An alternative strategy to prevent hypocalcemia by adding Zeolite A to the prepartum diet of dairy cows

by

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Abstract

Late gestation cows face several challenges related to nutritional needs and physiological and metabolic changes. Colostrogenesis in the last weeks before calving and subsequent milk production in early lactation increase calcium (Ca) requirements. Disruption of Ca homeostasis in the periparturient period leads to hypocalcemia. Although incidence of clinical hypocalcemia can be insignificant compared with other postpartum diseases, half of multiparous cows are affected by subclinical hypocalcemia (SCH). Calcium deficiency alters function of smooth muscle cells and immune response and interferes with several important physiological processes. Cows that experience hypocalcemia are predisposed to postpartum disorders, poor reproductive efficiency, decreased milk production, and increased risk of early culling. A balanced diet before calving can prevent hypocalcemia and improve Ca availability during the periparturient period, maintaining Ca homeostasis. Prevention of hypocalcemia is the most practical and economical approach to minimize its effects. Two strategies demonstrated to increase concentration of Ca after calving are to create a negative dietary cation-anion difference (DCAD) or include synthetic zeolite A to prepartum diets. A study was conducted to compare blood mineral concentrations after parturition, postpartum health, reproductive efficiency, and milk production of cows supplemented with either anionic salts (AS) or synthetic zeolite A (SZA) in the prepartum period. Primiparous (AS = 232, SZA = 223) and multiparous (AS = 325, SZA = 322) dry cows were randomly assigned at enrollment to be supplemented with AS or SZA and were evaluated daily for health disorders and health treatments during the first 60 days in milk (DIM). Cows were monitored until 150 DIM to determine reproductive outcomes and removal from the herd. Blood samples were collected at 0, 48, and 72 h after calving to determine concentrations of Ca, magnesium (Mg), and phosphorus (P) from a subgroup of cows. Four milk tests were conducted during the study and milk production

traits were evaluated. Prepartum supplementation with SZA resulted in greater Ca concentrations at parturition and 48 h after parturition compared with supplementation with AS. Phosphorus and Mg concentrations were less at parturition for SZA compared with AS cows at parturition. At 72 h after parturition, no treatment differences were detected for Mg, but P concentration was greater for SZA than AS cows. Multiparous AS cows were more likely to be treated for clinical hypocalcemia (recumbency) than multiparous SZA cows. Multiparous AS cows had greater morbidity of health disorders than primiparous AS cows, but no parity differences were detected in SZA cows. Among primiparous cows, AS cows were more likely to become pregnant by 150 DIM than SZA cows. More multiparous cows supplemented with AS tended to be removed from the herd by mid-lactation compared with SZA cows. Urine pH was decreased for AS than SZA cows. No significant differences in milk traits were detected between treatments. These findings indicate that supplementation with SZA before calving can serve as an alternative prevention method to AS to reduce hypocalcemia and improve postpartum health, while not compromising milk production traits or reproductive risk.

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Chapter 1 - Review of Literature

Introduction

The transition period in dairy cows is defined as the 3 wk before and after parturition. This period is associated with challenges to achieve nutritional needs and many physiological and metabolic changes in the cow. During the last 3 prepartum wk, the demand for calcium (Ca) increases in cows because of colostrogenesis and lactogenesis. Function of the immune system is affected, and cows are susceptible to disease (Goff and Horst, 1997; Oetzel and Goff, 2009; Martinez et al., 2012). Failing to maintain and meet Ca requirements can lead a metabolic disorder called parturient hypocalcemia, also referred to as milk fever (DeGaris and Lean, 2008). Jerseys and Guernseys are most susceptible to milk fever, whereas Holsteins and Brown Swiss have moderate susceptibility, and Ayrshires and Milking Shorthorns are least susceptible (Oetzel and Goff, 2009).

Nearly half of multiparous cows develop subclinical hypocalcemia (SCH) after calving (Reinhardt et al., 2011; Oetzel, 2017), and 3 to 7% develop clinical milk fever (DeGaris and Lean, 2008). Oetzel and Goff (2009) reported that 75% of milk fever cases occur by 24 h after calving, 12% in between 24 and 48 h after calving, and 6% during the parturition process. Cows with hypocalcemia also are predisposed to displaced abomasum, ketosis, uterine diseases, mastitis, decreased milk yield, lower reproduction risks, and other illnesses. Disease and metabolic problems lead to increased culling risk in the herd and negative effects on economics of the dairy herd (Oetzel and Goff, 2009; Chapinal et al., 2011, 2012; Seifi et al., 2011; Goff, 2014; Martinez et al., 2014; Santos and Ribeiro, 2014).

A nutrient balanced diet formulated for prepartum cows can effectively prevent hypocalcemia and increase Ca availability during the periparturient period by maintaining Ca

homeostasis. Two different approaches related to diet formulation currently in use are to: (1) create a negative dietary cation-anion difference (DCAD) or (2) add synthetic zeolite A to the prepartum diet to bind up Ca in the gut (Oetzel and Goff, 2009).

Previous researchers demonstrated that feeding a diet to produce a lower DCAD would improve postpartum Ca homeostasis (Ender et al., 1971; Dishington, 1975). Results of a meta-analysis consisting of 43 studies (Santos et al., 2019) demonstrated that reducing prepartum DCAD from +200 to -100 mEq/kg increased total Ca on the day of parturition, decreased prepartum dry matter intake (DMI), increased postpartum DMI, reduced the risk of hypocalcemia, and decreased the incidence of retained fetal membranes and metritis.

Adding synthetic zeolite A to the diet is an alternative approach to increase Ca during the periparturient period. Unlike making the prepartum diet more acidic by controlling the dietary DCAD, zeolite does not acidify the diet. After binding up Ca in the gut, zeolite is excreted in the feces, thus, stimulating Ca resorption from bone. Mobilization of Ca from the bones improves Ca status by parturition (Thilsing et al., 2006, 2007; Pallesen et al., 2008). Kerwin et al. (2019) demonstrated that prepartum feeding of synthetic zeolite A improved Ca status on the day of parturition, decreased prepartum DMI, increased postpartum DMI, decreased incidence of postpartum SCH, and eliminated postpartum clinical hypocalcemia compared with a control.

The objectives of this review of literature are to provide a better understanding of Ca homeostasis and its disorders, hypocalcemia and its clinical stages, treatments for hypocalcemia, carry-over effects of hypocalcemia during lactation, and prevention of Ca disorders during the transition period.

Calcium Homeostasis

Goff (2000) reported that total blood Ca (tCa) concentration in an adult cow normally ranges from 8.5 to 10 mg/dL (2.1 to 2.5 mmol/L). Approximately 50% of the tCa is bound to proteins in the plasma. Ionized Ca (iCa) is the biologically active form of the tCa, and its concentration has the most physiological relevance. The iCa represents approximately 40 to 45% of the tCa (Goff, 1999). When blood pH is more acidic, the iCa concentration gets closer to 45% of tCa, and when blood pH is more alkalogenic, the iCa concentration gets closer to the 40%. Even though the iCa is more relevant for Ca determination, the tCa is adequate to diagnose Ca health disorders.

The Ca plasma pool of a 600-kg cow consists of approximately 3 g, the extracellular pool is approximately 8 to 9 g of Ca, and Ca within the skeleton is approximately 7.8 to 8.5 kg (Goff, 2000). Before parturition, the daily Ca requirement is about 30 g, 15 g for fetal development, and 15 g for fecal and urinary loss. After parturition, the Ca requirement exceeds 50 g because colostrum and milk production increases the demand for Ca (Goff, 1999, 2000; DeGaris and Lean, 2008). Low Ca availability triggers Ca homeostatic mechanisms to maintain an average Ca concentration. In a normal physiological process, this Ca demand only can be regulated by increasing Ca absorption from the rumen or intestines. Calcium mobilization from tissues occurs, principally from bone Ca reserves, when circulating blood Ca concentrations are inadequate. Whenever blood Ca changes, body homeostatic mechanisms reestablish normal Ca concentrations. This process includes calcitonin (secreted when blood Ca concentrations are elevated) or parathyroid hormone (PTH; released by the parathyroid gland when blood Ca concentration is low). Whenever PTH increases, the body reacts to increase Ca mobilization from bone (Goff et al., 1991; Goff, 1999, 2000, 2006; DeGaris and Lean, 2008; Oetzel and Goff, 2009). Binding to

PTH receptors in bone, PTH activates osteoclasts to mobilize Ca from bone. This mechanism, known as lactational osteoporosis, results in skeletal Ca losses of 9 to 13% of skeletal Ca, but the same amount is recovered later in the lactation. In addition, 99% of Ca reserves in the body are in bones, and mobilization of Ca from bone has a variable onset. Mobilization of Ca from bone is slower in older cows and cows fed large amounts of Ca in their prepartum diet or fed alkalogenic salts (Ramberg et al., 1984; Goff, 2006; DeGaris and Lean, 2008).

Cows depend more on gut absorption than bone resorption to maintain normocalcemia (DeGaris and Lean, 2008). For this to occur, another important hormone comes into play, 1,25-dihydroxyvitamin D_3 (active form of the vitamin D_3; calcitriol), is converted from the vitamin D_3 in the kidneys. Parathyroid hormone activates this conversion and works by stimulating increased efficiency of Ca absorption in the intestines to replace the Ca excreted into colostrum and milk. Parathyroid hormone also activates renal tubular reabsorption of Ca, even though the amount of Ca recovered from urinary Ca is small. The time needed for the body to regulate Ca concentrations is approximately 1 to 2 d, a similar period in which dairy cows develop SCH during the first few days after calving (Oetzel, 2013). When all of these processes fail to increase blood Ca to produce normocalcemia, milk fever can occur (Goff, 2006; DeGaris and Lean, 2008).

Factors Affecting Ca Homeostasis

Many factors can alter physiological functions to maintain Ca homeostasis, decreasing tCa concentrations, and inducing milk fever (Goff, 2006; DeGaris and Lean, 2008; Oetzel and Goff, 2009). A discussion of these factors ensues.

