Effects of Feeding Synthetic Zeolite A and Negative Dietary Cation-Anion Difference Diets Prepartum on Mineral Metabolism of Multiparous Holstein Cows

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Abstract

The periparturient period is characterized by increased demand for calcium in dairy cows. For this reason, different nutritional strategies have been adopted for use to prevent hypocalcemia. A common nutritional strategy is feeding a negative dietary cation-anion difference **(DCAD)** prepartum. Recent research has focused on using other prepartum dietary strategies to improve postpartum blood calcium concentrations. The objective of this study was to determine the effects of feeding synthetic zeolite A, a negative DCAD diet, or a control diet during the close-up period on peripartal mineral metabolism. Multiparous Holstein cows (n = 121) were enrolled 3 weeks prior to expected date of parturition and randomly assigned to 1 of 3 different prepartum diets with low potassium corn silage: control (CON; +190.24 mEq/kg; n = 40), negative DCAD (DCAD; -64.71 mEq/kg; n = 41), or a diet supplemented with sodium aluminum silicate (**XZ**; +277.40 mEq/kg with 500 g/day X-Zelit, Protekta Inc., Lucknow, Ontario, CA/Vilofoss, Fredericia, DK; n = 40). Cows were fed a common postpartum diet. Blood, urine, and saliva samples were collected prior to the start of treatments, daily beginning 14 days before parturition (D-14) until parturition (D0), and on days 1, 2, 3, 6, 9, 12, 15, 18, 21, 35, and 49 postpartum. Cows fed XZ had increased total calcium concentrations **(tCa)** compared to CON prepartum ($P < 0.05$). On D0, cows fed XZ had the highest tCa concentrations, and cows fed DCAD had increased tCa concentrations compared to CON ($P < 0.01$). These differences were maintained on D1 ($p < 0.01$). On D2, cows fed XZ continued to exhibit the highest tCa concentrations, but no differences occurred between cows fed with DCAD and CON. Phosphorus **(P)** concentrations were decreased in XZ cows compared to CON and DCAD cows during the prepartum period ($P < 0.05$), and this difference was maintained through D1 postpartum. On D6, D9, and D12, P concentrations were increased in cows fed XZ compared to DCAD and CON diets ($P < 0.05$). On D0, cows fed with CON had the highest magnesium concentration ($P < 0.05$).

Introduction

The periparturient period is between 3 weeks before to 3 weeks after parturition and is characterized by a high demand for calcium **(Ca)**. Previous research indicates the presence of a high incidence of clinical (**CH**; 5%) and subclinical (**SCH**; 25% in primiparous and 50% in multiparous cows) hypocalcemia in the US (Reinhardt et al., 2011). Clinical ([tCa] < 1.4 mmol/L) and subclinical ([tCa] ≤ 2.0 mmol/L) hypocalcemia are associated with the occurrence of numerous diseases during lactation, including ketosis, displaced abomasum, metritis, and mastitis, which lead to a decrease in milk production, low pregnancy rates, and higher culling risk (DeGaris and Lean, 2008).

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The regulation of blood Ca concentrations is a tightly regulated process that is influenced by several hormones. Typically, as Ca concentrations decrease, parathyroid hormone **(PTH)** is released from the parathyroid gland into the bloodstream (DeGaris and Lean, 2008). Parathyroid hormone increases Ca mobilization from bones as well as regulates the hydroxylation of 25-hydroxycholecalciferol to 1.25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), leading to increased intestinal absorption of Ca and increased renal tubular reabsorption of Ca (DeGaris and Lean, 2008).

In addition to hormonal regulation of Ca metabolism, different minerals have been demonstrated to influence Ca concentrations. Circulating Mg concentrations are maintained between 0.75 to 1 mmol/L, but when its concentration is below the normal range, the risk of developing hypocalcemia increases because of a reduction in PTH secretion in response to low Ca concentrations. Additionally, tissue sensitivity to PTH has been demonstrated to be reduced (Goff, 2008). Previous research has also demonstrated that feeding prepartum diets high in P concentrations increases the incidence of hypocalcemia (Lean et al., 2006). Like Ca, P is a critical structural component of bone tissue, and is found in numerous other tissues in the body (Goldsweig and Carpenter, 2015). In a recent study, researchers demonstrated that feeding restricted dietary P during the last 4 weeks of gestation enhanced Ca metabolism in early lactation due to increased bone mobilization (Wächter et al., 2022). Phosphorous metabolism is regulated by fibroblast growth factor 23 **(FGF23)**, produced by the osteoblasts/ osteocytes in the bone, in conjunction with PTH regulates phosphate reabsorption by the kidneys (Goldsweig and Carpenter, 2015). In fact, dietary P is a key regulator of FGF23 section. When dietary P is decreased, serum FGF23 is decreased and this has been proposed to be independent of $1,25(OH)_2D_3$ (Yu et al., 2005). These researchers demonstrated that diets high in Ca and P normalize serum P concentrations in mice that lack the vitamin D receptor.

Numerous studies have demonstrated that feeding diets with a low dietary cation-anion difference **(DCAD)** 21 days prior to the expected due date improved postpartum Ca homeostasis. Negative DCAD diets work by inducing metabolic acidosis, allowing bone to accept hydrogen ions which allow calcium to be liberated from the bone and into the circulation (Charbonneau et al., 2006). Further, the metabolic acidosis generated by DCAD increases the sensitivity of the bone tissue to PTH (Goff et al., 1991). Another approach to increasing peripartum blood Ca concentrations is the use of synthetic zeolite in prepartum diets. Zeolite A is a sodium aluminum silicate molecule that binds dietary Ca, but if the concentration of "free" Ca is low, it can bind to other cations, such as Mg, and P, decreasing the absorption of these minerals by the gut and leading to the activation of calcium homeostatic mechanisms (Thilsing et al., 2006). In fact, recent research demonstrated that prepartum cows fed with zeolite A had approximately 0.4 mmol/L higher blood calcium concentrations compared to the control-fed cows on the first day of lactation (Kerwin et al., 2019). Additionally, P concentrations were decreased during the feeding period through 2 days of lactation in this study. Previous in vivo and in vitro experiments using synthetic zeolite A have also demonstrated large reductions in P concentrations (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Grabherr et al., 2009; Kerwin et al., 2019; Crookenden et al., 2020). However, previous work has yet to demonstrate where in the cow the P is going.

