984; Zimpel et al., 2018). Acidogenic diets are characterized by having a negative dietary cation-anion difference **(DCAD)**. The DCAD is expressed in milliequivalents per kilogram of dry matter (mEq/kg DM) and decreases with addition of a greater amount of anions (Cl^- and SO_4^{2-}) than cations ($Na^+$and K^+) in the diet (Ender et al., 1971). A negative DCAD results in the dietary anions being absorbed in the digestive tract, which tends to decrease the plasma strong ion difference, causing a state of metabolic acidosis (Constable et al., 2017). While in metabolic

acidosis, renal production of 1,25 dihydroxyvitamin D is increased, and Ca metabolism is stimulated by Ca resorption from bones and Ca absorption from the intestines (Constable et al., 2017). Therefore, addition of dietary anions to achieve a negative DCAD aids in prevention of hypocalcemia in periparturient dairy cows.

A potential decrease in DMI is an important factor to consider when adding acidogenic products to a ration, especially considering that DMI is generally decreased as calving nears. Feeding anion sources, such as chlorides and sulfates (Goff, 2008), may result in reduced feed palatability (Oetzel and Barmore, 1993). Charbonneau et al. (2006) found, after analyzing 22 studies with 75 different treatments, that DMI decreased by an average of 1.3 kg DM/d (11.3%) when DCAD was lowered by 300 mEq/kg DM.

Polyhalite mineral ($K_2SO_4 \cdot 2CaSO_4 \cdot MgSO_4 \cdot 2H_2O$) is an acidogenic product that is found natural and abundant in rock salt formations (Peryt et al., 1998; Wollmann et al., 2008; Ferreira et al., 2019). Polyhalite was formed when an ancient sea, the Zechstein Sea, evaporated approximately 260 million years ago, and is now mined by Sirius Minerals PLC (Scarborough, England). Based on the empirical formula of polyhalite, this mineral contains 2 equivalents of K^+ and 8 equivalents of SO_4^{2-}, which are two ions that affect the DCAD. Based on these equivalents, polyhalite has a cation-anion difference of -9,000 mEq/kg DM, which suggests polyhalite should be highly acidogenic.

The dietary addition of an acidogenic product typically results in lower urine pH due to induced metabolic acidosis. Ferreira et al. (2019) completed a preliminary study that evaluated the use of polyhalite mineral as an acidogenic product for pre-partum dairy cow diets. For their study, the authors hypothesized that dietary inclusion of polyhalite would decrease urine pH in pre-partum cows. As hypothesized, the urine pH values of the close-up cows were statistically

lower than the baseline urine pH values obtained during the far-off period (Ferreira et al., 2019). A drawback of the study was it did not evaluate if feeding polyhalite affected DMI or mineral metabolism (e.g., Ca, Mg).

With no previous studies evaluating the effect of polyhalite mineral on DMI or mineral metabolism, we hypothesized that including polyhalite as an acidogenic product in the diets of pre-partum and non-lactating dairy cows would reduce urine pH by inducing metabolic acidosis and stimulate mineral metabolism. Therefore, the objectives of this study were to determine if pre-partum feeding of polyhalite reduces urine pH of pre-partum non-lactating dairy cows, affects DMI during the pre- and post-partum period, and alters Ca and Mg concentrations in serum and urine.

2.3 MATERIALS AND METHODS

2.3.1 Animals, Housing, and Diets

The trial was conducted from July 2018 to September 2019. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of Virginia Tech. Forty pregnant and non-lactating Holstein cows [739±84 kg of BW] and 22±5 d before their expected calving date **(ECD)** at the beginning of the experiment], entering their second or greater lactation, were randomly assigned to 1 of 4 experimental diets in a randomized complete block design with 10 blocks. Cows were assigned to blocks based on their ECD.

At 35 d before ECD, cows were transferred from pasture to a compost-bedded pack barn. Cows were fed once daily (1200 h) using a Calan gate feeding system (American Calan Inc., Northwood, NH). At 35 d before ECD, cows were trained for 14 d to locate their assigned door.

Cows were fed a far-off diet throughout the 2-wk training period. Beginning at 21 d before ECD and extending until calving, cows were fed the close-up experimental diets. The experimental diets were formulated to include 48% corn silage, 25% grass hay, and 27% concentrate (DM basis). The concentrates were formulated to include: no acidogenic product **(CON)**, 200 g/day of polyhalite **(LOW)**, 400 g/day of polyhalite **(HIGH)**, or 250 g/day of calcium chloride **(CHL)**. Both acidogenic products were incorporated into pelleted concentrates at a commercial feed mill (Big Spring Mill Inc., Elliston, VA). Aiming to achieve a DCAD between -100 and -200 mEq/kg DM, the granular and free-flowing polyhalite product was incorporated into the pelleted concentrates for the LOW and HIGH diets.

Concentrate pellets were mixed with corn silage and grass hay and delivered ad libitum (~5% refusals) as a TMR. A vertical mixer (NDEco FS600, Sioux Falls, SD) was used to mix the corn silage and grass hay. The forage mix and the pellets were weighed separately on a portable scale (OHAUS, Parsippany, NJ) and then mixed for 2 min in a cement mixer (Muller Machinery Company, Inc., Metuchen, NJ). Cows were offered approximately 17.3 kg of TMR (DM basis) each day. The amount of feed offered and refused was recorded daily.

After calving, cows stayed in the same housing system until 28 d postpartum. After calving, cows were fed a common lactating diet. The amount of feed offered and refused was recorded daily. Lactating cows were distinguished from non-lactating cows by wearing plastic chain-like necklaces. Lactating cows were milked twice daily (0100 and 1300 h), and milk weights were automatically recorded at each milking. Milk fat and true protein concentrations were determined using an automated milk analyzer (Afimilk Ltd., Kibbutz Afikim, Israel).

2.3.2 Sample Collection and Analysis

Before feeding on d -21, -14, -7, 0 relative to ECD and d 7 and 28 after calving, urine and blood samples were collected. Urine samples were collected after vulva stimulation, and urine pH was determined immediately after collection using a portable pH meter (Hanna Checker; Hanna Instruments; Woonsocket, RI) that was calibrated immediately before each use. After collection, urine samples were filtered through grade 1 qualitative filter paper (Whatman, GE Healthcare Bio-sciences, Pittsburgh, PA) and stored at -20°C until mineral analysis. Blood samples were collected into 10-mL vacutainer tubes by venipuncture of the coccygeal vessel. After 1 h of blood clotting, blood samples were centrifuged at 3,000 × *g* for 30 min at 20°C, and serum was collected and stored at -20°C until mineral analysis.