Age

As cows increase in age, their capacity to maintain efficient Ca mobilization from bone decreases. The capacity of converting vitamin D_3 to 1,25-dihydroxyvitamin D_3 in the kidneys also

decreases. Most PTH lack of responsiveness is caused by the reduced number of PTH receptors in target tissues (Goff, 2000, 2006).

Metabolic Alkalosis

Another factor is the acid-base status of the cow at parturition. A cow in a metabolic alkalosis at the time of parturition has its physiological function of the PTH altered, dysregulating the Ca homeostasis. The presumption is that metabolic alkalosis somehow disrupts the integrity of PTH receptors in target tissues (Horst et al., 1994). In addition, during metabolic alkalosis, PTH does not bind to receptors in the kidneys, leading to failed reabsorption of renal Ca and conversion of 1,25-dihydroxyvitamin D_3 from the vitamin D_3. Metabolic alkalosis is mainly a result of diets being fed with more cations than anions (positive DCAD), which also increases blood pH (Goff, 2006).

Hypophosphatemia

Normal phosphorus (P) concentration ranges from 4 to 8 mg/dL (1.3 to 2.6 mmol/L). As Mg, P can alter Ca homeostasis. Cows fed diets with less P than the physiological requirements will develop hypophosphatemia, resulting in inappetence, unthriftiness, compromised milk production, and reproductive failure. Cows with chronic hypophosphatemia have P ranging from 2 to 3.5 mg/dL (0.6 to 1.1 mmol/L), and recumbent cows have less than 1 mg/dL (0.3 mmol/L). A cow with hypophosphatemia usually also has hypocalcemia, hypomagnesemia, and in a few cases, hypoglycemia. Dairy cows usually have P concentration below the normal range at parturition, and if hypocalcemic, P is approximately 1 to 2 mg/dL (0.3 to 0.6 mmol/L). Regardless of its similarities with Ca homeostasis, P does not stimulate PTH release or 1,25-dihydroxy vitamin D_3 conversion from Vitamin D_3, but it can do the opposite. Blood P concentration at approximately 6.19 mg/dL (2 mmol/L) inhibits the PTH-stimulated conversion of vitamin D_3 to 1,25-

dihydroxyvitamin D_3 in the kidney, thus reducing the availability of 1,25-dihydroxyvitamin D_3 in the intestines to promote Ca absorption and transport (Goff, 2006). Prepartum cows fed low dietary Ca will have a greater tCa concentration at parturition and decreased risk of milk fever (Kichura et al., 1982; Peterson et al., 2005; Lean et al., 2006; Cohrs et al., 2018). Peterson et al. (2005) found that prepartum feeding of P [0.21% of dietary dry matter (DM)] to cows resulted in greater energy-corrected milk and less SCH than feeding a larger amount (0.44%) of P. In another study, Cohrs et al. (2018) observed no milk fever in a group of prepartum cows fed P (0.152%), but 3 of 9 cows had milk fever when fed 0.283% P.

Hypomagnesemia

Deficiency of Mg can affect the Ca homeostasis by decreasing the secretion of PTH and reduce the responsiveness of the PTH receptors to PTH, in the presence or absence of metabolic alkalosis (Rude et al., 1978). Normal concentrations of Mg ranges from 1.8 to 2.4 mg/dL (0.75 to 1.0 mmol/L), and is vital for nerve conduction, muscle function, and bone formation. Depending on its deficiency or excess, Mg can also cause recumbency, hyperexcitability, tetany, convulsion, and death (Goff, 1999).

Clinical Symptoms of Hypocalcemia

Diagnosing SCH is not easy because cows do not display any clinical signs. If not diagnosed or treated, cows may thenl display clinical hypocalcemia (CH) with all its symptoms and effects (Oetzel and Miller, 2012).

Subclinical Hypocalcemia

Subclinical hypocalcemia is the first stage when blood Ca concentrations are less than normal. Subclinical hypocalcemia consists of a tCa concentration ≤ 8.59 mg/dL (or ≤ 2.14 mmol/L) without clinical signs (e.g., recumbency, lethargy, hypothermia, and rumen atony;

Martinez et al., 2012; Caixeta et al., 2017). Incidence of SCH increases with parity, with approximately 73% of cows having SCH in their third or greater lactation during the first 3 DIM (Caixeta et al., 2015). Even though cold ears may be used to diagnose SCH, one study (Venjakob et al., 2017) found that this technique is not adequate, considering the ambient temperature is a leading factor in this thermoregulation process.

Clinical Hypocalcemia

Clinical HypocalcemiIn Stage 1, cows are not recumbent still able to stand, even though it is possible to recognize clinical signs. The tCa concentration for this stage usually ranges from 5.5 to 7.5 mg/dL (1.37 to 1.87 mmol/L; Oetzel and Goff, 2009; Oetzel, 2017). Cow first present hypersensitivity, excitability, and cold extremities. In addition, they may shift their weight back and forth, be hypothermic, start to show ataxia, some tremors of the flanks and triceps, twitching ears, and reduced rumen activity. They are usually restless. If untreated, Stage 2 will begin.

Stage 2 begins when the cow is unable to remain standing, resulting in sternal recumbency. The tCa at Stage 2 ranges from 3.5 to 6.5 mg/dL (0.87 to 1.62 mmol/L; Oetzel and Goff, 2009). This begins a dangerous point in which cows are depressed and partially paralyzed. Heart rate increases with low intensity of the sounds because of poor cardiac muscle contractility. Blood perfusion in the extremities becomes poor causing coldness upon palpation, and the body temperature decreases to 35.6 to 37.8 °C. Because of the smooth muscle paralysis, clinical signs lead to gastrointestinal hypomotility and atony, mild bloat, and decreased rumen contractions. Even the pupillae muscle goes into atony, making the pupils to be constantly dilated and unresponsive to light. Stage 2 often lasts for 1 to 12 h, and if not treated, Stage 3 ensues.

Cows tCa in Stage 3 ranges from as low as 1 to 2.5 mg/dL (0.25 to 0.62 mmol/L; Oetzel and Goff, 2009; Oetzel, 2017). At stage 3, cows are laterally recumbent, often with their heads turned toward their flank. If the head were extended, a curve in the neck is easy to identify. Cows begin to lose consciousness and go into a coma. At this stage, muscles are completely flaccid, and unresponsive to any stimulation. Heart rate may accelerate to as much as 120 bpm without any detectable peripheral pulse. Cows can have irreversible musculoskeletal damage. If not treated in a few hours, cows may not survive. Even after administering Ca intravenously, cows can develop further complications. Diseases such as toxic mastitis, metritis, or any other systemic toxic condition, calving paralysis syndrome, or a traumatic injury, could be subsequently diagnosed.

Treatments for Hypocalcemia

Different treatment regimens are possible and successful depending on the stage of hypocalcemia. When the cow is able to remain standing, an efficient treatment is to provide slowly absorbed. A good approach may consist of an oral Ca supplement. Use of Ca chloride (4 g) increases tCa concentration by 30 min after administration. Oral supplementation is equivalent to approximately one-half that amount when given intravenously. A bottle of Ca contains approximately 8 to 11 g of Ca, which approximates the entire amount of Ca in the extracellular pool of an adult dairy cow. A Ca bolus is a great option because it eliminates the risk of aspiration and the strong taste found in a Ca gel. Absorption of Ca can occur by two different means. The 1,25-dihydroxyvitamin D_3 mediates the first by transporting Ca across the intestinal cells into the extracellular space. The second way is based on passive transport with a concentration dependence, diffusing Ca into the blood once the Ca concentration in the blood is less than the amount in the intestinal tract mucosa cells (Goff, 1999; Oetzel and Goff, 2009; Oetzel and Miller, 2012; Oetzel, 2017).

Cows in Stage 2 or 3 should receive intravenous treatment as soon as possible. This treatment method is adequate because it quickly replaces the Ca needed in the blood concentration pool and supplies 1 to 2 g of Ca that can be lost by 30 min after treatment (Oetzel and Goff, 2009; Oetzel, 2017). Intravenous treatment during Stage 1 is more than what is needed at this stage. Once standing, intravenous treatment increases tCa concentration to a peak in a few minutes. Unnecessary high amounts of Ca, however, change the ability of the body to maintain Ca homeostasis. In less than 24 h, cows may return to a hypocalcemic status, but at that point, it can endure for 48 h or more (Blanc et al., 2014; Oetzel, 2017). Effective follow-up treatments to intravenous Ca administration should consist of using either oral Ca drenches, Ca boluses, or subcutaneous Ca administration because approximately 38% of cows may return to the former Ca deficits in less than 24 h. Providing slowly absorbing Ca helps the cow to recover. Complete recovery can take up to 3 d (Oetzel, 2017).

Prevention of Hypocalcemia

Many strategies to control milk fever in dairy cows are described in the literature, most of which involve prepartum diet management. Three main strategies exist to prevent parturient hypocalcemia: (1) feeding low Ca diets; (2) adding products such as zeolite to the diet to absorb Ca; or (3) feeding a diet that produces a negative DCAD during the last 3 wk before calving (Pallesen et al., 2008).

Low Ca Diets

Feeding prepartum diets that are low in Ca (< 20 g/d of absorbable Ca) stimulate Ca homeostatic mechanisms before calving, and thus reduce the potential for milk fever at parturition (Wiggers et al., 1975; Kichura et al., 1982; Thilsing-Hansen et al., 2002b; Goff, 2006). In a study with 16 dairy cows, Green et al. (1981) found that 8 d was insufficient time to have Ca

concentration in the required range at parturition. In a previous study, Wiggers et al. (1975) concluded that low Ca diets must be fed for at least 10 to 14 d before parturition to be effective. The low Ca strategy can be achieved by replacing high Ca rich feed ingredients, like alfalfa, with low Ca feed ingredients, such as corn silage or grass hays. This change reduces dietary cations and Ca itself (Goff, 2000). Difficulties in maintaining low concentrations of Ca using feeds available in many countries require different approaches to the prepartum diet (Thilsing-Hansen et al., 2002b).