Early and recent data studies demonstrate the benefits of feeding negative DCAD diets prepartum on Ca homeostasis postpartum

(Block, 1984; Goff et al., 1991; Goff et al., 1984; Leno et al., 2017; Martinez et al., 2018; Rodney et al., 2018; Lean et al., 2019; Santos et al., 2019). Additionally, several studies have demonstrated the benefit of feeding synthetic zeolite A supplementation prepartum on calcium homeostasis (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Pallesen et al., 2008; Grabherr et al., 2009; Kerwin et al., 2019). However, there are no studies that directly compare these two prepartum strategies. Therefore, the objectives of this study were to determine the effects of a negative DCAD diet and supplementation with synthetic zeolite A during the close-up period to multiparous Holstein cows on serum, saliva, urine, and fecal mineral status, plasma PTH concentrations, and whole blood serotonin **(5-HT)** concentrations. We hypothesized that feeding cows with a negative DCAD diet or zeolite 24 days prior to the expected calving date would result in improved serum calcium concentrations after parturition. Additionally, we aimed to determine where the P was going in the cows receiving the synthetic zeolite A prepartum.

Materials and Methods

All experimental procedures were approved by the College of Agriculture and Life Sciences Animal Care at the University of Wisconsin – Madison under protocol number A006169-01, and strictly followed. Multiparous Holstein cows between their second to sixth lactation (3.24 ± 0.10 average lactation parity) at University of Wisconsin-Madison Emmons Blaine Dairy Research Center (UW; Arlington, WI) from November 2019 to April 2020 were utilized for this study.

Experimental design, animals, and dietary treatments

A hundred and twenty-eight cows arranged in a randomized complete block design and balanced by parity were enrolled in the experiment, of which 121 remained in the final data set. Four animals were excluded because they received supplemental calcium postpartum, 2 cows received their respective dietary treatment for less than 14 days, and 1 cow remained on their respective dietary treatment for more than 35 days. Twenty-four days prior to the expected due date, cows were moved from the dry cow barn at Blaine Dairy to the close-up pen that is bedded with straw, and provided with ad libitum water. No more than 30 animals were allocated at the same time to the close-up pen. Cows were then blocked by expected due date and parity number and randomly assigned to 1 of 3 different total mixed rations **(TMR)** diets with low potassium corn silage: control (**CON**; +190.24 mEq/kg; n = 40), negative DCAD (**DCAD**, -64.95 mEq/kg;n = 41; Ultra Chlor; Vita Plus, Lake Mills, WI, USA), and a diet containing sodium aluminum silicate zeolite (**XZ**; +278.41 mEq/kg, fed at 3.3% DM, targeting 500 g/day as fed; n = 40; X-Zelit, Protekta Inc., Lucknow, ON, Canada/Vilofoss, Graasten, Denmark).

Total mixed rations were formulated to meet the nutrient requirements of prepartum Holstein cows and provide 13.5 kg of DMI, considering 5% of refusals using NRC 2001 models (Tables 1 and 2). During the close-up period, cows were housed in a single freestall pen with 15 Insentec Roughage Intake Control **(RIC)** gates (RIC System, Holofarm group, Netherlands) and fed once a day. The 15 RIC gates in the pen were randomly assigned to receive 1 of the 3 different diets, resulting in 5 RIC gates for each treatment so that a group of 10 cows had access to a group of 5 RIC gates that

all contain the same diet (Weld and Armentano, 2018). Cows each had an electronic ear tag (FDX EID TAG, Allflex, USA) that allowed them to have access to the specific feeders of the diet to which they were assigned. Weekly TMR was collected and sent to Dairyland laboratories, Inc. (Arcadia, WI) for wet chemistry analysis.

Blood, saliva, and urine sampling

Blood, saliva, and urine samples were collected daily before feeding from enrollment until calving (D0), and subsequently on days 1, 2, 3, 6, 9, 12, 15, 18, 21, 35, and 49 postpartum. Blood samples were collected from the coccygeal vein using 20-gauge Vacutainer needles (Greiner Bio-One GmbH, Kremsmuenster, Austria; Exelint International, Co., Redondo Beach, CA). Whole blood was collected into 10-mL BD Vacutainer K2 EDTA Plus (Becton, Dickinson, and Company), 10-mL BD Vacutainer Serum (Becton, Dickinson, and Company), and 10-mL Lithium Heparin 158 USP Units (Becton, Dickinson, and Company) blood collection tubes and inverted gently. Immediately after inversion, 3 to 4 mL of whole blood was transferred from the 10-mL Vacutainer K2 EDTA Plus tube to a 5 mL Eppendorf preloaded with approximately 35 mg of ascorbic acid (10 mg/mL) to prevent oxidation of serotonin, and frozen at -20°C until further analysis (Connelly et al., 2021). Blood samples collected into serum tubes were kept at room temperature and allowed to clot prior to centrifugation. Serum and plasma samples were harvested after centrifugation at 3,000 x *g* for 20 min at 4°C. Samples were allocated into triplicate aliquots and stored at -80°C until further analysis.

Saliva samples were collected using Salivette tubes (Sarstedt, Aktiengesellschaft & Co, Nümbrecht, Germany). Each tube contained a cotton swab clipped with a surgical clamp, and cows were allowed to chew it (Riek et al., 2019). Then, the cotton swab was placed back into the tube and centrifuged at 1,000 x g for 10 min at 4°C. Saliva samples (approximately 1 to 2 mL per cotton swab) were stored at -20°C until further analysis. Prior to analyzing saliva, samples were scored from 1 to 4 according to their clarity to classify samples that contained only saliva or ruminal fluid, allowing us to control for this in our statistical analysis model (Figure 1). Acetic acid (0.5M) was then added to samples using a 1:2 dilution and centrifuged at 13,000 g for 12 min at 4°C and the supernatant was separated and stored at -20°C for further analysis.

Urine samples were collected into 5 mL Eppendorf by gently stimulating the area between the udder and the vulva. Urine pH was measured using an electronic pH meter (Horiba LaquaTwin Compact pH Meter, Chicago, IL, USA) to verify DCAD dietary adjustments. The urine pH average during the prepartum period was 8.42 ± 0.03, 6.22 ± 0.03, and 8.40 ± 0.03, for CON, DCAD, and XZ, respectively. During the postpartum period, urine pH's were 8.35 ± 0.02, 8.26 ± 0.02, and 8.36 ± 0.02 for CON, DCAD, and XZ, respectively. After measuring pH, urine samples were frozen at -20°C until further analysis. Acetic acid (0.5M) was added to samples in a 1:2 dilution and centrifuged at 13,000 x *g* for 12 min at 4°C and the supernatant was separated and used for mineral analysis.

