PREPARTUM DIETARY MANAGEMENT OF HYPOCALCEMIA THROUGH THE USE OF A SYNTHETIC ZEOLITE A

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INTRODUCTION

Around the time of parturition, it is common for dairy cows to experience decreased blood Ca due to a decrease in dry matter intake (**DMI**) and an increase in Ca demand for colostrogenesis and subsequent milk production. Hypocalcemia ensues when blood Ca drops below normal ranges, and is associated with an increased risk of developing other health disorders (Kimura et al., 2006; Chapinal et al., 2011; Martinez et al., 2012), decreased milk production (Chapinal et al., 2012), and reduced reproductive performance (Chapinal et al., 2012; Martinez et al., 2012; Caixeta et al., 2017).

One strategy for preventing hypocalcemia is to feed a Ca-deficient (< 20 g/d available Ca) prepartum diet, which will cause a slight decrease in blood Ca and stimulate parathyroid hormone (**PTH**) secretion (Goings et al., 1974; Kichura et al., 1982). Parathyroid hormone mobilizes skeletal Ca when blood Ca concentrations drop and increases Ca absorption efficiency in the small intestine and Ca reabsorption in the kidney (Goff, 2006). Due to Ca concentrations found in common feedstuffs, it is difficult to formulate a prepartum diet sufficiently low in Ca to result in homeostatic changes (Horst et al., 2005). However, the effects of a low Ca dietary approach can be actualized by decreasing the bioavailability of dietary Ca through the use of a Ca binding agent. In an *in vitro* study, Thilsing et al. (2006) showed that synthetic zeolite A, a sodium aluminum silicate, has the capacity to bind to Ca, P and Mg in rumen fluid at varying pH levels. However, the binding capacity of zeolite A to microminerals that may play an important role in combating oxidative stress has not been investigated. Previous studies have shown that feeding zeolite A during the prepartum period results in improved Ca status during the periparturient period and is hypothesized to be a result of actualizing a low Ca prepartum diet (Thilsing-Hansen et al., 2002; Thilsing et al., 2007; Pallesen et al., 2008). Only one study has observed the effects of feeding zeolite A for more than 2 weeks. Despite observing improved Ca status on the day after calving in this study, cow numbers were limited as only 9 Jersey cows were fed zeolite A (Thilsing-Hansen and Jørgensen, 2001). Subsequent studies limited zeolite A supplementation to the 2 weeks prior to expected calving (Thilsing et al., 2007; Pallesen et al., 2008; Grabherr et al., 2009). It is common for North American commercial dairy farms to utilize a 2-group dry cow strategy such that animals move to a close-up pen 21 to 28 d prior to expected calving. Despite demonstrating improved blood Ca status, further research is needed to investigate the effects of feeding zeolite A on intake and performance as well as the potential effects on immune function and reproductive performance that may result from altering blood mineral status.

The objective of this study was to determine the effects of feeding zeolite A for 3 weeks prior to expected calving to multiparous Holstein cows on serum mineral status, plasma oxidant status, DMI, milk production, and reproductive performance. We hypothesized that zeolite-fed cows would have improved Ca status during the postpartum period, resulting in improved postpartum outcomes.

EXPERIMENTAL DESIGN

All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee prior to the onset of the experiment. Cows (n = 55) were enrolled in a completely randomized design study, with randomization restricted to balance for parity group (entering 2nd vs. 3rd and greater lactation) and previous 305-d mature equivalent milk production. Cows were fed a control diet beginning at 28 d prior to expected parturition for a covariate (pretreatment) period of 7 d. Animals were housed in individual tie-stalls with individual feed bins. At 21 d prior to expected parturition, cows were assigned to 1 of 2 treatment groups, Control (**CON**) or Experimental (**EXP**). Diets were identical except for the addition of synthetic zeolite A (X-Zelit, Protekta Inc., Lucknow, Ontario, Canada/Vilofoss, Graasten, Denmark) in the EXP diet. After parturition, all cows were fed the same fresh cow diet and managed similarly until 28 DIM.