Dietary Cation-Anion Difference (DCAD)

Many studies demonstrated that adding specific salts or acids to the prepartum diet reduces hypocalcemia in dairy cows. This approach leads to stimulated bone resorption of Ca and activation of 1,25-dihydroxyvitamin D_3, functions that are directly dependent on PTH secretion (Ender et al., 1971; Dishington, 1975; Block, 1984; Goff et al., 1991). Metabolic alkalosis status reduces the ability for PTH to bind to its receptors in specific target tissues such as bone and kidney. The DCAD strategy focuses on acidifying the blood, restoring Ca homeostasis (Goff, 2000). Blood pH is determined by the number of anions and cations absorbed from the diet. The difference between these two ions is usually expressed in mEq per 100 g or per kg of the diet (Goff, 2000). To calculate DCAD, values of anions are subtracted from those of cations so DCAD = $(Na^+ + K^+) - (Cl^- + S^{--})$. This is the most used equation, cited first by Ender et al. (1971) and later used by others (Dishington, 1975; Block, 1984). Two other equations were proposed, considering more anions and cations, and specificities for each of them. One equation came from Goff and Horst (1997), DCAD = $(0.38\ Ca^{++} + 0.3\ Mg^{++} + Na^+ + K^+) - (Cl^- + S^{--})$. The second from Goff (2000), DCAD = $(0.15\ Ca^{++} + 0.15\ Mg^{++} + Na^+ + K^+) - (Cl^- + 0.25\ S^{--} + 0.5\ P^{---})$. Thilsing-Hansen et al. (2002) in its review also cited two other equations, DCAD = $(Na^+ + K^+) - (Cl^-)$ and DCAD = (Ca

228 + Mg + Na + K) − (Cl + SO_4 + H_2PO_4 + HPO_4). The main cations in feed ingredients are sodium,
229 potassium, calcium, and magnesium, and the main anions are chloride, sulfate, and phosphate.
230 Ammonium should be considered for a complete equation, considering it contributes to the blood
231 cation content (Goff, 2000). In an elegant meta-analysis, Santos et al. (2019) compared the
232 difference in the performance of dairy cows when reducing the DCAD from +200 to -100 mEq/kg.
233 In addition, the latter suggested that a negative DCAD should not be < -150 mEq/kg of the DM.
234 They concluded that reducing the DCAD would lessen the incidence of milk fever in parous cows,
235 and metritis and retained fetal membranes in all cows. Parous cows subsequently produced more
236 milk, milk fat, and milk protein.

237 When applying a negative DCAD diet, which acidifies the blood, urinary pH should be
238 monitored (Goff, 2000). When urinary pH decreases, blood pH becomes more acidified. In a
239 DCAD strategy, urine pH should range between 6.2 and 6.8 in Holsteins and 5.8 and 6.3 in Jerseys
240 for better prevention of milk fever. When urine pH ranges from 5.0 to 5.5, an uncompensated
241 metabolic acidosis induced by the anionic salts reduces DMI. Urine samples should be collected
242 48 h or more after a ration change. Urine samples provide the best pH estimate when collected 6
243 to 9 h after feeding (Goff, 2000). A negative DCAD induces a compensated acidosis by 4 to 5 d
244 (Goff, 2006).

245 **Zeolite A**

246 Adding Zeolite A (sodium aluminum silicate) to the prepartum feed is another dietary
247 method to prevent hypocalcemia (Jørgensen et al., 2003; Thilsing-Hansen et al., 2003; Goff, 2006;
248 Oetzel and Goff, 2009; Kerwin et al., 2019). Zeolite has a capacity to bind dietary Ca, Mg, and P,
249 precluding their absorption (Thilsing-Hansen et al., 2002a; Thilsing et al., 2006) and excreting Ca
250 in the feces (Goff, 2006). Thus, Zeolite promotes Ca homeostasis by inducing a Ca deficiency,

increasing PTH secretion, and facilitating resorption of Ca from tissue stores. Previous studies (Thilsing et al., 2007; Pallesen et al., 2008; Kerwin et al., 2019) found that prepartum feeding of Zeolite A (0.5 kg/d) increased tCa concentration at parturition and during the first few days after calving, reduced the incidence of parturient hypocalcemia in multiparous cows, and provided better postpartum health in all cows. Zeolite A also seems to reduce postpartum inflammation by decreasing expression of immune mediators in neutrophils (Crookenden et al., 2020). Kerwin et al. (2019) reported that in prepartum cows fed Zeolite A, Mg concentration was less than that observed in the control, but remained in the normal range. Pallesen et al. (2008) proposed that hypophosphatemia induced by the Zeolite A might have an essential role in Ca balance and prevention of milk fever. Kerwin et al. (2019) also found that a greater proportion of prepartum cows fed Zeolite A was pregnant by 150 DIM compared with controls.

Hypocalcemia Effects on Postpartum Health and Performance, and Estimated Cost Related of Disease

Cows that develop SCH during the first 3 DIM have reduced numbers and function of neutrophils (Martinez et al., 2012). This immune suppression is one of the main contributors to the increased risk of postpartum diseases (Goff and Horst, 1997; Chapinal et al., 2011, 2012; Seifi et al., 2011; Martinez et al., 2012). Immune suppression is also associated with early lactation negative energy balance (NEB; Ribeiro et al., 2013). Changes in body condition score (BCS) can serve as a proxy to estimate NEB. Negative energy balance is reflected by increased concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB), and these markers are associated with postpartum health disorders (Chapinal et al., 2012; Weber et al., 2013). Poor DMI is another cause of postpartum health disorders associated with NEB as well as reduced BCS (Grummer et al., 2004; Hammon et al., 2006). Cows that gain BCS during the dry period have

274 better postpartum reproductive, health, and performance risk (Chebel et al., 2018). Ribeiro et al.
275 (2013) found that cows with SCH lost more BCS between postpartum d 7 and 35 and had overall
276 smaller BCS during the study than cows with normocalcemia.

277 **Uterine disease**

278 Uterine disease is a common term referring to complications resulting from retained fetal
279 membranes. Retained fetal membranes (RFM) are characterized as ailure of the placenta to detach
280 from the uterine wall by 12 h after parturition. Inflammation of the uterus of metritis (MET) is
281 diagnosed as fetid red or pink discharge by 21 d after calving. Decreased blood tCa concentration
282 affects fertility (Santos and Ribeiro, 2014) because of reduced neutrophil function (Martinez et al.,
283 2012). Cows affected by decreased neutrophil function may develop RFM (Kimura et al., 2002)
284 and MET (Martinez et al., 2012). Cows that develop uterine diseases experience more NEB,
285 greater NEFA, and BHB concentrations during the periparturient period (Galvão et al., 2010).
286 Martinez et al. (2012) demonstrated that cows with SCH had a numerical increase in rectal
287 temperature compared with cows with normocalcemia, with or without MET. Nonetheless, cows
288 with SCH and MET display significantly greater rectal temperatures than cows with MET and
289 normocalcemia.

290 In the latter study (Martinez et al., 2012), 97% cows that develop metritis were diagnosed
291 with SCH, and cows with SCH tended to have poorer pregnancy risk and 15 greater days open
292 than normocalcemic cows. Cows with MET can have reduced conception risk by approximately
293 20% (Borsberry and Dobson, 1989). In another study (Chapinal et al., 2012), cows with SCH also
294 had lower odds of becoming pregnant at the first AI. Increasing the number of days cows are
295 exposed to a negative prepartum DCAD diet improved days open, tended to improve odds of
296 pregnancy, and reduced culling risk (DeGaris et al., 2010). Furthermore, cows with SCH are less

likely to express signs of estrus by 60 d after calving (Caixeta et al., 2017; Rodríguez et al., 2017) and more likely to have a delayed pregnancy (Martinez et al., 2012). Guard (2008) estimated the cost of RP and MET as $315 per case. McArt et al. (2015) estimated a total cost of MET of $386. In another study, Lima et al. (2019) compared the cost of MET when treatment included two different antibiotics, ampicillin and ceftiofur, and the estimated cost were $344 and $410, respectively, when withheld milk was discarded, and $267 and $406, respectively, when withheld milk was fed to calves.

Displaced Abomasum

Displaced abomasum (DA) occurs when the abomasum is enlarged with gas or fluid and moves to the right or left of the abdominal cavity (Coppock, 1974). Constable et al. (1991) considered right displaced abomasum (RDA) as the mirrored image of the left displaced abomasum (LDA) with no volvulus. Abomasal volvulus is an evolution of the process. Cows with SCH have increased todds of having a displaced abomasum by 3.7% compared with normocalcemic cows (Rodríguez et al., 2017). Cows with SCH may have abomasal muscular tone similar to cows with CH (Daniel, 1983; Hansen et al., 2003). Jørgensen et al. (1998) reported that cows with hypocalcemia had reduced rumen contractions by approximately 40%. Frequency and amplitude of rumen contractions and intestinal motility are identified clearly when tCa is approximately 6.49 mg/dL (1.62 mmol/L; Ramberg CF et al., 1967) and occurs more quickly when blood tCa concentration decreases to 4.01 mg/dL (1.0 mmol/L; Jørgensen et al., 1998). Smooth and skeletal muscle contraction is correlated with tCa. Cows with SCH have decreased rumination (Hansen et al., 2003; Goff et al., 2020) and jaw movements compared with normocalcemic cows during the first 3 DIM (Hansen et al., 2003) and this decrease is probably one reason for decreased DMI, which may facilitate a subsequent DA. McArt et al. (2015) estimated the cost of a DA at

320 $792 per case, including the indirect costs of $295 associated with losses in milk, fertility, and
321 culling.