Digestibility of minerals

Fecal samples were collected over 3 days at 8-hour intervals covering a 4 hour clock period (Cook et al., 2016) to make a composite sample per cow in a subset of 24 cows enrolled in the study (n=8 per treatment). Samples were obtained rectally with clean palpation sleeves and placed in a plastic container, one per cow. After collection, samples were frozen at -20°C until further analysis. The TMR and orts samples

were collected on the same week as the feces collection. Samples were thawed and dried in a forced-air oven at 60°C for 72 hours, ground to pass through a 1-mm Wiley screen and submitted for iNDF and mineral analysis by wet chemistry (Dairyland laboratories Inc., Arcadia, WI). Additionally, dried and ground fecal samples were also analyzed for water extractable P concentrations (University of Wisconsin-Madison, Soil and Forage Laboratory, Madison WI).

Laboratory analysis

Serum, saliva, and urine tCa concentrations were determined using a colorimetric Ca assay (700550, Cayman Chemical) per the manufacturer's instructions as previously described (Laporta et al., 2015). Serum tCa intra- and interassay coefficients of variation **(CV)** were 1.91% and 8.51%, respectively. Saliva tCa intra- and interassay CV were 2.48% and 10.06%, respectively. Urine tCa intra- and interassay CV were 2.47% and 6.88%, respectively. Urine Ca concentrations were corrected to creatinine concentration to correct urine volume. Creatinine concentrations were measured using coloria metric assay (DICT-500, QuantiChrom Creatinine Assay Kit, BioAssay Systems), as described previously (Slater et al., 2018) with intra- and interassay CV of 3.48% and 16.83%, respectively.

Inorganic serum, saliva, and urine P concentrations were determined with ammonium molybdate (C274-01, Catachem Inc., Oxford, Connecticut) using a Chemwell analyzer (Awareness, Palm City, FL, USA). Mili-Q water was added into diluted saliva samples prior to analysis, resulting in a final 1:40 dilution. Serum, saliva and urine Mg concentrations were determined using xylidyl blue (C355-01, Catachem Inc., Oxford, Connecticut) using a Chemwell analyzer (Awareness, Palm City, FL, USA).

Statistical analysis

Prepartum and postpartum data were analyzed separately. Prepartum data was restricted to 14 d before calving until D0. A baseline measurement was determined using the first sample collected from each cow before the start of treatment and was used as a covariate for the respective analyses. Data were analyzed by lactation number, with lactation number 2 and lactation number 3 or higher analyzed separately. All statistical analyses were conducted using the MIXED procedure of SAS (version 9.4 SAS Institute Inc.). Fixed terms in the model for blood and urine variables were treatment, day, lactation, covariate, the interaction of treatment X day, and interaction of treatment X lactation. For saliva variables, the same variables were used as fixed terms with the addition of salivary grade. Two separate analyses were used to fit the proper covariance structure per sampling day. The day was considered the repeated measure in both analyses. The first analysis included the prepartum period with daily sampling, and to account for autocorrelated errors, the ar(1) structure was utilized. Due to different sampling timeframes during the postpartum period, the spatial power structure was utilized as a covariance structure. The random statement in all models included cow. Data were analyzed for normality, and when the assumption failed, data were transformed. Transformations were based on diagnostics plots and overall model fit. If transformations were necessary, analysis was performed and the p-value is shown, but the values in graphs or tables are shown from untransformed data. Statistical significance was declared if $P \leq 0.05$, with tendencies for differences declared at $0.05 < P \leq 0.1$.

Results

Blood, saliva, and urinary mineral concentrations

Serum total Ca concentrations for cows fed the three prepartum diets are presented in Figure 2A. Cows fed the XZ diet had the highest serum Ca concentrations during the prepartum period of the three diets. On D0, D1 and D2 cows fed the XZ diet (2.20 ± 0.03 mmol/L, 2.18 ± 0.03 mmol/L, and 2.27 ± 0.03 mmol/L, respectively) continued to maintain the highest Ca concentrations compared to DCAD (1.94 ± 0.03 mmol/L, 1.98 ± 0.03 mmol/L, and 2.17 ± 0.03 mmol/L, respectively) and CON diets (1.82 ± 0.03 mmol/L, 1.86 ± 0.03 mmol/L, and 2.08 ± 0.03 mmol/L, respectively). After D3, there were no differences in serum Ca concentrations between treatments, except on D9 when cows fed the CON diet had the highest serum Ca concentrations (2.47 ± 0.03 mmol/L) and cows fed the XZ diet had the lowest serum Ca concentrations (2.38 ± 0.03 mmol/L), while cows fed the DCAD diet did not differ from either treatment (2.39 ± 0.03 mmol/L). Cows in their second lactation had higher serum Ca concentrations compared to cows in their third or higher lactation in both the prepartum (2.44 ± 0.01 mmol/L and 2.40 ± 0.01 mmol/L, respectively) and postpartum (2.40 ± 0.01 mmol/L and 2.35 ± 0.01 mmol/L, respectively) periods.

Salivary Ca concentrations are presented in Figure 1B. Animals fed the XZ diet had the highest salivary Ca concentrations compared to DCAD and CON during the prepartum period (1.70 ± 0.04 mmol/L, 1.41 ± 0.04 mmol/L, and 1.43 ± 0.04 mmol/L, respectively). There was a prepartum treatment by lactation effect for cows fed the XZ diet having the highest salivary Ca concentrations even in their second or third or greater lactations (6.92 ± 0.25 mmol/L and 6.65 ± 0.18 mmol/L, respectively). Cows fed the DCAD diet in their second lactation had the lowest salivary Ca concentrations (5.26 ± 0.24 mmol/L). A prepartum and postpartum effect of salivary Ca grade was observed, with the highest concentrations being detected in grade 4 (2.18 ± 0.05 mmol/L and 3.82 ± 0.10 mmol/L, respectively), followed by grade 3 (1.89 ± 0.06 mmol/L and 2.81 ± 0.07 mmol/L, respectively), grade 2 (1.09 ± 0.04 mmol/L and 1.36 ± 0.05 mmol/L, respectively), and grade 1 (0.88 ± 0.03 mmol/L and 0.85 ± 0.06 mmol/L, respectively).