Total concentrations of Ca and Mg in urine and serum were determined following a procedure provided by Dr. Jesse P. Goff (Iowa State University). First, a stock solution of 0.36 M $LaCl_3$ was prepared by reacting La_2O_3 with HCl as follows: 1) 150 mL of deionized water were added to a 1-L Erlenmeyer flask, 2) while stirring, 200 mL of concentrated HCl were added, 3) 58.64 g of La_2O_3 were very slowly added while stirring, 4) once solvation was apparent, 150 mL of distilled water were added, and the solution was stirred for at least 1 hour, and 5) once solvation was apparent, the solution was filtered through grade 1 qualitative filter paper, transferred into a 1-L volumetric Erlenmeyer flask, and was brought to volume with distilled water. Urine and serum samples were diluted in stock solution to a 0.5% and 0.1%, respectively. Diluted samples were analyzed for Ca and Mg using inductively coupled plasma optical emission spectrometry (Spectro Arcos II ICP-AES, SPECTRO Analytical Instruments GmbH, Germany) using 318 and 280 µm of wavelength for Ca and Mg, respectively. The concentrations of Ca and Mg in urine were normalized using urinary creatinine concentration,

which were determined using a colorimetric assay kit (500701 Cayman Chemical, Ann Arbor, MI) after a 1:20 dilution.

Samples of feed ingredients and feed refusals were collected weekly and composited. All samples were dried in a forced-air oven (55°C) until constant weight and ground to pass through a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ). Ash concentration was determined after burning feed samples in a furnace (Thermolyne 30400, Barnstead International, Dubuque, IA) for 3 h at 550°C. Crude protein concentration was calculated as percent N x 6.25 after combustion analysis using a Vario El Cube CN analyzer (Elementar Americas Inc., Mount Laurel, NJ). The concentration of NDF was determined using the Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY) with sodium sulfite and α-amylase (Ankom Technology). Acid detergent fiber and lignin concentrations were determined sequentially. After determining ADF weights, residues were incubated for 3 h with 72% sulfuric acid in a 4-L jar that was placed in a DaisyII incubator (Ankom Technology). Starch concentration was determined using the acetate buffer method of Hall (2009) with α-amylase from *Bacillus licheniformis* (FAA, Ankom Technology) and amyloglucosidase from *Aspergillus niger* (E-AMGDF, Megazyme International, Wicklow, Ireland).

Health events around parturition (d -21 to d 28 relative to ECD) were recorded in PC-Dart (Dairy Record Management Systems; Raleigh, NC) by the farm crew and included clinical hypocalcemia, ketosis, displaced abomasum, metritis, and mastitis. These data were extracted once the experiment was concluded. Cows with clinical hypocalcemia were defined as those receiving an intravenous infusion of Ca solution.

2.3.3 Statistical Analysis

All variables were analyzed using the PROC MIXED procedure of SAS (SAS version 9.4, SAS Institute Inc., Cary, NC) using repeated measures; cow was the subject. The statistical model for evaluating urine pH in the pre-partum period included the effects of block [random; degrees of freedom (df) = 9], diet (fixed; df = 3), block x diet (random; df = 27); day (fixed; df = 3), day x diet interaction (fixed; df = 9), and the random residual error. The statistical model for evaluating DMI in the pre-partum period included the effects of block (random; df = 9), diet (fixed; df = 3), block x diet (random; df = 27); day (fixed; df = 20), day x diet interaction (fixed; df = 60), and the random residual error. The statistical model for evaluating DMI and milk yield in the post-partum period both included the effects of block (random; df = 9), diet (fixed; df = 3), block x diet (random; df = 27); day (fixed; df = 27), day x diet interaction (fixed; df = 81), and the random residual error. The statistical model for evaluating Mg and Ca concentrations in serum and urine included the effects of block (random; df = 9), diet (df = 3), block x diet (random; df = 27); day (fixed; df = 5), day x diet interaction (fixed; df = 15), and random residual error. Differences between main effects and interactions between main effects were declared at $P < 0.05$. Two of the 40 cows did not complete the experiment due to insufficient amounts of CON and LOW concentrates. Partial data collected from the two removed cows were excluded from statistical analysis.

2.4 RESULTS

After switching from far-off diets to close-up experimental diets, urine pH decreased in cows consuming HIGH and CHL diets but did not decrease in cows consuming CON and LOW diets ($P < 0.01$; Figure 1). Dry matter intake did not differ among diets in the pre-calving period

(P < 0.11) but decreased as cows approached their calving date (P < 0.01; Figure 2). Based on the lack of interaction (P > 0.67), the magnitude of the decrease in dry matter intake as cows approached their calving date did not differ among diets.

Serum total Ca concentration did not differ among diets (P < 0.83; Figure 3). Serum Ca concentration decreased the day of calving (P < 0.01), and this decrease was similar among diets (P > 0.51). Serum Mg concentration differed among diets, with cows consuming HIGH diet having the greatest Mg concentration throughout the majority of the experiment (P < 0.01; Figure 4). Serum Mg concentration increased on day of calving, decreased on d 7 after calving, then increased after d 7 after calving (P < 0.01). This trend in serum Mg concentration was similar among diets (P > 0.75).

The Ca concentration in urine was greater in cows consuming HIGH and CHL diets than in cows consuming CON and LOW diets (P < 0.01; Figure 5). The Ca concentration in urine stayed constantly low throughout the experiment for cows consuming LOW and CON diets, whereas for cows consuming HIGH and CHL diets the Ca concentration in urine decreased at calving (P < 0.01). The concentration of Mg in urine did not differ among diets (P > 0.46; Figure 6) and did not differ by day relative to calving (P > 0.07). There was also a lack of diet by day interaction for Mg concentration in urine (P > 0.54).

Dry matter intake did not differ among diets in the post-partum period (P > 0.68; Figure 7). Although DMI increased by day relative to calving (P < 0.01), this increase was similar among diets (P > 0.51). Milk yield did not differ among diets (P > 0.78; Figure 8). Milk yield increased by day relative to calving (P < 0.01), but milk yield increased similarly among diets (P > 0.06). Milk true protein concentrations were 2.60%, 2.81%, 2.80%, and 2.64% for CON,

LOW, HIGH, and CHL diets, respectively. Milk fat concentrations were 3.92%, 4.26%, 4.03%, and 3.87% for CON, LOW, HIGH, and CHL diets, respectively.