322 **Lameness**

323 Lameness is a clinical health disorder in animals with impaired locomotion, no matter the
324 cause (Archer et al., 2010). Juarez et al. (2003) considered lameness to be one of the three main
325 problems affecting dairy cows, only after infertility and mastitis. Cows with tCa imbalance may
326 have impaired immune function and greater risk of developing health disorders (Martinez et al.,
327 2012). In addition to unbalanced nutrition and trauma, infection is also an essential factor that
328 triggers lameness (Endres, 2017). Cows with SCH also have increased risk of developing lameness
329 (Neves et al., 2017). Lameness is responsible for increased costs related to decreased milk
330 production, increased treatments (Esslemont and Kossaibati, 1996; Rajala-Schultz et al., 1999a;
331 Warnick et al., 2001; Chapinal et al., 2011), reduced fertility (Hernandez et al., 2001), and
332 increased culling risk (Sprecher et al., 1997; Warnick et al., 2001). Lameness is typically scored
333 by observing the mobility of the cow whenwalking using a scale from 1 to 5; with 1 being normal
334 and 5 not being able to touch the ground with one or more feet (Sprecher et al., 1997). In one study
335 (Espejo et al., 2006), lameness had an average prevalence of 24.6% in dairy herds, starting at
336 12.8% for first-lactation cows and increasing by about 8% for each successive lactation. In another
337 study, (Blackie and Maclaurin 2019) reported a prevalence of 38%. A greater incidence of
338 lameness is observed when cows are housed in free stalls with mattress surfaces than those bedded
339 with sand (Cook et al., 2004; Espejo et al., 2006). Cha et al. (2010) reported an estimated a range
340 in cost of lameness from $121 to $216, depending on the type of lameness, averaging $157 per
341 case. The latter authors also compared their results with an older study (Enting et al., 1997), and
342 after converting to US$, the estimated cost was $155.

Mastitis

Mastitis is inflammation of milk-secretory tissue in the udder, which can be assessed by somatic cell count. Mastitis is the most common disease affecting dairy cows, which results in significant losses in revenue (Rajala-Schultz et al., 1999b; Juarez et al., 2003; Cha et al., 2011; Rodríguez et al., 2017). Immune suppression caused by hypocalcemia makes cows more susceptible to mastitis (Martinez et al., 2012; Santos and Ribeiro, 2014). Cows with mastitis and SCH have fewer neutrophils than cows with normocalcemia (Martinez et al., 2014). Dairy herds have a clinical mastitis incidence of approximately 24.8%, and usually, approximately 72.8% of infected cows recover and remain in the herd. Less than 5% of cows diagnosed with mastitis die (NAHMS, 2014). Cows that contract mastitis are usually highly productive multiparous cows, producing approximately 2.6 kg more milk per day than healthy cows. Cows that contract mastitis have an estimated milk loss of 1,200 kg per lactation (Wilson et al., 2004).

In a meta-analysis that evaluated how mastitis affects reproductive performance, Dolecheck et al. (2019) reported that mastitis increases days to first service, insemination per conception, and days open from insemination to conception, but decreases the odds of pregnancy per insemination at first service. Treatment of mastitis is responsible for most antibiotic use on dairy farms (Redding et al., 2019). Cost of mastitis is estimated at $134 for gram-positive cases, $211 for gram-negative cases, and $95 for other cases (Cha et al., 2011). In another study (Bar et al., 2008), a clinical case was estimated at $179. Both of the previous studies accounted for treatment, milk losses, reproduction losses, and other indirect factors.

Prepartum Nutritional Strategies Focusing on Improving Periparturient Ca Status

Several researchers have evaluated prepartum nutritional strategies in order to improve postpartum Ca status and health and lactation performance of dairy cows. Most researchwas designed to understand further how manipulating the DCAD in prepartum diets affects Ca after calving. Goff (2004) demonstrated the correlation of milk fever and urinary pH based on the following equation: DCAD = (Na + K) – (Cl + 0.6 S).

In 2006, a meta-analysis (Charbonneau et al.) consisting of 22 studies and 5 different DCAD equations demonstrated how prepartum DCAD affects periparturient Ca status. In addition, reducing prepartum DCAD +300 to 0 mEq/kg increased periparturient Ca concentration (0.78 mg/dL for tCa and 0.48mg/dL for iCa), reduced risk of CH, and decreased postpartum health disorders, urinary pH, and DMI.

Martinez et al. (2012) evaluated how a prepartum diet consisting of -94 mEq/kg DCAD influenced postpartum Ca and immune response of Holstein cows. Cows with SCH (blood tCa concentration ≤ 8.59 mg/dL [≤ 2.14 mmol/L]) had reduced neutrophil function and increased risk of uterine diseases. They concluded that the risk of metritis was reduced by 22% for each 1 mg/dL of tCa. Cows with SCH also had a greater risk for other postpartum diseases and impaired reproductive performance.

In a study aimed to induce milk fever in order to evaluate physiological responses, Martinez et al. (2014) fed a positive DCAD (160 mEq/kg) in the prepartum period. Cows with SCH (iCa concentration ≤ 0.80 mmol/L) had reduced DMI, rumen contractions, and neutrophil function. These findings suggest that the increased risk of postpartum diseases may be caused by decreased immune responses mediated by reduced cytosolic iCa concentrations.

386 Valldecabres et al. (2019) explored how various factors are associated with SCH in
387 prepartum cows fed a negative DCAD (-176 mEq/kg) diet. Two different thresholds were used to
388 define SCH: < 8.02 mg/dL (< 2.00 mmol/L) and < 8.82 mg/dL (< 2.12 mmol/L). Jersey and
389 Holstein crossbreed cows were used in the study. Cows were divided by previous 305-d mature-
390 equivalent milk yield into percentiles (25^{th}, interquartile, and 75^{th}). Both thresholds were
391 associated with SCH in parous cows. Parity was associated with SCH. Jersey cows calving male
392 calves were more likely to have SCH (< 8.82 mg/dL threshold) compared with Jersey cows calving
393 female calves. In addition, cows with milk yield above the 75^{th} percentile had a greater risk of
394 SCH. Jersey cows were more likely to have Ca concentration < 8.02 mg/dL than Holstein-
395 crossbreed cows.
396 Santos et al. (2019) predicted that reducing the DCAD from +200 to -100 mEq/kg in a
397 prepartum diet would increase postpartum serum Ca concentration, decrease milk fever incidence
398 and uterine disease, and increase milk production of parous cows. Reducing the DCAD resulted
399 in decreased prepartum DMI, but increased postpartum DMI.
400 Kerwin et al. (2019) investigated the effects of Ca status of prepartum Hlstein cows
401 supplemented with Zeolite A (0.5 kg/d). Cows supplemented with Zeolite A had improved Ca
402 status at calving and during the first 3 d after calving. In addition, supplementation with Zeolite A
403 decreased the proportion of cows with SCH (8.59 mg/dL; 2.14 mmol/L). Crookenden et al. (2020)
404 demonstrated that supplementation with Zeolite A increased Ca status at calving and altered
405 postpartum neutrophil gene expression. Cows supplemented with Zeolite A had an average tCa
406 concentration on the day of 8.82 mg/dL (2.2 mmol/L). On the other hand, Ca concentration of
407 control cows was 8.02 mg/dL (2.00 mmol/L). Supplementation with Zeolite A reduced
408 concentration of interleukin-6, a cytokine that delays neutrophil apoptosis in the onset of

409 inflammation. These findings suggest that Ca availability may decrease inflammation by
410 downregulating expression of immune mediators.

411 A recent publication demonstrated that dietary Ca may influence Ca status in diets
412 formulated to reduce DCAD (Ryan et al., 2020). The following diets were evaluated in the study:
413 DCAD = -240 mEq/kg with low dietary Ca (0.4% DM); DCAD = -240 mEq/kg with high dietary
414 Ca (2.0% DM); and DCAD = +6 mEq/kg with low dietary Ca (0.4% DM). The diet with more
415 dietary Ca (2% DM) resulted in greater endometrial glandular epithelial height, more endometrial
416 epithelial cells per gland, decreased plasma haptoglobin concentration, and increased glutathione
417 peroxidase activity compared with the similar DCAD diet with lower dietary DM Ca. The authors
418 of the study concluded that the inclusion of greater dietary Ca in negative DCAD diets improved
419 periparturient immune response of cows.

420 Although several studies were conducted to evaluate how strategies or factors are
421 associated with Ca concentration after calving, more research is needed in this area. Further
422 research is warranted to determine thresholds of Ca concentrations at different times after calving
423 and the relationship of concentration of other minerals with Ca homeostasis. In addition, studies
424 evaluating the economics of implementing nutritional strategies to alter Ca concentration are
425 needed.

426 ## Conclusions

427 As dairy cows age, many factors can affect Ca homeostasis and cause hypocalcemia.
428 Hypocalcemia affects a large percentage of the herd as subclinical cases. Cows that experience
429 hypocalcemia are prone to other postpartum health disorders. These diseases decrease milk
430 production throughout lactation, reduce fertility, increase treatment frequency and cost, increase
431 culling risk, and increase other indirect costs related to these problems. Different clinical stages of

hypocalcemia should be treated appropriately; otherwise, the disease can become more severe. To identify SCH, blood tCa concentration is a useful measurement, but it is costly and requires laboratory measures that render it impractical. The best approach to control hypocalcemia is by prevention.

The negative DCAD strategy or adding Zeolite A to prepartum diets is the most common approach that effectively prevents hypocalcemia, resulting in greater blood tCa concentration before and after parturition. The Ca increased at parturition decreases the incidence of SCH and can eliminate CH after calving, improve postpartum health status and DM intake, and decrease the number of treatments. Hypocalcemia and Ca status affecting dairy cows have been investigated for more than 70 yr. Nevertheless, many questions remain to be answered for a better performance of a dairy herd. Prevention remains the most practical and economically viable approach.