Serum Mg concentrations are presented in Figure 3A. Cows fed the XZ diet had the lowest serum Mg concentrations during the prepartum period ($P < 0.01$; 1.09 ± 0.02 mmol/L, 1.10 ± 0.02 mmol/L, and 1.00 ± 0.02 mmol/L for CON, DCAD, and XZ diets, respectively). On D0, cows fed XZ had the lowest serum Mg concentrations (1.06 ± 0.03 mmol/L) of all treatment groups, cows fed the CON diet had the highest (1.32 ± 0.03 mmol/L), and DCAD fed cows exhibited intermediate serum Mg concentrations (1.18 ± 0.03 mmol/L). On D1, cows fed the XZ diet had the lowest serum Mg concentrations (1.10 ± 0.04 mmol/L), with no differences observed between CON and DCAD (1.25 ± 0.04 mmol/L and 1.18 ± 0.04 mmol/L, respectively) or between XZ and DCAD. Beginning on D6, cows fed XZ had the highest serum Mg concentrations, and this pattern continued until D49.

Salivary Mg concentrations are presented in Figure 3B. Cows fed the XZ diet had higher salivary Mg concentrations compared to DCAD and CON during the prepartum (3.17 ± 0.07 mmol/L, 2.66 ± 0.07 mmol/L, and 2.57 ± 0.07 mmol/L, respectively). There was an effect of salivary grade on Mg concentrations in both the prepartum and postpartum periods, with the highest Mg concentrations detected in grade 4 (5.11 ± 0.08 mmol/L and 6.92 ± 0.24

mmol/L, respectively), followed by grade 3 (3.97 ± 0.10 mmol/L and 5.74 ± 0.16 mmol/L, respectively), grade 2 (1.58 ± 0.07 mmol/L and 2.45 ± 0.12 mmol/L, respectively), and the lowest concentration being detected in grade 1 samples (0.53 ± 0.05 mmol/L and 0.72 ± 0.16 mmol/L, respetively).

Serum P concentrations are presented in Figure 4A. Cows fed XZ had the lowest serum P concentrations during the entire prepartum period ($P < 0.01$; 1.67 ± 0.03 mmol/L, 1.67± 0.02 mmol/L, and 0.95 ± 0.03 mmol/L for CON, DCAD, and XZ diets, respectively). On D0, cows fed XZ had the lowest serum P concentrations (0.66 ± 0.05 mmol/L), and no differences were detected between DCAD and CON diets (1.11 ± 0.04 mmol/L and 1.02 ± 0.05 mmol/L, respectively). Beginning on D6, cows fed XZ had the highest serum P concentrations, and this pattern continued until D21.

Salivary P concentrations according to treatment and day are presented in Figure 4B. Cows fed the XZ diet had the lowest salivary P concentrations during the prepartum period ($P < 0.01$; 6.22 ± 0.18 mmol/L), and saliva from cows fed the CON diet had decreased concentrations compared to the DCAD cows (8.52 ± 0.21 mmol/L and 10.02 ± 0.18 mmol/L, respectively). During the postpartum period, cows fed the CON diet had the lowest salivary P concentrations ($P < 0.01$; 9.09 ± 0.21 mmol/L), but no differences were detected between DCAD and XZ diets (9.90 ± 0.19 mmol/L and 9.90 ± 0.18 mmol/L, respectively). There was an effect of salivary grade during the prepartum period, with the highest concentrations observed in grade 4 (8.68 ± 0.18 mmol/L), followed by grade 2 (8.36 ± 0.16 mmol/L) which did not differ from grade 4 and 1 (8.17 ± 0.12 mmol/L), and with the lowest concentrations detected in grade 3 (7.80 ± 0.22 mmol/L), which did not differ from grade 1.There was an effect of salivary grade during the postpartum period, with the highest concentrations in grade 4 (10.84 ± 0.30 mmol/L), followed by grade 3 (9.96 ± 0.20 mmol/L), and grades 2 and 1 which did not differ (8.96 ± 0.16 mmol/L and 8.76 ± 0.18 mmol/L, respectively).

Prepartum mineral digestibility is provided in Table 3. There were no differences among treatments for Ca, P, Mg, K, and S digestibilities. Urine P was higher for DCAD versus CON and X2 during the prepartum period but no differences occurred during the postpartum period (Figure 5).

Blood PTH concentrations

Plasma PTH concentrations according to pre and postpartum are presented in Table 4. There was no treatment effect on plasma PTH during the prepartum ($P = 0.59$) or the postpartum ($P = 0.37$) periods. However, there was an effect of the day during the prepartum period ($P < 0.01$), with the highest PTH concentrations occurring on D0 (183.15 ± 6.45 pg/mL).

Discussion

The main objective of this study was to determine the effects of a negative DCAD diet and supplementation with synthetic zeolite A during the close-up period on serum, saliva, urine, and fecal mineral concentrations, and plasma PTH concentrations in multiparous Holstein cows. Previous studies had shown similar results for Ca concentrations when cows were supplemented with synthetic zeolite A or received a negative DCAD diet during the close-up period, however, there are no studies comparing these two different approaches (Block, 1984; Goff et al., 1991; Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Martinez et al., 2018; Kerwin et al., 2019; Santos et al., 2019). Further, several studies using synthetic zeolite

A supplementation have demonstrated decreases in circulating P concentrations, but there is a gap in knowledge about where the ingested P was going in the body (Thilsing-Hansen et al., 2002; Pallensen et al., 2008; Grabherr et al., 2009; Kerwin et al., 2019).

On average, cows receiving prepartum XZ had higher serum Ca concentrations than cows fed the DCAD and CON diets. Previous studies demonstrate similar findings for serum Ca concentrations when feeding synthetic zeolite A during the close-up period (Thilsing-Hansen and Jørgensen, 2001; Thilsing-Hansen et al., 2002; Pallesen et al., 2008; Grabherr et al., 2009; Kerwin et al., 2019). However, these experiments did not compare to the feeding of negative DCAD diets. As observed previously, our study demonstrated that cows fed negative DCAD diets also had improved postpartum Ca concentrations compared to CON (Leno et al., 2017; Lean et al., 2019; Santos et al., 2019).