2.5 DISCUSSION

Polyhalite is a mineral that contains potassium and sulfate; 2 of the 4 major ions used when calculating DCAD. Although potassium is a cation, there is a greater percentage of sulfate than potassium in the chemical composition of polyhalite (12% and 19%, respectively), resulting in acidogenic properties. Based on these properties, Ferreira et al. (2019) suggested polyhalite would be a suitable addition to the diets of close-up, non-lactating cows to prevent both clinical and subclinical hypocalcemia after calving by inducing a metabolic acidosis. In this study, urine pH at -21 d for cows consuming the far-off diet was 7.86, then decreased once fed HIGH diet to a pH of 6.15, 6.44, and 6.04 for -14, -7, and 0 d, respectively (Figure 1). Urine pH for cows consuming CHL diet decreased similarly with values of 8.26, 5.40, 5.85, and 5.21 for -21, -14, -7, and 0 d, respectively. Our results agree with the findings of Ferreira et al. (2019), as polyhalite was shown to induce a metabolic acidosis.

In our study, we found that DMI was not depressed in cows consuming acidogenic products relative to cows consuming the CON diet. Block (1984) achieved similar results with no difference in DMI when comparing cows fed a positive vs. negative DCAD. Although, palatability is a major concern when feeding acidogenic diets, as it can lead to a decrease in DMI. Calcium chloride, for example, can be very harsh on the mucosal lining of the gastrointestinal tract due to its acidic properties (Thilsing-Hansen et al., 2002). If a cow has oral irritation or lesions, cows will likely have a decrease in DMI due to the acidity of the acidogenic product. A recent meta-analysis on the effects of prepartum DCAD found that decreasing

prepartum DCAD from 200 to -100 mEq/kg predicted a 0.4-kg/d DMI reduction in parous cows (Santos et al., 2019). Further analysis revealed that including an acidogenic product decreased DMI regardless of anionic source used (salt or commercial product). Zimpel et al. (2018) found that a decrease in prepartum DMI was not caused by palatability issues, but rather the acid-base state of the cow.

We found that serum Ca concentrations were similar among diets (Figure 3). Santos et al. (2018) found contradictory results, in which reducing the DCAD of prepartum diets resulted in improvement of total Ca concentrations in blood on the day of calving and days following calving. Our results agree with the typical drop in serum Ca concentrations on the day of calving, as all diets significantly decreased the day of calving (Figure 3). This drop of Ca concentration in serum is due to the large Ca requirement during lactation, as Ca concentration is decreased through the output of milk faster than Ca can be absorbed through the intestines and resorbed through the kidneys. The averages of serum Ca concentrations on the day of calving were 7.53, 8.16, 7.43, and 7.48 mg/dL for cows consuming CON, LOW, HIGH, and CHL diets, respectively. The thresholds for subclinical and clinical hypocalcemia are < 8 mg/dL and < 5 mg/dL, respectively (Goff, 2008; NRC, 2001). Therefore, only cows consuming LOW diet averaged serum Ca concentrations greater than the subclinical hypocalcemia threshold.

We observed greater Ca concentration in urine in HIGH and CHL vs. CON and LOW diets in the weeks leading to the day of calving (Figure 5). These results agree with Zimpel et al. (2018), which found that excretion of Ca in urine was influenced by DCAD and was not associated with dietary Ca. In our study, Mg concentration in urine yielded statistically insignificant results, however, the average values throughout the experiment for cows consuming CON diet were the most numerically variable compared to the other 3 treatments receiving

acidogenic products. This suggests that there is a slight trend in reducing Mg concentration variability in urine with the use of acidogenic products.

Potassium can decrease magnesium absorption in the rumen (Martens and Schweigel, 2000). This is concerning regarding polyhalite use an acidogenic product, as it contains a significant amount of potassium. Mg concentration in serum < 1.8 mg/dL suggests that dietary Mg absorption is inadequate, and that hypomagnesemia could be contributing to the prevalence of hypocalcemia (Goff, 2008). Only one average value reached close to this threshold with 1.99 mg/dL for cows consuming CON diet (Figure 4). These results suggest that the amount of potassium in polyhalite did not have an effect on Mg absorption.

Acidogenic diets fed throughout the close-up pre-partum period have been known to increase milk yield post-partum (DeGroot et al., 2010). We found that milk yield was similar among diets, which is contradictory to results from a recent study consisting of 4 treatments similar to our study, with three negative DCAD treatments and one positive DCAD treatment (DeGroot et al., 2010). In the study by DeGroot et al. (2010), cows fed acidogenic diets prepartum had a significantly greater milk yield compared to cows fed the control diet. There were no clinical cases of hypocalcemia observed in that study. Chapinal et al. (2012) found that the incidence of clinical hypocalcemia can lead to a decrease in milk production.

Cows consuming CHL diets had a hypocalcemia prevalence of 20%, while CON, LOW, and HIGH diets had prevalences of 0%, 11.1%, and 10%, respectively (Table 4). One possible explanation for greater prevalence of clinical hypocalcemia in CHL diet is cows with higher parity tend to be more susceptible to hypocalcemia (Table 5). Klingbeil (2015) found that multiparous cows have a greater Ca output because they produce a greater amount of colostrum compared to primiparous cows, which could contribute to their susceptibility to hypocalcemia.

However, a recent cross-sectional study found that although there was an increase in prevalence of hypocalcemia in multiparous cows with increasing age, there was no difference in colostrum yield (Venjakob et al., 2017). This parity-related hypocalcemia prevalence increase could be a consequence of delayed Ca mobilization due to a reduction in active osteoclasts and osteoblasts, resulting in reduced bone remodeling (Goff, 2014).

One possible recommendation of future research is the inclusion of PTH measurement as an additional variable in assessing the prevalence of clinical hypocalcemia with the use of polyhalite mineral. Clinical hypocalcemia causes an increase in PTH, as change in serum calcium concentration is sensed by the calcium-sensing receptor, which is located in parathyroid cells (Goodman, 2008). Additional measurements of serum Ca concentration on d 2 and d 3 after calving could be included in future studies to increase detection of both clinical and subclinical hypocalcemia, as both clinical and subclinical hypocalcemia are typically detected between 24 h to 72 h after calving (Megahed, 2017). Another major improvement to this study would be to increase the number of cows participating in the study, as a Latin square design is not possible for a short-term transition cow study. A greater number of cows would increase statistical power, giving the study more statistically sound results.