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Chapter 2 - Comparison of Blood Mineral Concentrations, Postpartum Health, and Reproductive Outcomes of Dairy Cows Supplemented before Parturition with Anionic Salts or Synthetic Zeolite A

ABSTRACT

Objective: The objectives were to compare blood mineral concentrations after calving, postpartum health, reproductive outcomes, and milk production of cows supplemented with either anionic salts (AS) or synthetic zeolite A (SZA) before calving.

Materials and Methods: Primiparous (AS = 232, SZA = 223) and multiparous (AS = 325, SZA = 322) cows (n = 1,102) were assigned randomly to be supplemented with AS or SZA during the prepartum period. Cows were evaluated daily for diagnosis and treatment of health disorders from calving until 60 DIM. Cows were monitored until 150 DIM to determine reproductive efficiency and removal from the herd. In a subgroup of cows, blood samples were collected at 0, 48, and 72 h after calving to determine Ca, Mg, and P status. During the study, four milk tests were conducted, and milk production traits were evaluated.

Results and Discussion: Supplementation with SZA increased ($P < 0.01$) Ca concentrations at parturition and 48 h after parturition compared with supplementation with AS. At calving, P and Mg concentrations were decreased ($P \leq 0.02$) for SZA. In contrast, at 72 h after calving, no treatment differences were detected for Mg, and SZA had greater ($P < 0.01$) P concentrations than AS cows. Among multiparous cows, AS cows were more likely to be treated ($P < 0.01$) for milk fever (recumbency) than SZA cows. No differences in morbidity of health disorders during the first 60 DIM were detected between primiparous and multiparous SZA cows.

In contrast, among AS, multiparous cows had greater ($P < 0.01$) morbidity than primiparous cows. Among primiparous cows, AS cows were more likely to become pregnant by 150 DIM than SZA cows. More cows supplemented with AS tended to be removed from the herd by mid-lactation compared with SZA cows. Urine pH was greater ($P < 0.01$) for SZA cows than AS cows. Milk traits did not differbetween dietary treatments.

Implications and Applications: These findings indicate that supplementation with SZA before calving can serve as an alternative prevention method to anionic salts to reduce hypocalcemia and improve postpartum health, while not compromising milk production.

Key words: disease; transition period; dairy cow; hypocalcemia.

INTRODUCTION

Several research studies have described negative carryover effects of postpartum disorders on reproductive performance. In eight compiled experiments (Santos et al., 2010), cows with postpartum disorders by 60 d after calving were less likely to become pregnant at first AI compared with cows not diagnosed with diseases. Considering that peripartum hypocalcemia is associated with postpartum disorders (Martinez et al., 2012) and decreased reproductive efficiency (Caixeta et al., 2017), several researchers have investigated strategies to increase blood Ca during the periparturient period to increase overall efficiency of dairy cows.

It is well documented that prepartum supplementation with anionic salts changes the dietary cation-anion difference (DCAD), increases postpartum Ca concentrations, and improves postpartum health of dairy cows (Santos et al., 2019). Multiparous cows supplemented with anionic salts for 21 d before calving are expected to have less risk of hypocalcemia than nonsupplemented multiparous cows (Santos et al., 2019). A recent study demonstrated that another strategy utilized during the last weeks of gestation could reduce the risk of hypocalcemia during

752 the periparturient period (Kerwin et al., 2019). Parous cows supplemented with synthetic zeolite
753 A for 21 d before calving had reduced postpartum hypocalcemia than cows not supplemented with
754 the feed additive (Kerwin et al., 2019). Synthetic zeolite A binds dietary Ca, decreases its
755 absorption, and alters Ca concentration in the blood (Thilsing et al., 2006).

756 Although evidence supports that prepartum supplementation with anionic salts or synthetic
757 zeolite A effectively reduces proportion of cows with hypocalcemia, no studies have compared
758 both strategies in the same experiment. Moreover, it is unknown how these strategies are
759 comparable regarding improving postpartum health, reproductive efficiency, and production.

760 The first objective of this experiment was to compare incidence of postpartum health,
761 reproductive performance, and milk production of cows after calving and prepartum
762 supplementation with anionic salts or synthetic zeolite A. The second objective was to compare
763 mineral statuses at and after calving of cows submitted to the prepartum treatments (anionic salts
764 or synthetic zeolite A).

765 # MATERIALS AND METHODS

766 ## Cows, Facilities, and Treatments

767 This study was approved by the Kansas State University's Institutional Animal Care and
768 Use Committee (application number 4323). Holstein-Jersey crossbred cows from a commercial
769 dairy farm located in southwest Kansas were used in this experiment. Cows were housed in dry
770 lot pens in the far-off pen (60 to 21 days before parturition). In the close-up period (3 to 4 weeks
771 before parturition), cows were housed in dry lot pens equipped with fans and shade available at
772 the feed bunk area. Lactating cows were housed in free-stall barns equipped with fans. Enrollment
773 period in the study was from July through October 2019. Primiparous (n = 455) and multiparous
774 cows (n = 647) were assigned randomly to treatments based upon date of movement from the far-

775 off to the close-up pen and monitored until 150 DIM. Moves from the far-off to the close-up pen
776 were based on the dairy standard operation procedures, which consisted of weekly movement of
777 cows that were at least at 250 d of gestation. Treatments consisted of supplementation with (1)
778 anionic salts (**AS**; primiparous = 232 and multiparous = 325; Bio-Chlor, Church & Dwight Co.
779 Inc., Princeton, NJ); or (2) synthetic zeolite A (**SZA**; primiparous = 223 and multiparous = 322;
780 X-Zelit, Protekta Inc., Lucknow, ON, Canada/Vilofoss, Graasten, Denmark) in the close-up pen.
781 Cows in both treatments were managed in a similar manner, except diets fed during the prepartum
782 period (either supplementation with AS or SZA). The diets fed to close-up cows had similar
783 ingredients (Table 1), only varying the type of supplement (AS or SZA) and concentration of
784 dietary Ca (CaCO3 supplementation). To ensure cows were offered the correct diet, our research
785 team assisted farm staff responsible for mixing the diets daily. Research personnel weighed anionic
786 salts and synthetic zeolite A using a digital scale before adding the supplements to the mixer
787 wagon. The SZA diet was formulated to provide 500 g X-Zelit per cow. The AS diet contained
788 5.1% of DM of Bio-Chlor in order to achieve approximately −100 mEq/kg of DM. Dietary Ca was
789 included in the AS diet to ensure optimal uterine health and fertility after calving (Ryan et al.,
790 2020). Amount of AS or SZA needed in each diet was calculated by the on-farm feeding
791 management software EZ Feed (DHI Provo, Provo, UT). Prepartum cows were fed a TMR once
792 daily. When cows achieved 264 days of gestation, they were moved to similar close-up pens that
793 were closer to the maternity barn, where they were fed the same diet (supplementation with AS or
794 SZA). All close-up pens were identical and contained similar stocking density, number of
795 headlocks, fans, and space per cow. Cows in the prepartum pens were monitored hourly for signs
796 of calving and calving assistance was provided when no progress was observed 60 min after

appearance of amniotic sac or feet. All cows were moved to free-stall barns after calving, milked twice daily, and fed twice daily the same postpartum diet.

Blood Sampling, BCS, and Urine pH

In a subgroup of cows (AS = 32; SZA = 29), blood samples were collected by 2 h after calving, and at 48 h and 72 h after calving. Samples were collected from a coccygeal vessel into evacuated tubes without anticoagulant (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Tubes were stored in ice until centrifugation (1,200 × *g* for 15 min at 20 °C) to harvest plasma. Plasma was aliquoted into microcentrifuge tubes and stored at -10 °C until analysis. Plasma samples were submitted to the Kansas State Veterinary Diagnostic Laboratory for analysis of concentrations of total Ca, P, and Mg using the Cobas Ca Gen 2 kit (Roche Diagnostics, Indianapolis, IN). In another subgroup of cows (AS = 118, SZA = 103), BCS was assessed at enrollment, calving, and 21 DIM. Body condition score was assessed by 1 person on a scale of 1 (severe under conditioning) to 5 (severe over conditioning) in 0.25 increments (Ferguson et al., 1994). In a subgroup of cows (AS = 32; SZA = 32), urine was sampled in the prepartum pen after manually stimulating the perineal area of cows that were at least 10 d on their respective treatment diet. Using a portable digital pH meter, urine pH was determined.

Health-Related Events, Culling, and Milk Production Data

Information related to calving, such as stillbirth (born dead or died by 12 h after parturition), twinning, calf sex, and hour and date of calving were recorded by farm personnel and input into the on-farm management software (Dairy Comp; Valley Ag Software, Tulare, CA). Cows were monitored daily for health disorders, which consisted of milk fever, uterine disease (retained placenta and metritis), mastitis, lameness, pneumonia, and digestive problems. Farm personnel, who were monitored by research personnel, conducted diagnosis of diseases and health

820 treatments. Farm personnel was blinded to treatment groups. Disease and treatment events also
821 were recorded in the on-farm software and retrieved from the records when they occurred during
822 the first 60 DIM. Culling (dead or sold) that occurred during the first 150 DIM were extracted
823 from the on-farm software. The herd was enrolled in the milk recording DHIA program. Because
824 only 4 milk yield tests were conducted during the study period, not all cows were tested, and each
825 milk test comprised of a different subpopulation of cows. Similar to a previous report, each test
826 information was limited based on DIM and ECM was calculated using milk, fat, and protein yield
827 (Mendonça et al., 2014).