On average, serum P concentrations were 52% lower in the XZ group, compared to DCAD and CON during the prepartum period. Similar results were observed when cows were supplemented with synthetic zeolite A prepartum in previous experiments (Thilsing-Hansen et al., 2002; Pallesen et al., 2008; Grabherr et al., 2009; Kerwin et al., 2019). This is attributed to the reduced P availability for the cow when the diet is supplemented with zeolite A (Thilsing et al., 2007; Kerwin et al., 2019). Zeolites are commonly used in ion exchange reactions and are well known for their ability to take up cations. For example, in an in vitro study, zeolite was demonstrated to have extensive binding to P, in addition to the expected binding to Ca (Iglezakis, 2004; Thilsing et al., 2006). This study suggested that induced hypophosphatemia can influence Ca homeostasis due to the increased efficiency of intestinal receptors for $1,25(OH)_2D$ decreasing the incidence of hypocalcemia (Thilsing et al., 2006). Elevated P concentrations (> 6.19 mg/dL) have been shown to inhibit the conversion of 25-hydroxyvitamin D to $1,25(OH)_2D$; therefore, the cow would not be able to produce the hormone necessary for the activation of intestinal Ca transport (Goff, 2006).

The decrease in plasma P concentrations after zeolite supplementation has been previously demonstrated, but there is a lack of knowledge surrounding the metabolism of P during supplementation (Thilsing et al., 2006, 2007; Kerwin et al., 2019). The aluminum present in the zeolite molecule is released in a low pH environment and binds to P, forming a non-absorbable aluminum phosphate complex in the small intestine (Cook et al., 1982; Thilsing et al., 2007). Decreased dietary P absorption is reflected by the lower plasma P concentrations, likely because the main site of P absorption is in the proximal small intestine (Thilsing et al., 2007). Further, Thilsing-Hansen and colleagues (2002) suggested that the lower plasma P concentrations after zeolite supplementation was due to an increase in circulating PTH and an increase in salivary and renal phosphate excretion. However, contrary to Thilsing-Hansen et al., (2002), we observed that the XZ group had the lowest salivary P concentrations during the prepartum period, similar to plasma P concentrations. Additionally, salivary P concentrations increased postpartum in a similar pattern to the changes in plasma P concentrations, after cessation of XZ supplementation. This suggests that P was being excreted elsewhere. Upon analysis of fecal samples for water extractable P, we determined that P concentrations were increased in the feces from cows on the XZ diets compared to the CON and DCAD diets.

Similar to previous studies, plasma Mg concentrations were lower in the XZ group compared to DCAD and CON during the

prepartum until D1 (Thilsing-Hansen et al., 2002; Grabherr et al., 2009; Kerwin et al., 2019). However, in our study, Mg concentrations were within the normal range for dairy cows in all treatments (1.82-2.43 mM) (Goff, 1999). An *In vitro* study demonstrated that zeolite has a high affinity to bind to Ca, but when the "free" Ca from the solution decreases, zeolite will bind to other 2+ ions, such as Mg (Thilsing et al., 2006). Control of plasma Mg levels is not as refined as for Ca, and Mg control is a result of a balance between ruminal and intestinal absorption of Mg and renal excretion of Mg (Thilsing-Hansen et al., 2002). Hypomagnesemia can affect Ca homeostasis in two ways: reducing the PTH secretion in response to hypocalcemia, and reducing the tissue sensitivity to PTH (Goff, 2006).

The main hormone responsible for the mobilization of Ca from bones during a decrease in Ca levels in the blood is PTH. Parathyroid hormone also controls the renal tubular resorption of Ca (Goff, 2006). As PTH concentrations increase, the secretion of $1,25(OH)_2D$ from the kidneys increases as well, and $1,25(OH)_2$ D is responsible for the stimulation of absorption of dietary Ca in the intestine (Goff, 2006). Therefore in this study, we expected that PTH concentrations would be increased for XZ and DCAD groups due to the increase in Ca concentrations in early lactation. However, there were no differences in PTH detected (Table 4). Thilsing-Hansen and colleagues (2002) detected an increased $1,25(OH)_2D$ around calving for cows supplemented with zeolite A, which indicates an increase in PTH secretion. While we did not measure $1,25(OH)_2D$, our data are not consistent with the previous experiments where PTH concentrations were increased. It is possible that an alternative pathway is being stimulated.

Conclusion

In conclusion, multiparous Holstein cows fed a negative DCAD diet or supplemented with synthetic zeolite A during the close-up period resulted in improved Ca concentrations in early lactation compared to the CON diet. However, cows fed with the XZ diet had a lower P concentration in circulation and in the saliva, proving that the synthetic zeolite A molecule has a higher affinity for P. Further studies are necessary to better understand to where the P is being driven.

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Table 1. Dietary ingredient composition of experimental prepartum diets fed to Holstein multiparous cows 24 d prior to expected calving date.

Ingredient	Diet[1] (kg/cow/day)		
	CON	DCAD	XZ
Corn silage	7.37	7.37	7.37
Haylage	1.81	1.81	1.81
Wheat straw	2.04	2.04	2.04
Soybean meal, 48% CP	1.16	0.91	1.07
Oat hulls	0.59	0.39	0.14
Urea 46%	0.07	0.00	0.07
Blood meal	0.04	0.10	0.09
Ultra-Chlor[2]	-	0.50	-
X-Zelit[3]	-	-	0.49
RUP and mineral mix[4]	13.37	13.37	13.37

[1]Prepartum cows starting at 248 d of gestation were fed diets.

[2]Dried condensed, extracted glutamic acid fermentation product; dried condensed corn, fermentation solubles; processed grain by-products; magnesium chloride. CP: 42.31%, Cl: 7.9%, S: 3.13%,DCAD: -337.87 meq/100g.

[3]80% Synthetic aluminum silicate,20% wheat starch.

[4]Calculated based on the chemical analysis of dietary ingredients.

Table 2. Analyzed nutrient composition (mean ± SD; % of DM unless otherwise noted) for experimental prepartum diets fed to Holstein multiparous cows 24 d prior to the expected calving date.