2.6 CONCLUSIONS

Polyhalite mineral contained chemical properties effective in reducing DCAD, which we found to lead to a successful induction of metabolic acidosis reflected by a decrease in urine pH in HIGH diet. Polyhalite mineral also effectively stimulated Ca metabolism mechanisms, which was reflected by an increase in Ca urine output in HIGH diet. In addition, polyhalite did not reduce DMI during the pre- or post-partum periods in both LOW and HIGH diets. Based on this

study, polyhalite should be fed to close-up dairy cows at a dose of approximately 400 g/cow/day or more to induce a metabolic acidosis and stimulate Ca metabolism to aid in clinical hypocalcemia prevention after calving while not affecting DMI.

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Table 1. Chemical composition of far-off cow diet (%, DM basis) fed at -35 d to -22 d relative to ECD

Nutrient	Far-off cow diet
Ash,%	5.4
CP,%	10.8
NDF,%	48.7
Starch,%	15.5

Table 2. Ingredient and chemical composition of close-up experimental diets (%, DM basis) fed at -21 d to -1 d relative to ECD

	Treatment			
Item	CON	LOW	HIGH	CHL
Ingredient				
Corn silage	46.8	46.8	46.8	46.8
Grass hay	30.5	30.5	30.5	30.5
Soybean meal	11.8	11.8	11.8	11.8
Cottonseed hulls	4.1	4.1	4.1	4.1
Blood meal	1.6	1.6	1.6	1.6
Polyhalite	-	1.83	3.66	-
Calcium carbonate	3.66	1.83	-	1.42
Calcium chloride (dihydrate)	-	-	-	2.24
Rumen-protected choline	0.49	0.49	0.49	0.49
Salt	0.20	0.20	0.20	0.20
Chromium product (0.04%)	0.15	0.15	0.15	0.15
Yeast	0.11	0.11	0.11	0.11
Rumen-protected methionine	0.12	0.12	0.12	0.12
Binder	0.08	0.08	0.08	0.08
Trace minerals product	0.06	0.06	0.06	0.06
Selenium product	0.06	0.06	0.06	0.06
Vitamin E	0.11	0.11	0.11	0.11
Vitamin ADE mix	0.10	0.10	0.10	0.10
Trace mineral mix 2	0.02	0.02	0.02	0.02
Monensin product	0.002	0.002	0.002	0.002
…				
Nutrient				
Ash,%	7.2	7.6	8.0	7.7
CP,%	14.0	14.1	13.5	13.7
NDF,%	38.8	38.5	38.5	39.2
Starch,%	18.7	18.8	19.8	18.9
Ca,%	1.50	1.14	0.78	1.46
Mg,%	0.25	0.32	0.36	0.24
Na,%	0.12	0.16	0.16	0.14
K,%	1.66	1.86	1.94	1.45
Cl,%	1.12	0.37	0.39	1.69
S,%	0.21	0.64	0.98	0.25
DCAD, mEq/kg DM*	33	42	-150	-198

*DCAD = ($Na^+$and K^+) - (Cl^- and SO_4^{2-})

Table 3. Chemical composition of standard lactating diet (%, DM basis) fed at 0 d to 28 d relative to calving

Nutrient	Lactating diet
Ash,%	5.2
CP,%	15.0
NDF,%	29.4
Starch,%	26.6

Table 4. Health events and observations of cows in each experimental diet; no acidogenic product (CON), 200 g/day of polyhalite (LOW), 400 g/day of polyhalite (HIGH), or 250 g/day of calcium chloride (CHL)

	Diet			
Disease	CON (n=9)	LOW (n=9)	HIGH (n=10)	CHL (n=10)
Clinical hypocalcemia[1]	0	1	1	2
Hypocalcemic observations[2]	1	3	5	2
Clinical ketosis[3]	2	1	1	2
Displaced abomasum[4]	0	1	1	0
Metritis[5]	1	0	0	2
Clinical mastitis[6]	1	0	0	1

[1]A metabolic disease caused by low blood calcium level causing a flaccid paralysis (recumbency) within 72 hours after calving. This condition was treated with intravenous calcium and oral calcium.

[2]Cold ears, wobbly walk, or sunken eyes observed on day of calving.

[3]Elevated ketone bodies found in urine (dark purple ketostix) or blood (BHB >2.5 mmol/L on Precision xtra meter), as well as reduced milk production and loss of appetite (no rumen contractions on physical exam).

[4]The abomasum moves from the floor of the ventral right abdomen to an abnormal location on the left side (a gas ping was located between the 9th-13th rib on a line from the hook bone to the elbow) of the abdomen.

[5]Infection (red to brown fetid discharge) and inflammation of the uterus, with evidence of systemic signs (e.g., fever, decreased DMI, dehydration).

[6]Inflammation (swelling) of on one more of the mammary glands in the udder along with changes in milk appearance including flakes, clumps, or blood.

Table 5. Distribution of parity within each experimental diet; no acidogenic product (CON), 200 g/day of polyhalite (LOW), 400 g/day of polyhalite (HIGH), or 250 g/day of calcium chloride (CHL)

	Diet			
Parity	CON (n=9)	LOW (n=9)	HIGH (n=10)	CHL (n=10)
2	5	4	5	3
3	0	4	2	2
4	2	0	1	2
5	2	0	2	2
6	0	0	0	1
7	0	1	0	0
Average parity	**3.1**	**3.0**	**3.0**	**3.6**