828 **Disease Definition and Health Treatments**

829 A case of milk fever was defined as a cow with cold extremities, hypothermia, ataxia,
830 muscle tremors, and reduced ruminal contractions. Recumbent cows diagnosed with milk fever
831 were treated with i.v. Ca (500 mL of 23% Ca gluconate; Durvet, Blue Springs, MO) and an oral
832 bolus containing 43 g Ca. Non-recumbent cows diagnosed with milk fever were given oral drench
833 and an oral bolus containing 43 g Ca (Bovikalc; Boehringer Ingelheim Vetmedica Inc., St. Joseph,
834 MO). Cows with moderate depression and inappetence were characterized as undefined illness and
835 given supportive therapy (i.v. Ca and oral drench with propylene glycol). Retained placenta and
836 metritis were defined as failure of detachment of fetal membranes by 12 h after calving and
837 presence of red or brown watery fetid uterine discharge in the first 21 DIM, respectively. Cows
838 diagnosed with uterine disease were treated with parenteral antimicrobials. Gastrointestinal
839 hypomotility or atony, mild bloat, decreased ruminal contraction, or diarrhea was defined as
840 digestive problems and were treated with supportive therapy (i.v. Ca and oral drench with
841 propylene glycol). Cows diagnosed with clinical mastitis had a milk sample collected and
842 intramammary antimicrobial treatment was based on culture results. Recumbent cows with

abnormal respiration, inappetence, and rectal temperature ≥ 39.5 ˚C were characterized as respiratory problems and treated with parenteral antimicrobials.

Reproductive Management

All cows were submitted to the Double-Ovsynch program for first service (GnRH−7 d−$PGF_{2\alpha}$−3 d−GnRH−7 d−GnRH− 7 d−$PGF_{2\alpha}$−56 h−GnRH− 16 h−timed AI; Stevenson et al., 2018). The products used were 100 μg of gonadorelin diacetate tetrahydrate (Fertagyl; GnRH) and 500 μg of cloprostenol sodium (Estrumate; $PGF_{2\alpha}$, Merck Animal Health, Madison, NJ). Timed AI occurred at 77 ± 3 DIM. Pregnancy diagnosis was conducted at 36 ± 3 d after AI by transrectal ultrasonography, and pregnancy reconfirmation occurred at 134 ± 3 d by palpation per rectum. Nonpregnant cows were treated with $PGF_{2\alpha}$ at non-pregnancy diagnosis. Cows not reinseminated by 7 d after the not-pregnant diagnosis were enrolled in the Ovsynch program (Mendonça et al., 2019). Cows deemed ineligible to initiate a subsequent lactation were coded as do-not-breed in the on-farm software and were kept in the herd until milk production decreased to a specific level.

Statistical Analyses

Blood mineral concentrations were analyzed by ANOVA for repeated measures with the MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). Covariance structures [unstructured, compound symmetry, and autoregressive (1)] were tested in each model and chosen based on the lowest Akaike information criterion. The following variables were included in the repeat-measure analyses: treatment (AS vs. SZA), time relative to calving (0 vs. 48 vs. 72 h), parity at enrollment (primiparous vs. multiparous), interactions between treatment and time, treatment and parity, parity and time, and the three-way interaction of treatment, parity, and time.

Chronic subclinical hypocalcemia (**cSCH**) was defined when Ca ≤ 8.6 mg/dL in all 3 samples, as previously described (Caixeta et al., 2017). To investigate the proportion of cows with

cSCH by treatment and its impact on reproductive performance, logistic regression analysis was performed using the PROC GLIMMIX of SAS fitting a binomial distribution. For this analysis, models included treatment, parity, pregnancy status by 150 DIM, and the interactions between treatment and parity, treatment and pregnancy status, and parity and pregnancy status. Other dichotomous outcomes (sold, dead, culled, pregnant, submitted to AI, health disorders, and treatments) also were analyzed by logistic regression using the PROC GLIMMIX procedure using a binomial distribution. Such analyses included treatment, parity, and the interaction between treatment and parity as independent variables.

Continuous variables were analyzed by ANOVA using the PROC MIXED and the following variables were included: treatment, parity, and the interaction between treatment and parity. Considering that each milk test comprised of a different population of cows, data related to each test was analyzed separately, therefore, repeated measures was not used for any analysis of milk traits. Because of the limited number of cows tested between 5 and 34 DIM, no analyses were performed for the first monthly test. Number of cows with milk information of each test were 141, 289, 503, and 603 for the second, third, fourth, and fifth test, respectively.

In order to evaluate the effect of treatment on BCS, change of BCS during the dry period and early lactation were determined by differences in BCS between enrollment and calving, and between calving and 21 DIM, respectively. In all statistical analyses, independent variables and interactions with $P > 0.10$ were removed from the model using a stepwise backward elimination method. Statistical significance was defined as $P \leq 0.05$ and tendencies as $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

At enrollment, percentage of primiparous cows, days of gestation, and prior projected 305-d mature equivalent milk yield did not differ ($P \geq 0.56$) between treatments. In addition, durations

889 of the dry period and gestation, days spent in the close-up pen when experimental diets were fed,
890 and incidence of twinning did not differ ($P \geq 0.64$) between AS and SZA cows (Table 2), whereas
891 a tendency ($P = 0.08$) for more male calves born to cows fed SZA.s.

892 **Blood Mineral Concentration, BCS, and Urine pH**

893 Calcium concentrations at 0, 24 and 72 hours after parturition differed between treatments
894 ($P < 0.01$) and their interaction ($P < 0.01$) with time (upper panel, Figure 1). In addition, AS-treated
895 multiparous cows tended to have less Ca than AS primiparous cows (7.68 ± 0.31 vs. 8.29 ± 0.19
896 mg/dL), respectively, (treatment by parity interaction, $P = 0.10$), whereas no parity differences (P
897 $= 0.89$) were detected in SZA cows (8.64 ± 0.31 mg/dL). Parity ($P = 0.07$) and the interaction
898 between parity and time ($P = 0.09$) tended to be associated with Ca concentration. In the analysis
899 that evaluated proportion of cows with cSCH (all samples ≤ 8.6 mg/dL), treatment, parity, and
900 pregnancy status at 150 DIM were associated ($P \leq 0.04$) with cSCH (Table 3). Similar to previous
901 findings (Caixeta et al., 2017), cows with cSCH had impaired reproductive efficiency. In addition,
902 AS cows were more ($P < 0.01$) likely to have cSCH than SZA cows. These findings corroborate
903 previous experimental findings (Kerwin et al., 2019), in which supplementation with SZA
904 decreased proportion of cSCH after calving.

905 Similar to Ca, Mg concentration after calving was affected ($P = 0.02$) by the interaction
906 between parity and treatment. Although no differences in parity were detected ($P = 0.26$) in SZA
907 cows, AS multiparous cows had greater ($P = 0.02$) concentration of Mg than AS primiparous cows.
908 In addition, Mg concentration was affected ($P < 0.01$) by the interaction between treatment and
909 time (middle panel, Figure 1). This interaction was observed because SZA cows had lesser ($P <$
910 0.01) Mg concentration than AS cows until 48 h after calving, whereas no treatment differences
911 ($P = 0.55$) were observed at 72 h. Phosphorus concentration was associated ($P < 0.01$) with the

912 interaction between treatment and time (lower panel, Figure 1). At calving, SZA cows had lesser(P
913 < 0.01) concentration of P than AS cows. In contrast, at 48 and 72 h after calving, SZA cows had
914 greater ($P \leq 0.03$) P concentration than AS cows. Parity and the interaction between parity and
915 treatment did not affect ($P > 0.86$) P concentration. In all analyses of blood mineral concentrations,
916 no three-way interactions of treatment, parity, and time were detected.
917 Thilsing-Hansen et al. (2002) also reported that supplementation with SZA decreased
918 concentrations of Mg and P at calving, however, concentrations of these minerals increased by 1
919 wk after parturition. The current study provides further evidence that SZA binds to Mg and P, as
920 previously shown in an *in vitro* experiment (Thilsing et al., 2006). Nevertheless, homeostatic
921 mechanisms are likely triggered after calving in cows supplemented with SZA, resulting in
922 increased concentrations of Mg and P from 3 to 7 d after calving, as demonstrated by the current
923 study and previous reports (Thilsing-Hansen et al., 2002; Kerwin et al., 2019).
924 At enrollment, multiparous had greater ($P < 0.01$) BCS than primiparous cows (3.39 ± 0.03
925 vs. 3.23 ± 0.03). Body condition score at enrollment, however, was not different ($P \geq 0.72$) in cows
926 assigned to treatments. Change of BCS from enrollment to calving tended to be associated ($P =$
927 0.08) with treatment (SA = −0.08 ± 0.03; SZA = −0.16 ± 0.03). Parity and the interaction between
928 treatment and parity did not affect the change in prepartum BCS. From calving to 21 DIM,
929 multiparous cows lost more BCS than primiparous cows (−0.59 ± 0.03 vs. −0.47 ± 0.03).
930 Treatment and the interaction between treatment and parity were not associated with BCS change
931 after calving. Kerwin et al. (2019) showed that supplementation with SZA tended to reduce
932 prepartum DMI but no effects were observed on postpartum DMI. Prepartum diets formulated with
933 the goal to achieve negative DCAD are also expected to reduce prepartum DMI (Santos et al.
934 2019). Considering the difference in BCS change before calving, it is possible that SZA may have

reduced prepartum DMI compared with AS. Nonetheless, the difference in prepartum BCS change was subtle and did not have lasting effects after calving because postpartum BCS change did not differ between treatments. It is crucial to understand how inclusion of feed additives affects changes in prepartum BCS because of the relationship between BCS change in the dry period and subsequent postpartum performance (Chebel et al., 2018).

Urine pH was lower ($P < 0.01$) for AS compared with SZA cows (5.88 ± 0.12 vs. 7.69 ± 0.12). Anionic salts cows had a negative DCAD (−106 mEq/kg of DM) and SZA cows a positive DCAD (128 mEq/kg of DM). Goff (2000) reported that when utilizing a negative DCAD diet, urinary pH should be monitored. It is expected that blood pH is lower, if urinary pH is decreased. In order to prevent hypocalcemia when supplemeting prepartum cows with anionic salts, it is recommended to target urine pH between 6.2 and 6.8, and 5.8 and 6.3 for Holstein, and Jersey cows, respectively. Urine pH of SZA cows was 7.69 ± 0.12, which indicates that it was possible to increase calcium concentration in the first 48 h after calving without utilizing acidogenic salts.