Nutrient[1]	Treatment			*P*-value
	CON	DCAD	XZ	
DM, %	42.29 ± 0.45	41.99 ± 0.45	42.03 ± 0.45	0.87
CP	14.22 ± 0.12[b]	15.23 ± 0.12[a]	14.42 ± 0.12[b]	< 0.01
ADF	26.01 ± 0.20[ab]	25.57 ± 0.20[b]	26.28 ± 0.20[a]	0.05
NDF	38.48 ± 0.31	38.26 ± 0.31	38.32 ± 0.31	0.88
Lignin	10.25 ± 0.12[ab]	9.94 ± 0.12b	10.49 ± 0.12[a]	0.01
Starch	23.18 ± 0.31	23.54 ± 0.31	22.49 ± 0.31	0.06
Sugar	2.03 ± 0.03[b]	2.13 ± 0.03[a]	2.05 ± 0.03[b]	0.04
NFC[2]	39.49 ± 0.28[a]	38.12 ± 0.28[b]	38.92 ± 0.28[a]	< 0.01
Fat	3.33 ± 0.04[a]	2.65 ± 0.04[b]	3.34 ± 0.04[a]	< 0.01
Ash	8.13 ± 0.10[c]	9.36 ± 0.10[a]	8.83 ± 0.10[b]	< 0.01
Ca	0.78 ± 0.03	0.78 ± 0.03	0.79 ± 0.03	0.97
P	0.36 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.70
Mg	0.40 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.08
K	1.48 ± 0.02	1.45 ± 0.02	1.47 ± 0.02	0.70
S	0.24 ± 0.01[b]	0.29 ± 0.01[a]	0.26 ± 0.01[b]	< 0.01
Na	0.13 ± 0.01[b]	0.12 ± 0.01[b]	0.39 ± 0.01[a]	< 0.01
Cl	0.32 ± 0.01[b]	1.09 ± 0.01[a]	0.35 ± 0.01[b]	< 0.01
DCAD, mEq/kg of DM	190.24 ± 9.91[b]	-63.40 ± 9.91[c]	281.59 ± 9.91[a]	< 0.01

[1]Samples collected weekly for chemical analyses.
[2]Calculated using the equation DM – [CP + NDF + fat + ash – (NDF insoluble protein)].
[a,b,c]Treatment effects when treatment means differed significantly ($P \leq 0.05$).

Table 3. Least squares means and SE for prepartum minerals digestibility %.

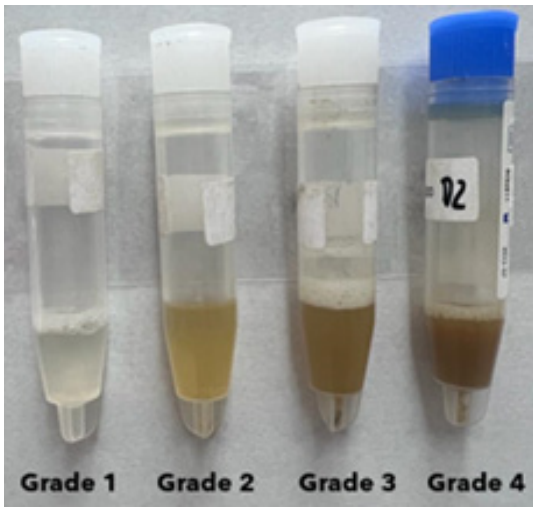
Mineral digestibility	Treatment[1]			*P*-value[2]			
	CON	DCAD	XZ	T	D	L[3]	T X L
Ca	-8.67 ± 11.82	2.28 ± 12.45	-0.82 ± 13.85	0.85	0.90	0.52	0.49
P	21.82 ± 4.59	21.34 ± 4.83	10.43 ± 5.38	0.22	0.63	0.87	0.19
Mg	-5.32 ± 6.75	-3.88 ± 7.11	-9.65 ± 7.91	0.84	0.98	0.78	0.79
K	87.85 ± 1.22	86.15 ± 1.28	87.82 ± 1.43	0.61	0.41	0.26	0.46
S	56.37 ± 1.57	61.02 ± 1.65	59.20 ± 1.84	0.25	0.52	0.37	0.28

[1]CON = control diet; DCAD = negative DCAD diet; XZ = control diet plus synthetic zeolite A
[2]T = treatment; D = day; and L = lactation.
[3]Lactation = cows entering second lactation versus third lactation and greater.

Table 4. Least squares means and SE for prepartum and postpartum PTH concentrations.

Variable	Treatment[1]			*P*-value[2]				
	CON	DCAD	XZ	T	D	L[3]	T X D	T X L
Prepartum								
PTH (pg/mL)[4]	165.23 ± 8.61	167.21 ± 8.14	164.35 ± 8.29	0.59	<0.01	0.29	0.28	0.95
Postpartum[5]								
PTH (pg/mL)	181.00 ± 12.85	162.26 ± 12.27	151.67 ± 12.54	0.37	0.07	0.54	0.66	0.88

[1]CON = control diet; DCAD = negative DCAD diet; and XZ = control diet plus synthetic zeolite A
[2]T = treatment; D = day; and L = lactation.
[3]Lactation = cows entering second lactation versus third lactation and greater.
[4]Data analyzed on D-3 to D0.
[5]Data analyzed on D1 to D3.



Grade 1 = clear sample, without coloration or debris;

Grade 2 = clear sample, with some coloration, and without debris;

Grade 3 = sample not clear and with some debris;

Grade 4 = sample completely cloudy and with a lot of debris.

Figure 1. Representative saliva samples by grade.

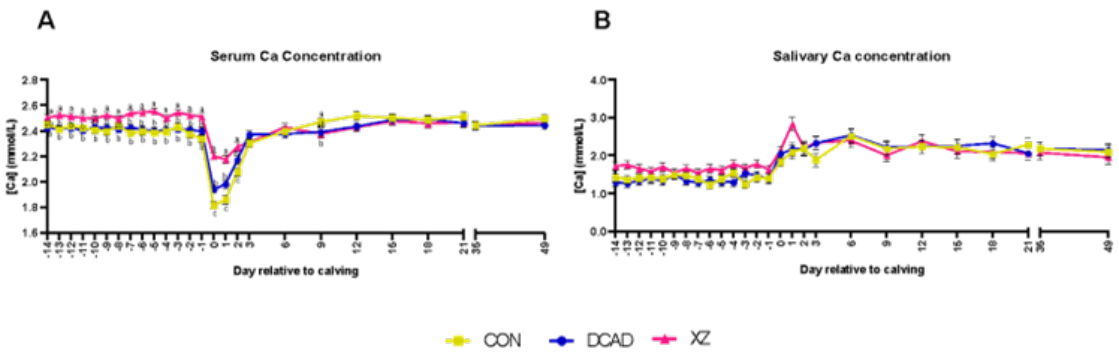


Figure 2. Least squares means and SE for prepartum and postpartum (A) serum Ca (mmol/L) and (B) salivary Ca (mmol/L) for multiparous cows fed with control (CON), negative DCAD (DCAD), or with supplementation of synthetic zeolite A (XZ) diet during the close-up period. Prepartum and postpartum data were analyzed separately. There was a treatment effect ($P < 0.01$), day effect ($P < 0.01$), lactation effect ($P < 0.01$), and treatment by day effect ($P < 0.01$) during the prepartum period, and a day effect ($P < 0.01$), lactation effect ($P < 0.01$), and treatment by day effect ($P < 0.01$) during the postpartum period for serum Ca. There was a treatment effect ($P < 0.01$), day effect ($P < 0.01$), grade effect ($P < 0.01$), and treatment by lactation effect ($P = 0.01$) during the prepartum period, and grade effect ($P < 0.01$) during the postpartum period. a, b, c Treatment by day effects at time points when treatment means differed significantly ($P \leq 0.05$).