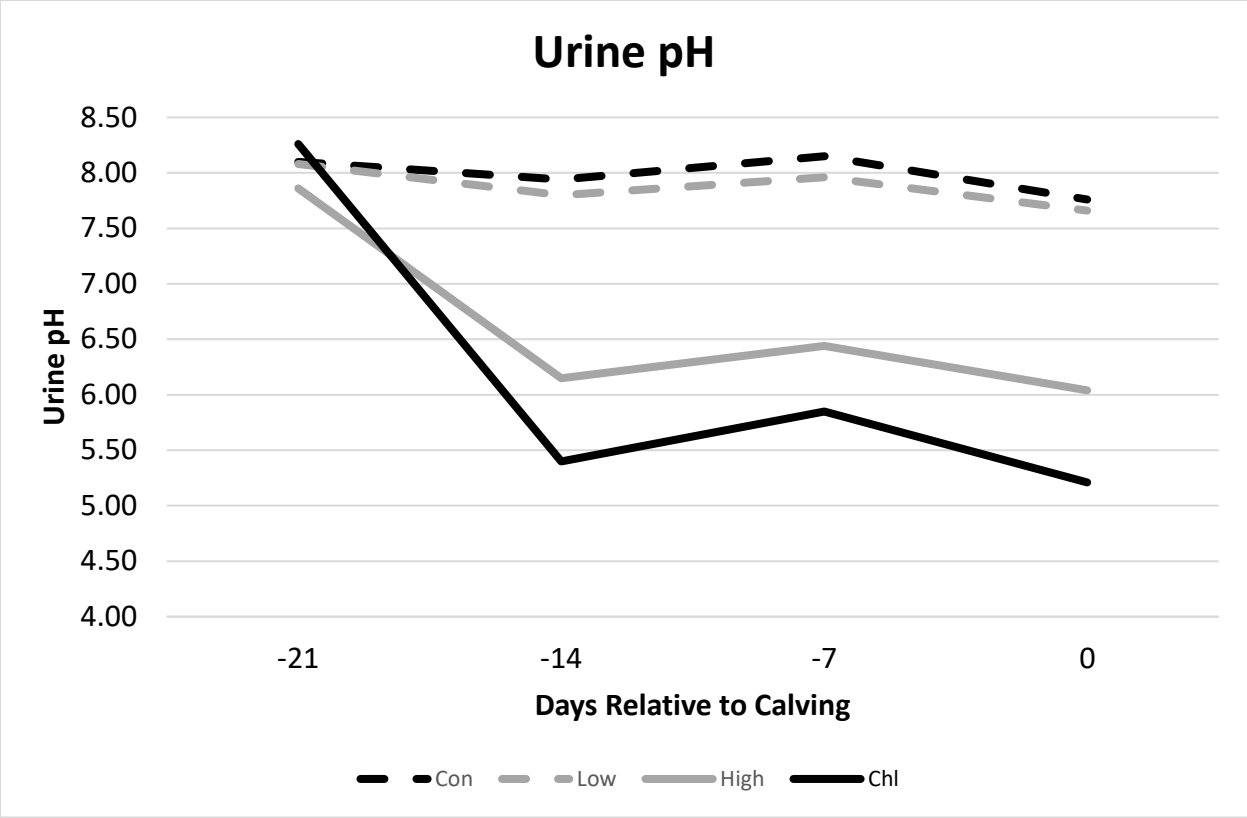

Figure 1. Urine pH of pre-partum non-lactating dairy cows fed diets consisting of no acidogenic product (CON), 200 g/day of polyhalite (LOW), 400 g/day of polyhalite (HIGH), or 250 g/day of calcium chloride (CHL). Standard error of the mean = 0.22.

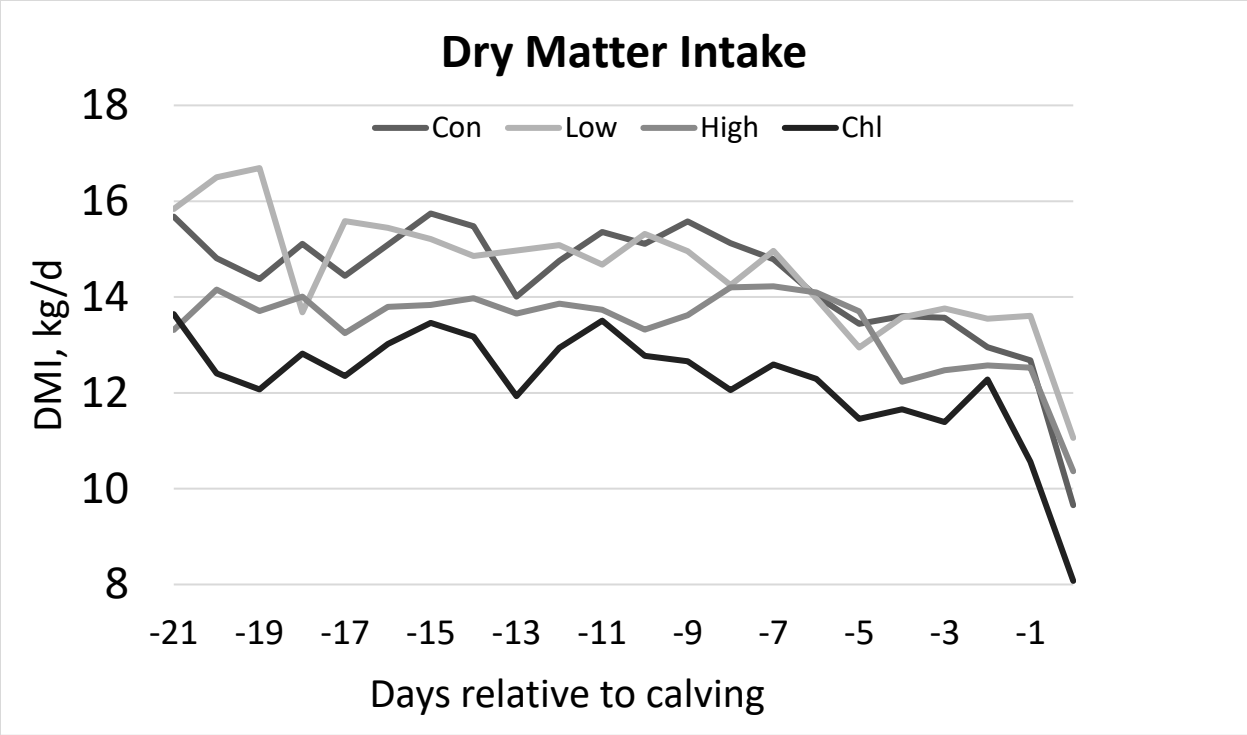

Figure 2. Dry matter intake of non-lactating pre-partum dairy cows fed diets consisting of no acidogenic product (CON), 200 g/day of polyhalite (LOW), 400 g/day of polyhalite (HIGH), or 250 g/day of calcium chloride (CHL). Standard error of the mean = 1.4.