Diseases and Health Treatments

Supplementation did not affect ($P = 0.97$) proportion of non-recumbent cows diagnosed with milk fever. Nonetheless, the interaction between treatment and parity tended ($P = 0.06$) to be associated with the proportion of recumbent cows diagnosed with milk fever (Table 4). Among multiparous cows, AS were more ($P < 0.01$) likely to be diagnosed with milk fever (recumbent) than SZA cows. In addition, the interaction between treatment and parity affected ($P = 0.03$) morbidity of health disorders and treatments during the first 60 DIM. This interaction occurred because AS multiparous cows had greater ($P < 0.01$) morbidity than AS primiparous cows. In contrast, no differences were detected ($P = 0.28$) between SZA primiparous and multiparous cows. Because a positive relationship exists between lactation number and risk of postpartum disorders

(Reinhardt et al., 2011; Oetzel and Miller, 2012), it is likely that increased periparturient Ca concentration is of utmost importance for older cows. In fact, others (Oetzel and Miller, 2012; Martinez et al., 2016) showed that supplementation with oral Ca after calving only increased milk production and reproductive performance in older cows, respectively. It is likely that increased Ca concentration achieved by supplementation of SZA instead of AS resulted in healthier older cows, with limited benefit to cows initiating the second lactation.

Considering the distinct Ca profile between AS and SZA cows, the lack of difference in uterine diseases between treatments is intriguing. Martinez et al. (2012) demonstrated the interplay of postpartum Ca status, neutrophil function, and risk of uterine disease. In the study conducted by Martinez et al. (2012), however, cows were not supplemented with SZA. Therefore, it is possible that the mechanism by which SZA increases Ca may not influence uterine health, despite evidence that supplementation with SZA alters neutrophil gene expression and function (Crookenden et al., 2020). No treatment differences were detected in proportion of cows with mastitis or culling during the first 60 DIM.

Reproductive Performance, Milk Production, and Culling in the First 150 DIM

Pregnancy per AI and proportion of cows pregnant at first AI did not differ between treatments (Table 5). Primiparous cows were more likely to become pregnant at first AI than multiparous cows. The interaction between treatment and parity was associated ($P < 0.01$) with proportion of cows pregnant by 150 DIM. Primiparous AS cows were more ($P < 0.01$) likely to be pregnant by 150 DIM than SZA cows (84.1 vs. 74.0%), but no differences were detected ($P = 0.19$) between AS and SZA multiparous cows (51.1 vs 56.2%). Martinez et al. (2016) reported that first-lactation cows supplemented with oral Ca after calving had poorer reproductive performance than non-supplemented cows. On the other hand, the opposite was observed for multiparous cows.

Findings from the current study and Martinez el al. (2016) suggest that increasing periparturient Ca concentrations may not improve reproductive efficiency in cows that do not have low concentrations of Ca at calving. Cows supplemented with AS tended ($P = 0.07$) to be more likely removed from the herd by 150 DIM than SZA cows. In addition, proportion of cows deemed do-not-breed by 150 DIM tended ($P = 0.07$) to be greater for AS than SZA cows. Increased Ca concentrations and improved postpartum health of SZA cows likely resulted in positive carryover effects on survival in the herd. To our knowledge, this is the first report to compare reproductive performance of cows after prepartum supplementation with AS or SZA.

As expected, multiparous cows had greater ($P \leq 0.03$) yield of milk and ECM than primiparous cows in all tests evaluated in the study. Except for the test between 95 to 124 DIM, in which the interaction between treatment and parity tended ($P = 0.10$) to affect yield of ECM, no differences were detected in milk, its components, and ECM between AS and SZA cows (Table 6). The tendency for the interaction was observed because SZA primiparous cows tended ($P = 0.08$) to have greater ECM yield than AS primiparous cows. Results from the current study suggest that supplementation with either AS or SZA is expected to result in similar yield of milk and components up to 150 DIM. Compared with controls, Kerwin et al. (2019) reported no differences in milk production of early lactation cows supplemented with SZA. Prepartum diets not supplemented with SZA, but designed to lower DCAD, reduced incidence of milk fever and increased milk yield after calving (Lean et al., 2019; Santos et al., 2019). The effects of including of acidogenic salts in prepartum diets on milk production remains unclear. Further experiments are warranted to compare milk yield of cows supplemented with AS or SZA.

IMPLICATIONS AND APPLICATIONS

Prepartum supplementation with SZA increased plasma Ca concentrations at and 48 h after parturition, and decreased Mg and P on the day of calving compared with cows supplemented with AS. Urine pH in the prepartum period was decreased for AS cows compared with SZA cows. Prepartum multiparous cows supplemented with SZA had improved postpartum health compared with AS supplementation. Despite no differences in P/AI at first AI, primiparous cows supplemented with SZA were less likely to be pregnant by 150 DIM compared with AS cows. Overall, SZA tended to reduce culling during the first 150 DIM compared with AS cows. No significant differences were detected in milk production by mid-lactation. Unfortunately, the current study did not evaluate a control group. Nonetheless, the findings from this experiment demonstrate that prepartum supplementation with SZA can be an effective strategy to increase Ca status after calving without compromising performance after calving. It is important to acknowledge that several previous reports have evaluated the effects of including acidogenic salts or changing DCAD in prepartum diets, however, few experiments have explored the effects of SZA. Therefore, the current study is important because it provides evidence that another strategy can be utilized to alter Ca metabolism and decrease proportion of cows with cSCH. Further studies are necessary to elucidate how AS or SZA may alter periparturient immune response of dairy cows because of differences in blood mineral profiles. Altogether, these results suggest that altering concentrations of blood minerals to maintain adequate Ca concentrations after calving may have carryover effects up to 150 DIM.

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Table 1. Composition of TMR offered to prepartum cows based on treatments.

	Treatment[1]	
Item	**AS**	**SZA**
Ingredient, % of DM		
Corn silage	45.0	47.4
Wheat straw	16.1	19.9
Cottonseed (whole)	6.1	6.9
Soybean (hulls)	8.8	5.1
Canola meal	11.2	13.4
Mineral premix[2,3]	6.6	2.4
Bio-Chlor[4]	5.1	-
X-Zelit[5]	-	4.9
Unical L[6]	1.1	-
DCAD, mEq/kg of DM	−106	128
Chemical composition, %		
CP	14.75	14.96
NDF	33.21	34.98
Starch	19.75	20.30
Sugar	4.20	3.63
Ca	2.53	0.57
P	0.43	0.36
Mg	0.42	0.44
K	1.22	1.23
S	0.41	0.26
Na	0.13	0.10
Cl	0.83	0.29

[1]Treatment consisted of supplementing prepartum diets with anionic salts (AS) or synthetic zeolite A (SZA).

[2]Mineral premix for SZA consisted of crude fat (min 8%), crude fiber (max 3%), ADF (max 2.3%), Ca (max 13%, min 8%), P (min 0.3%), salt (max 10%, min 5%), Se (min 12.9 ppm), and vitamin A (min 151,023 IU/kg).

[3]Mineral premix for AS consisted of CP (Min 40%), crude fiber (max 9%), ADF (max 8%), Na (min 1.2%), Na (max 1.8%), S (min 2.3%), chloride (Min), and Mg (min 1%); Countryside Feed LLC (Hillsboro, KS).

[4]Degradable protein and palatable anions; Church & Dwight Co., Inc. (Ewing, NJ).

[5]Sodium aluminum silicate; Protekta Inc. (ON, Canada/Vilofoss, Graasten, Denmark).

[6]Calcium carbonate; ILC Resources (Urbandale, IA).

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Table 2. Descriptive data of cows enrolled in the experiment.

	Treatment[1]		
Item	AS	SZA	*P*-value
Primiparous, %	41.7	40.9	0.80
Days after calving at dry-off	319.0 ± 1.7	315.8 ± 1.7	0.17
Days of gestation at dry-off	214.8 ± 0.5	214.6 ± 0.5	0.40
Days of gestation at enrollment	255.6 ± 0.2	255.7 ± 0.2	0.57
Days spent in the close-up pens[1]	19.9 ± 0.25	19.8 ± 0.3	0.98
Dry period length, d	60.6 ± 0.6	60.9 ± 0.6	0.64
Gestation length, d	275.4 ± 0.2	275.5 ± 0.2	0.71
Twinning, %	2.5	2.4	0.89
Male calves, %	46.4	51.7	0.08
305ME milk[2], kg	10,218 ± 85	10,189 ± 86	0.81

[1]Treatment consisted of supplementing prepartum diets with anionic salts (AS) or synthetic zeolite A (SZA).[2]Projected 305-d mature equivalent milk yield at enrollment.

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Table 3. Incidence of postpartum chronic subclinical hypocalcemia (cSCH)[1] of Holstein-Jersey cows according to treatment and pregnancy status at 150 DIM.

	Treatment (T)[2]		Pregnancy status (PS)		*P*-value				
Item	AS	SZA	Pregnant	Non-pregnant	T	Parity	PS	T × P	T × PS
cSCH, %	60.0	17.9	22.2	68.2	<0.01	0.04	0.03	0.98	0.14

[1]Chronic subclinical hypocalcemia was defined as cows having Ca $\leq$ 8.6 mg/dL in all 3 samples (0, 48, and 72 h after calving).

[2]Treatment consisted of supplementing prepartum diets with anionic salts (AS) or synthetic zeolite A (SZA).

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Table 4. Incidence of health disorders and health treatments, and removal from the herd during the first 60 DIM of Holstein-Jersey cows supplemented with anionic salts (AS) or synthetic zeolite A (SZA) in the prepartum period.