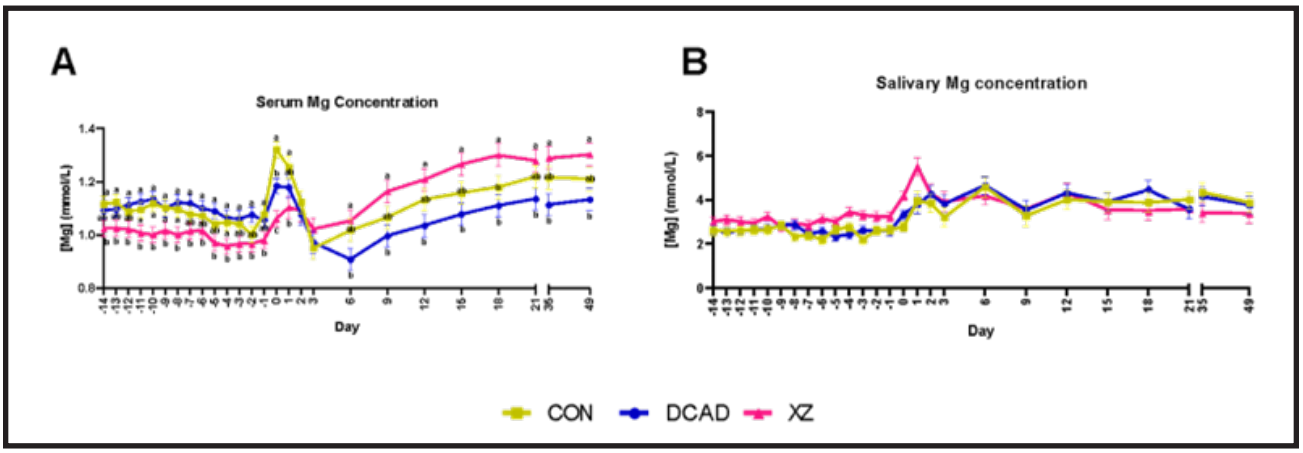


Figure 3. Least squares means and SE for prepartum and postpartum (A) serum Mg (mmol/L) and (B) salivary Mg (mmol/L) for multiparous cows fed with control (CON), negative DCAD (DCAD), or with supplementation of synthetic zeolite A (XZ) diet during the close-up period. Prepartum and postpartum data were analyzed separately. There was a treatment effect ($P < 0.01$), day effect ($P < 0.01$), and treatment by day effect ($P < 0.01$) during the prepartum period, and a treatment effect ($P < 0.01$), day effect ($P < 0.01$), treatment by day effect ($P < 0.01$), and treatment by lactation effect ($P = 0.01$) during the postpartum period for serum Mg. There was a treatment effect ($P < 0.01$), and grade effect ($P < 0.01$) during the prepartum period, and a grade effect ($P < 0.01$) during the postpartum period. [a,b,c] Treatment by day effects at time points when treatment means differed significantly ($P \leq 0.05$).

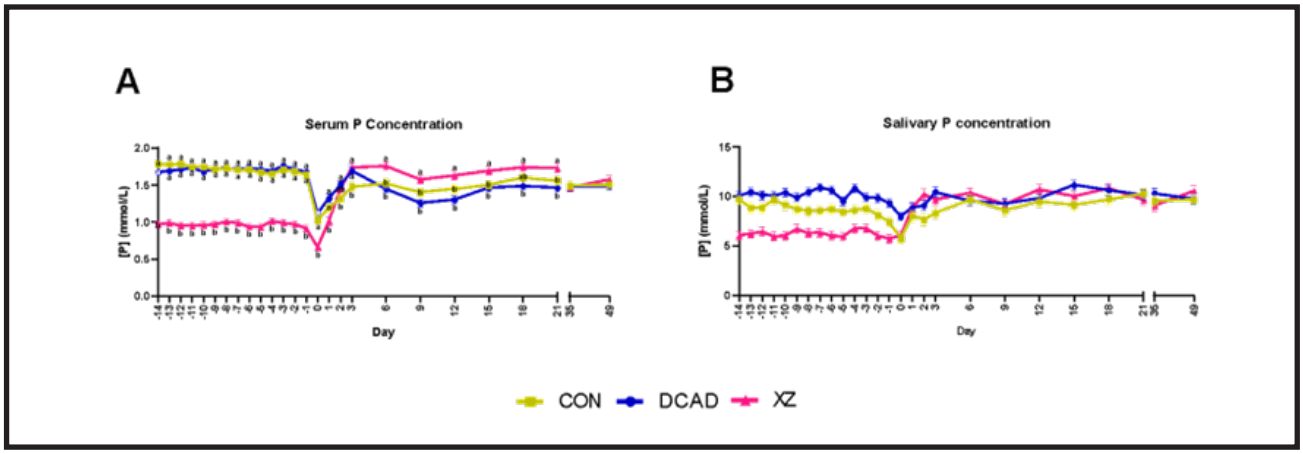


Figure 4. Least squares means and SE for prepartum and postpartum (A) serum P (mmol/L) and (B) salivary P (mmol/L) for multiparous cows fed with control (CON), negative DCAD (DCAD), or with supplementation of synthetic zeolite A (XZ) diet during the close-up period. Prepartum and postpartum data were analyzed separately. There was a treatment effect ($P < 0.01$), day effect ($P < 0.01$), lactation effect ($P < 0.01$), treatment by day effect ($P < 0.01$), and treatment by lactation effect ($P < 0.01$) during the prepartum period, and a treatment effect ($P < 0.01$), day effect ($P < 0.01$), lactation effect ($P < 0.01$), treatment by day effect ($P < 0.01$), and treatment by lactation effect ($P < 0.01$) during the postpartum period for serum P. There was a treatment effect ($P < 0.01$), day effect ($P < 0.01$), lactation effect ($P < 0.01$), grade effect ($P < 0.01$), and treatment by lactation effect ($P < 0.01$) during the prepartum, and treatment effect ($P < 0.01$), day effect ($P < 0.01$), lactation effect ($P < 0.01$), and grade effect ($P < 0.01$) during the postpartum period for salivary P. [a,b,c]Treatment by day effects at time points when treatment means differed significantly ($P \leq 0.05$).