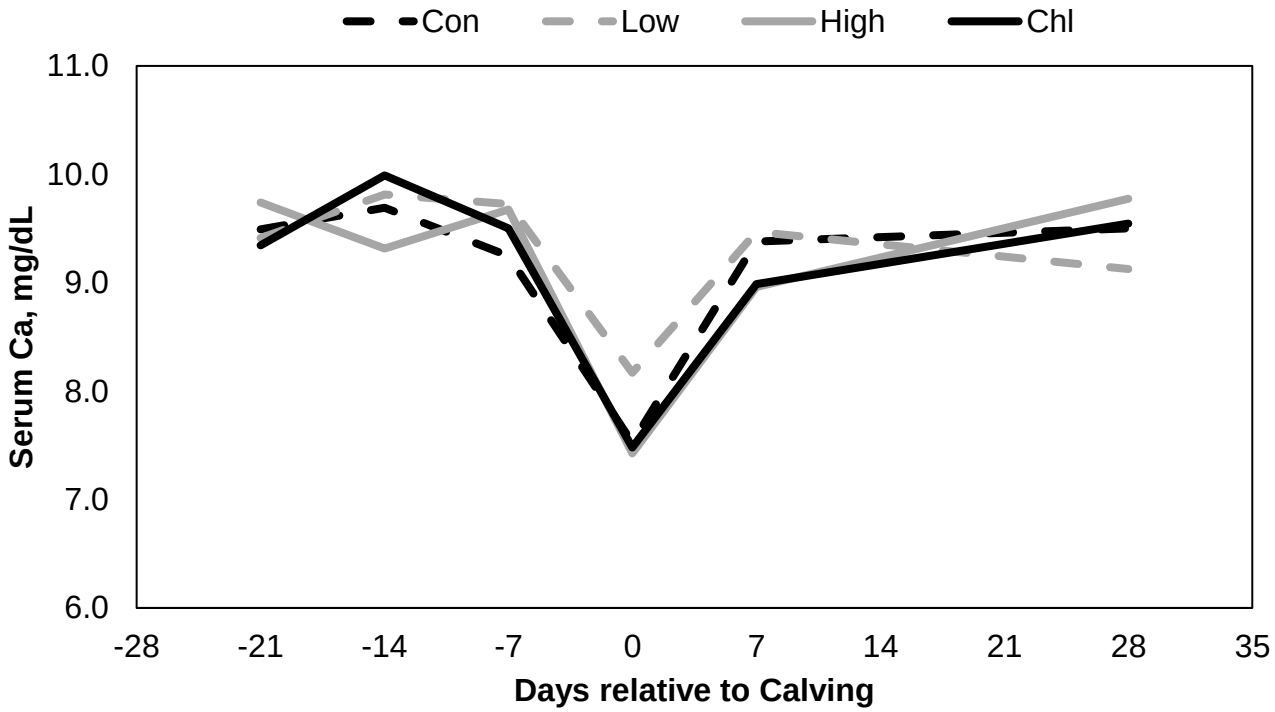

Figure 3. Serum Ca (mg/dL) concentrations of periparturient dairy cows fed diets consisting of no acidogenic product (CON), 200 g/day of polyhalite (LOW), 400 g/day of polyhalite (HIGH), or 250 g/day of calcium chloride (CHL). Standard error of the mean = 0.14.

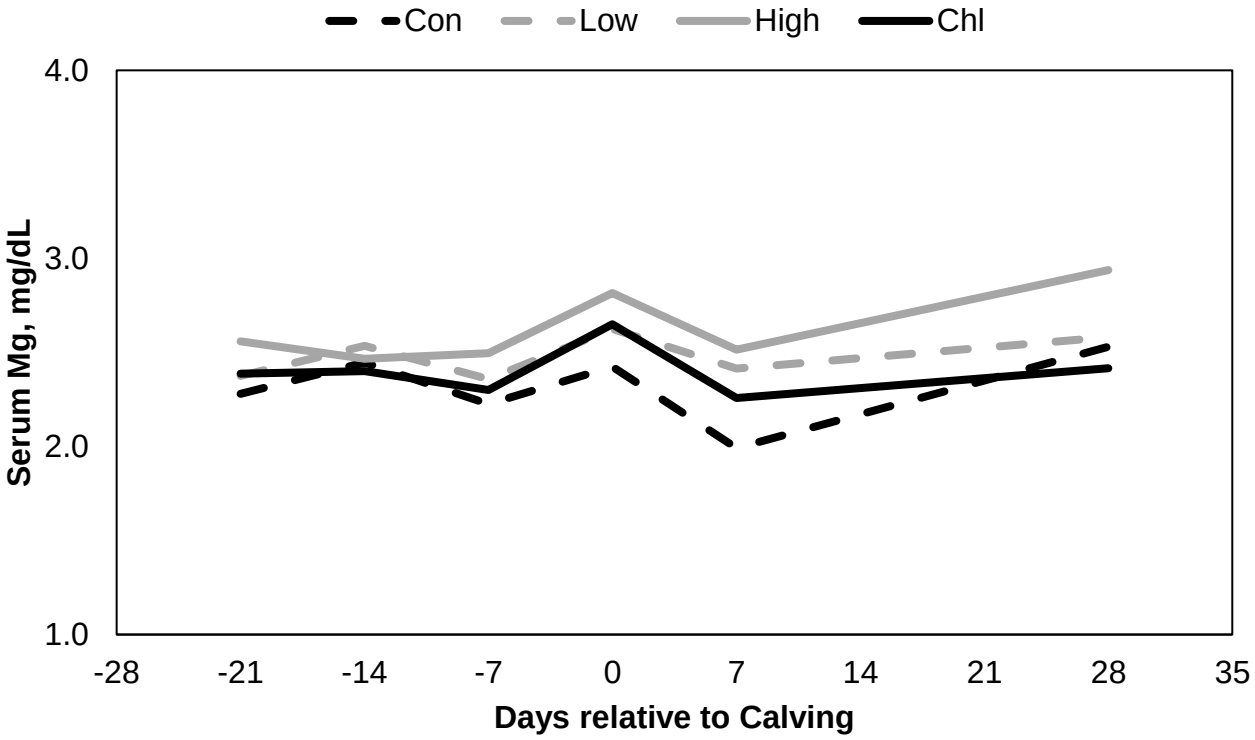

Figure 4. Serum Mg (mg/dL) concentrations of periparturient dairy cows fed diets consisting of no acidogenic product (CON), 200 g/day of polyhalite (LOW), 400 g/day of polyhalite (HIGH), or 250 g/day of calcium chloride (CHL). Standard error of the mean = 0.07.