	Treatment (T)[1]		*P*-value		
Item	AS	SZA	Treatment	Parity[2]	T × P
Health disorders and treatments					
Milk fever (recumbent), %	2.5	0.4	0.53	0.14	0.06
Milk fever (non-recumbent), %	1.4	1.5	0.97	0.05	0.98
Retained fetal membranes, %	2.7	1.7	0.26	0.93	0.80
Metritis, %	3.4	3.9	0.69	0.15	0.97
Mastitis, %	9.9	6.8	0.25	<0.01	0.35
Respiratory problems, %	3.4	5.5	0.09	0.29	0.80
Digestive problems, %	3.4	3.9	0.70	0.42	0.39
Undefined illness, %	16.2	16.2	0.69	<0.01	0.21
Antimicrobial treatment, %	10.8	11.7	0.61	0.47	0.48
Morbidity, %	32.9	31.7	0.88	<0.01	0.03
Removed from the herd by 60 DIM					
Sold, %	7.9	7.5	0.83	<0.01	0.98
Dead, %	4.3	2.8	0.16	0.01	0.42
Total removed, %	12.2	10.3	0.29	<0.01	0.58

[1]Treatment consisted of supplementing prepartum diets with anionic salts (AS) or synthetic zeolite A (SZA).[2]Parity = primiparous versus multiparous at study enrollment.

Table 5. Reproductive performance of cows and culling up to 150 DIM when fed anionic salts (AS) or synthetic zeolite A (SZA) in the prepartum period.

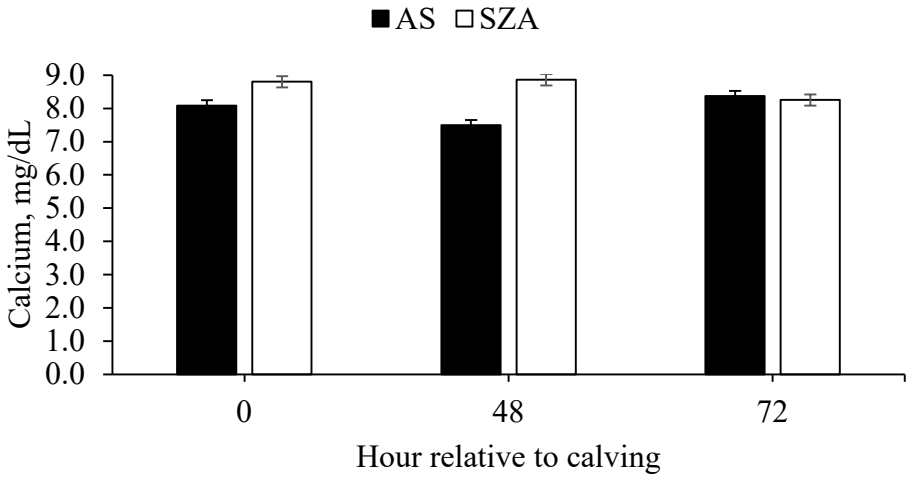
	Treatment (T)[1]		*P* – value		
Item	AS	SZA	T	Parity[2]	T × P
Reproductive outcomes					
Submitted to first service, %	79.2	82.6	0.13	<0.01	0.18
Pregnant at first service, %	45.6	42.8	0.36	<0.01	0.23
Pregnancy per AI at first service, %	58.3	52.7	0.13	<0.01	0.88
Pregnant at 150 DIM, %	64.8	63.5	0.15	<0.01	<0.01
Deemed do-not-breed by 150 DIM, %	7.7	8.1	0.07	<0.01	0.02
Removed from the herd by 150 DIM					
Sold, %	13.6	11.2	0.21	<0.01	0.45
Dead, %	5.0	3.5	0.20	<0.01	0.50
Total removed, %	18.7	14.7	0.07	<0.01	0.27

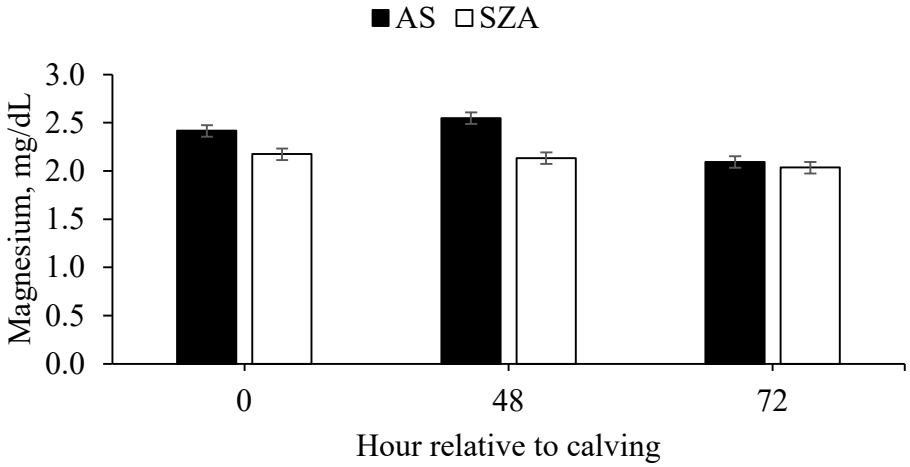
[1]Treatment consisted of supplementing prepartum diets with anionic salts (AS) or synthetic zeolite A (SZA).
[2]Parity = primiparous versus multiparous at study enrollment.

Table 6. Effects of supplementing prepartum diets with anionic salts (AS) or synthetic zeolite A (SZA) on lactation performance of Holstein-Jersey crossbred cows.

	Treatment (T)[1]		*P* – value		
Item[2]	AS	SZA	T	Parity[3]	T × P
Test between 35 and 64 DIM	(n = 137)	(n = 138)			
Fat, %	4.5 ± 0.08	4.5 ± 0.08	0.86	0.03	0.64
Protein, %	3.6 ± 0.04	3.5 ± 0.04	0.61	<0.01	0.69
Milk yield, kg/d	36.0 ± 0.69	37.2 ± 0.70	0.23	<0.01	0.81
ECM, kg/d	41.4 ± 0.62	42.4 ± 0.64	0.28	<0.01	0.82
Test between 65 and 94 DIM	(n = 141)	(n = 136)			
Fat, %	4.6 ± 0.08	4.5 ± 0.08	0.25	0.01	0.38
Protein, %	3.7 ± 0.03	3.6 ± 0.03	0.22	<0.01	0.29
Milk yield, kg/d	35.9 ± 0.62	35.5 ± 0.62	0.68	<0.01	0.90
ECM, kg/d	42.0 ± 0.63	41.1 ± 0.64	0.31	<0.01	0.85
Test between 95 and 124 DIM	(n = 243)	(n = 254)			
Fat, %	4.4 ± 0.06	4.4 ± 0.06	0.46	<0.01	0.48
Protein, %	3.7 ± 0.02	3.7 ± 0.02	0.26	<0.01	0.30
Milk yield, kg/d	34.7 ± 0.49	35.5 ± 0.48	0.22	<0.01	0.57
ECM, kg/d	40.1 ± 0.51	40.8 ± 0.51	0.35	<0.01	0.10
Test between 125 and 154 DIM	(n = 289)	(n = 307)			
Fat, %	4.4 ± 0.05	4.4 ± 0.05	0.58	<0.01	0.29
Protein, %	3.7 ± 0.02	3.7 ± 0.02	0.37	<0.01	0.05
Milk yield, kg/d	32.6 ± 0.43	32.8 ± 0.42	0.71	<0.01	0.13
ECM, kg/d	37.5 ± 0.45	37.9 ± 0.43	0.47	<0.01	0.40

[1]Treatment consisted of supplementing prepartum diets with anionic salts (AS) or synthetic zeolite A (SZA).
[2]Four milk tests were conducted during the study period. Not all cows were tested. Each test consisted of a different subpopulation of cows.
[3]Parity = primiparous versus multiparous at study enrollment.


AS SZA
Calcium, mg/dL
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
48
72
Hour relative to calving


AS SZA
Magnesium, mg/dL
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
48
72
Hour relative to calving

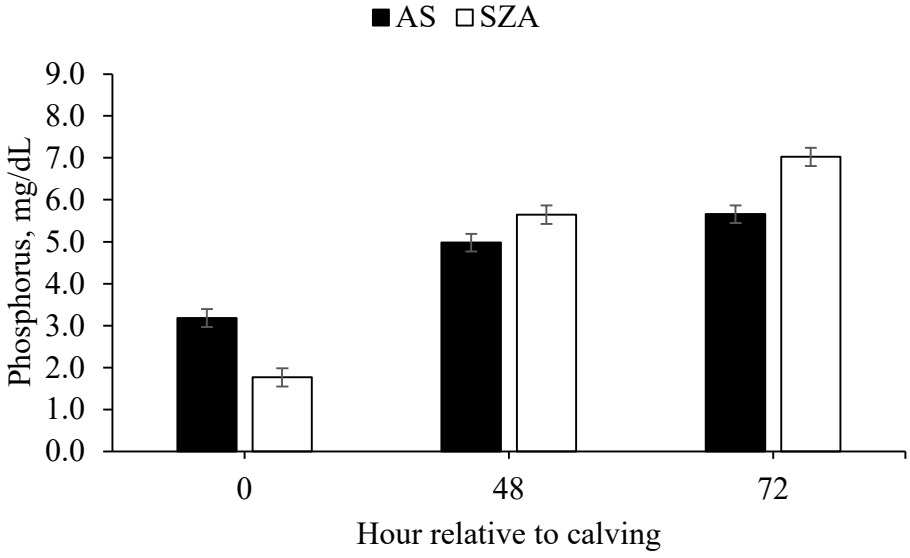

AS SZA
Phosphorus, mg/dL
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
48
72
Hour relative to calving

Figure 1. Blood mineral profile at 0, 48, and 72 h after calving of lactating dairy cows supplemented with anionic salts (AS) or synthetic zeolite A (SZA) during the last 3 wk of gestation. (upper panel) Calcium concentration: treatment ($P < 0.01$); time ($P = 0.14$); and, treatment by time ($P < 0.01$). (middle panel) Magnesium concentration: treatment ($P < 0.01$); time ($P < 0.01$); and, treatment by time ($P < 0.01$). (lower panel) Phosphorus concentration: treatment ($P = 0.31$); time ($P < 0.01$); and, treatment by time ($P < 0.01$).