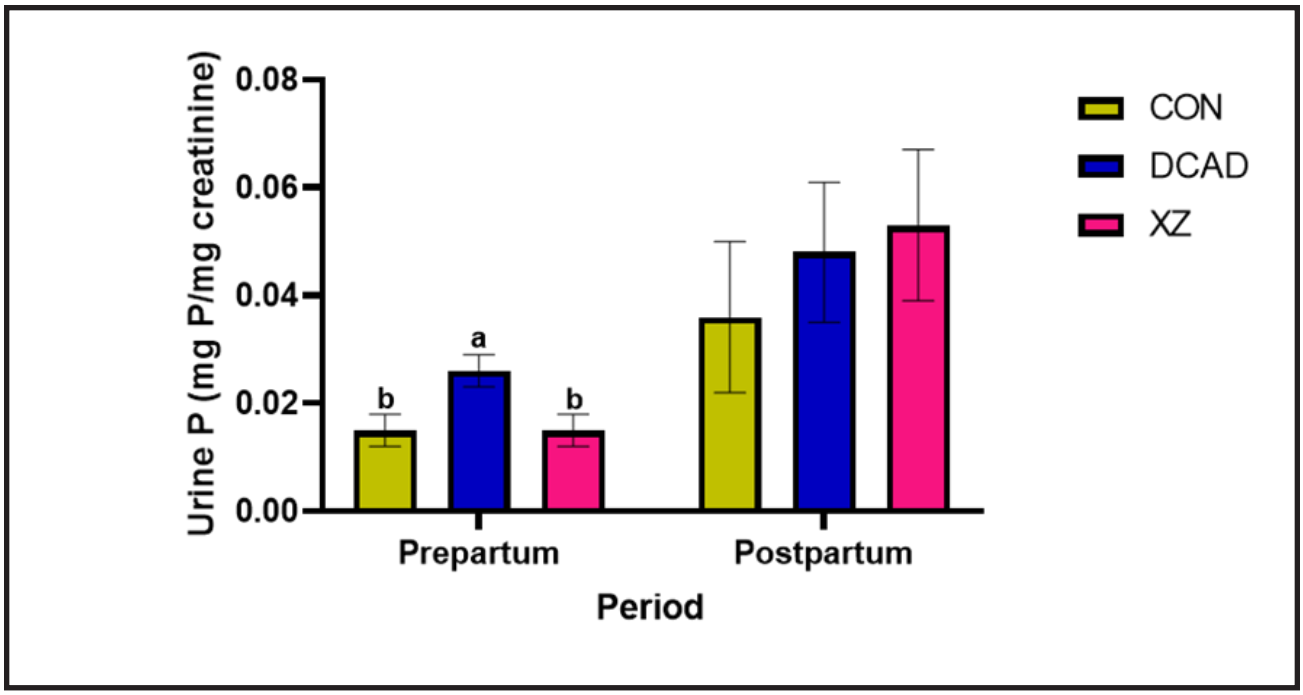


Figure 5. Least squares means and SE for urine P (mg/kg) in multiparous cows fed with control (CON), negative DCAD (DCAD), or with supplementation of synthetic zeolite A (XZ) diet during the close-up period.

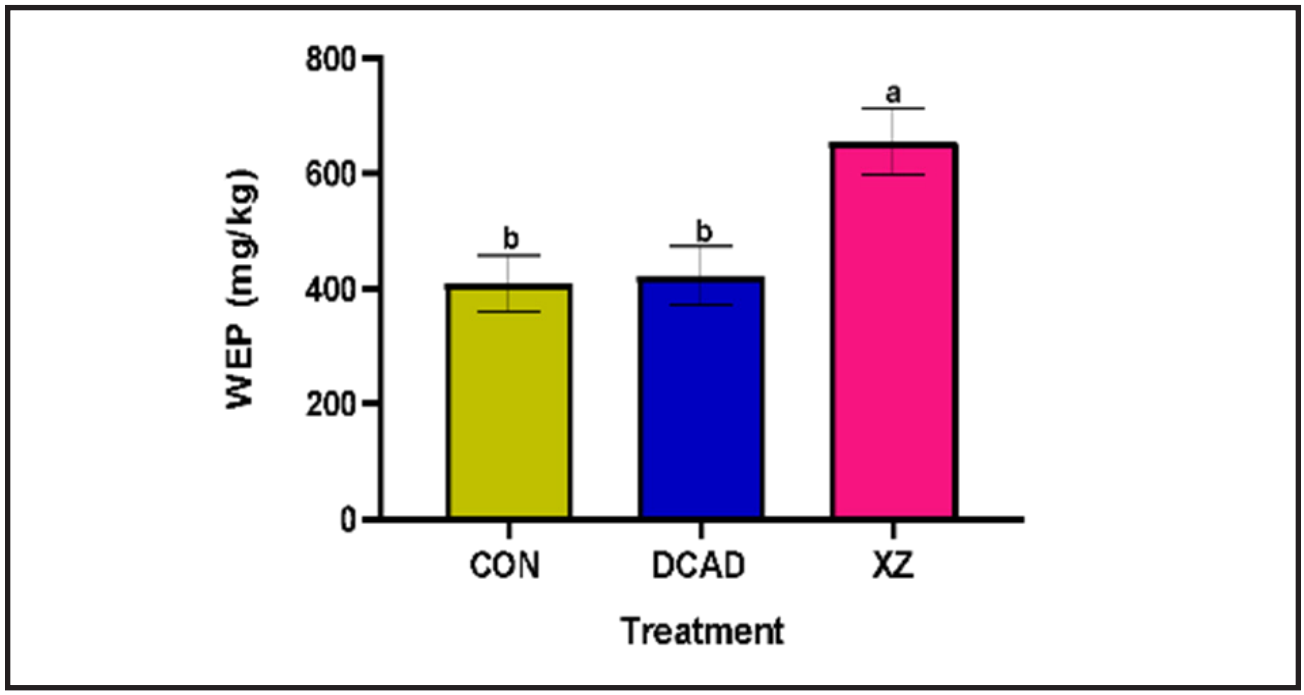


Figure 6. Least squares means and SE for WEP (mg/kg) in manure for multiparous cows fed with control (CON), negative DCAD (DCAD), or with supplementation of synthetic zeolite A (XZ) diet during the close-up period.