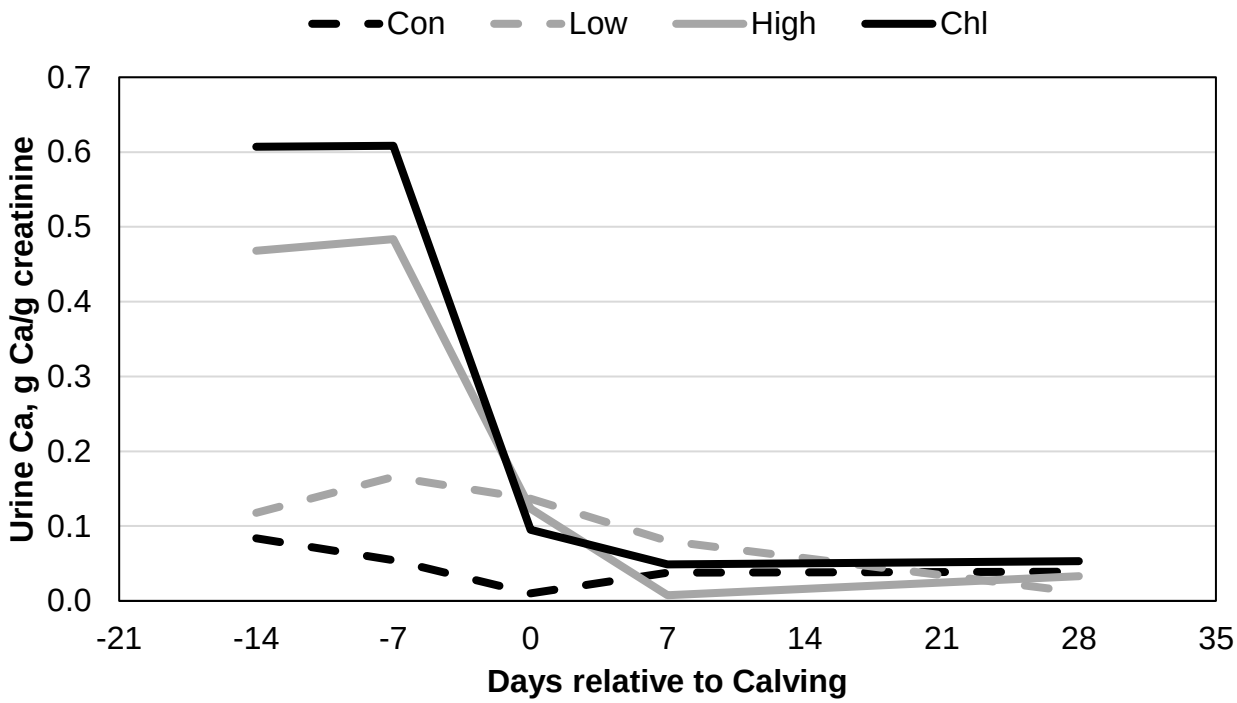

Figure 5. Urine Ca concentrations (g Ca/g creatinine) of periparturient dairy cows fed diets consisting of no acidogenic product (CON), 200 g/day of polyhalite (LOW), 400 g/day of polyhalite (HIGH), or 250 g/day of calcium chloride (CHL). Standard error of the mean = 0.04.

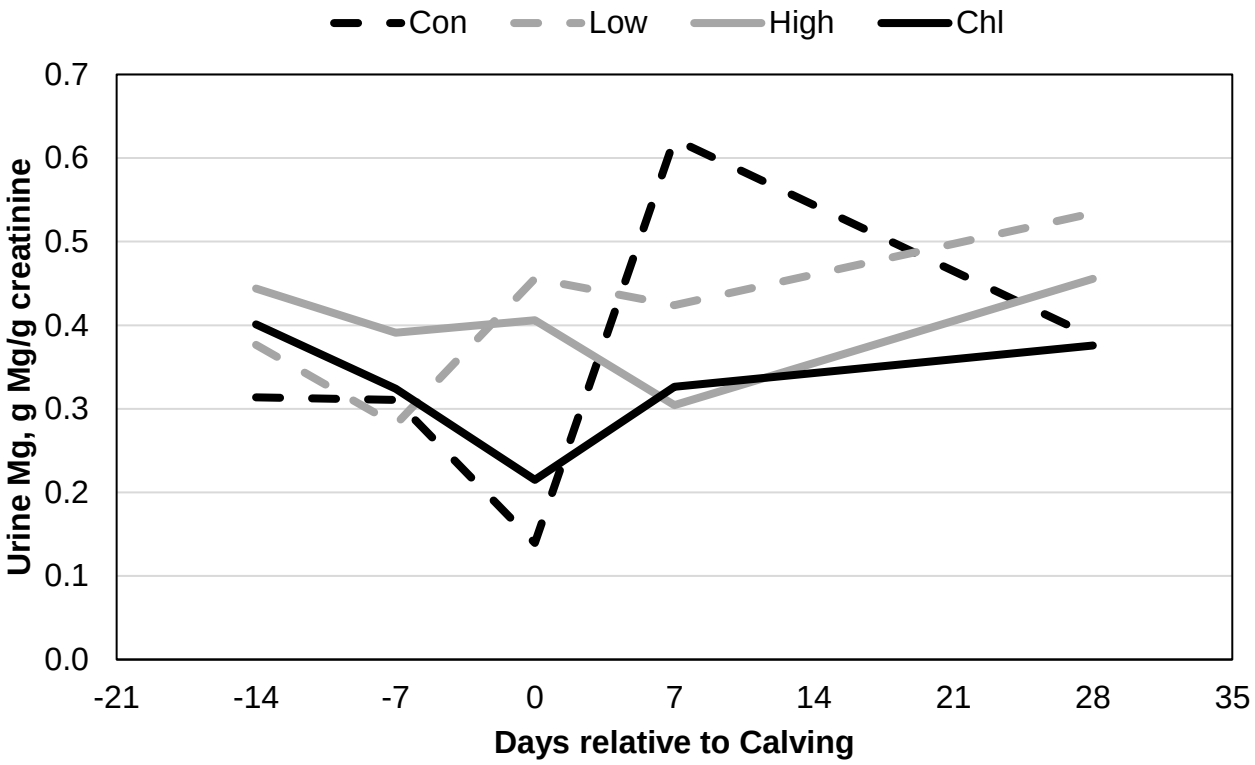

Figure 6. Urine Mg concentrations (g Mg/g creatinine) of periparturient dairy cows fed diets consisting of no acidogenic product (CON), 200 g/day of polyhalite (LOW), 400 g/day of polyhalite (HIGH), or 250 g/day of calcium chloride (CHL). Standard error of the mean = 0.12.

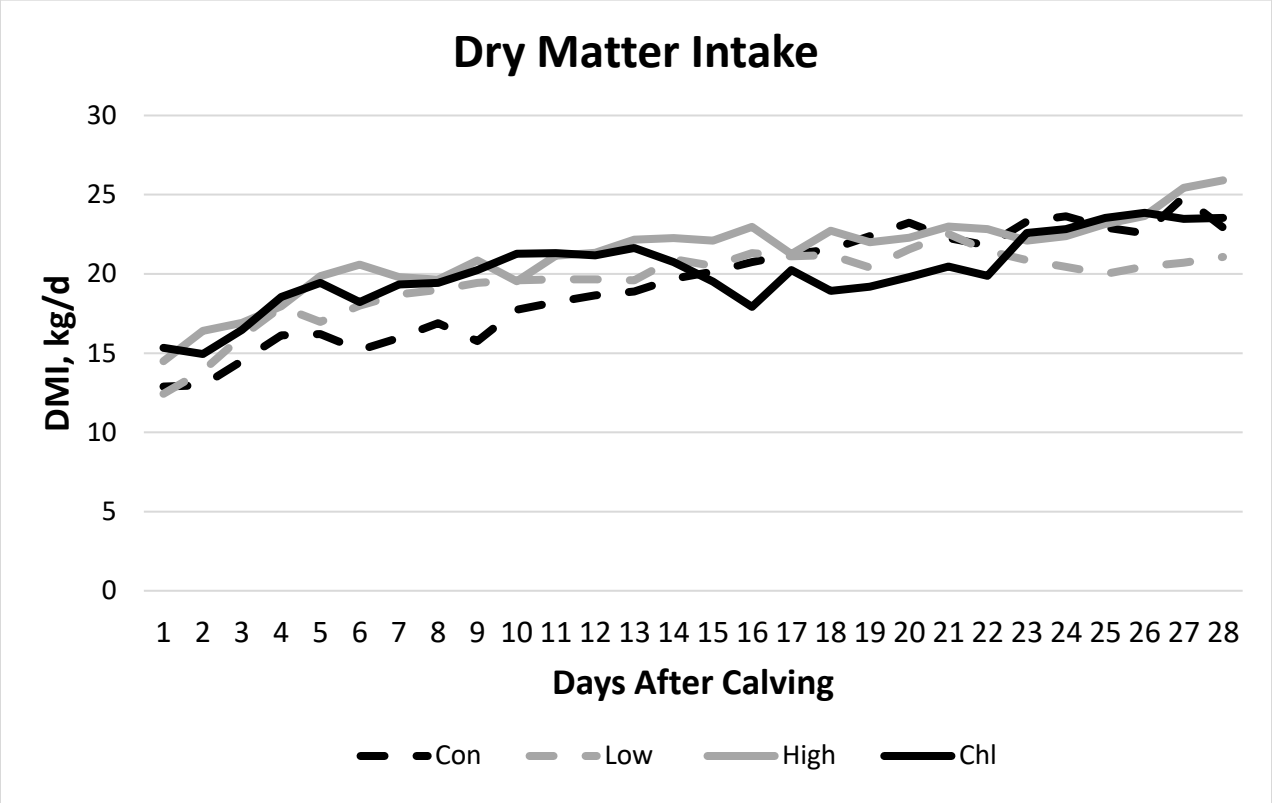

Figure 7. Dry matter intake of periparturient dairy cows fed diets consisting of no acidogenic product (CON), 200 g/day of polyhalite (LOW), 400 g/day of polyhalite (HIGH), or 250 g/day of calcium chloride (CHL) in the close-up pre-partum period. Standard error of the mean = 1.2.

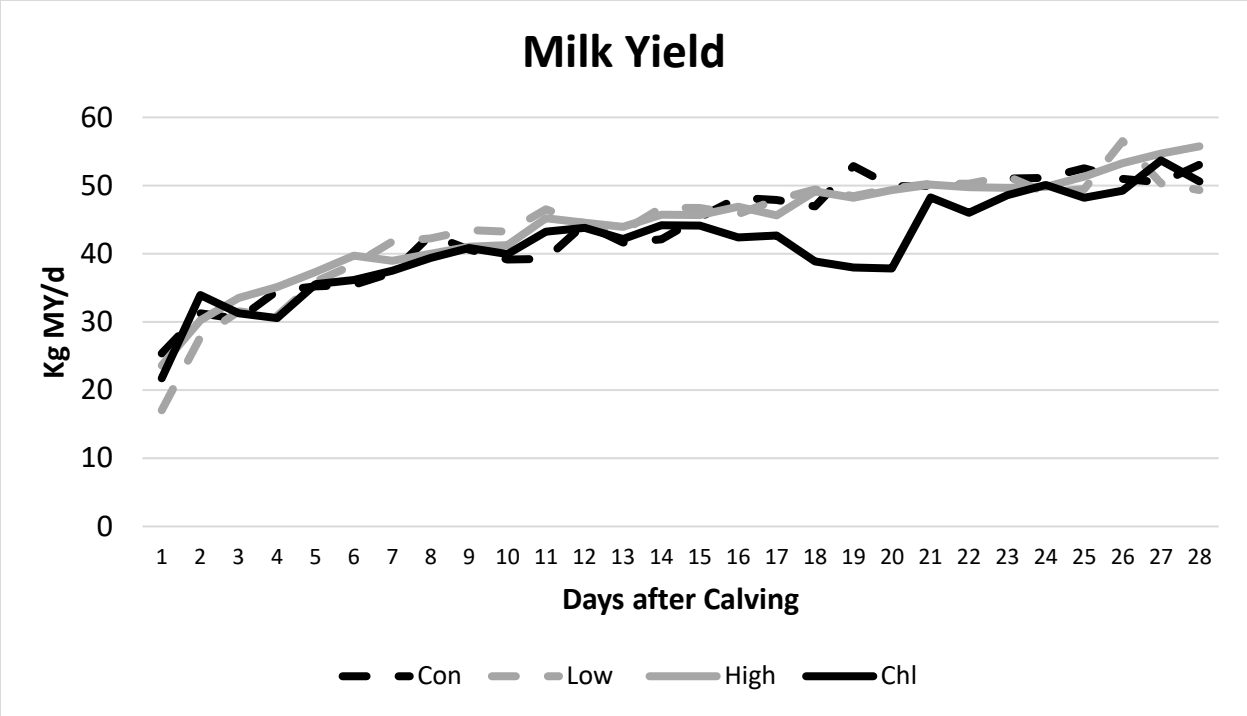

Figure 8. Milk yield of periparturient dairy cows fed diets consisting of no acidogenic product (CON), 200 g/day of polyhalite (LOW), 400 g/day of polyhalite (HIGH), or 250 g/day of calcium chloride (CHL) in the close-up pre-partum period. Standard error of the mean = 2.2.