Diets were formulated using the Cornell Net Carbohydrate and Protein System (**CNCPS**, version 6.55, Cornell University, Ithaca, NY). Ration ingredient composition and analyzed diet composition are presented in Table 1. In the EXP diet, zeolite A was fed at 3.3% of DM, targeting 500 g/d as fed. Cows were individually fed and the weight of feed delivered and refused was recorded to calculate daily feed intake. Weekly TMR samples and feed ingredient samples were collected for determination of DM and the as-fed inclusion rate of the ration ingredients were adjusted weekly based on the DM values. Forages and TMR samples were dried and ground; TMR samples were composited at 4-wk intervals and feed ingredients were composited over the course of the study. Composited samples were submitted to a commercial laboratory (Cumberland Valley Analytical Services, Waynesboro, PA) for wet chemistry analysis.

Body weight was measured and a BCS was assigned weekly for each cow from enrollment through 28 DIM. Rumination data were collected with HR tags (SCR Dairy, Netanya, Israel) in 2-h intervals from enrollment through 28 DIM. Colostrum was harvested within 2 h of calving, weighed, and sampled for determination of IgG concentration (Cornell University Animal Health Diagnostic Center, Ithaca, NY). All cows were milked 3×/d and daily milk weights were recorded. Milk samples were taken weekly at 3 consecutive milkings for wk 1 through 4 of lactation and analyzed for milk composition in the Barbano lab at Cornell University using Fourier transform mid-infrared (FTIR) techniques (Barbano et al., 2014).

Table 1. Formulated and analyzed (mean ± SD) diet composition of the prepartum diets and the common postpartum diet.

	Prepartum		
Item	**CON**	**EXP**	**Postpartum**
Ingredient, % of diet DM			
Corn silage	40.00	38.60	40.25
Alfalfa hay	—	—	3.58
Wheat straw	33.33	32.17	4.48
Alfalfa silage	—	—	10.74
Canola meal	8.33	8.03	5.37
Ground shelled corn	—	—	3.58
Steam flaked corn	—	—	6.27
Citrus pulp	3.33	3.24	2.02
Blood meal	1.67	1.62	—
Amino Plus[1]	6.67	6.47	6.14
Smartamine M[2]	0.03	0.05	—
LysAAmet[3]	—	—	1.77
Soybean hulls	5.00	4.85	3.74
Dried molasses	—	—	4.08
X-Zelit[4]	—	3.40	—
Urea	—	—	0.41
Other	1.6	1.56	7.53
Analyses, % of diet DM			
CP	13.6 ± 1.0	13.5 ± 0.7	16.4 ± 0.4
NDF	46.4 ± 1.4	46.0 ± 1.7	30.6 ± 2.8
Starch	16.8 ± 1.7	16.3 ± 0.3	26.1 ± 1.6
Sugar	3.2 ± 0.8	3.3 ± 0.4	3.3 ± 0.5
Fat	2.24 ± 0.13	2.25 ± 0.30	2.64 ± 0.24
Ash	6.12 ± 0.53	7.99 ± 0.36	9.14 ± 0.49
Ca	0.68 ± 0.05	0.65 ± 0.03	1.00 ± 0.07
P	0.39 ± 0.03	0.38 ± 0.02	0.38 ± 0.01
Mg	0.42 ± 0.05	0.42 ± 0.03	0.51 ± 0.04
DCAD, mEq/100 g of DM	11.03 ± 2.06	26.87 ± 1.71	40.75 ± 2.54
MP, g/kg of DM[5]	87.24	85.41	123.04

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[2] Adisseo, Antony, France.
[3] Perdue AgSolutions LLC, Salisbury, MD.
[4] Protekta, Inc., ON, Canada/Vilofoss, Graasten, Denmark.
[5] Metabolizable protein as predicted by the Cornell Net Carbohydrate and Protein System, based on analyzed forage composition.

Blood samples were collected 1×/wk from enrollment until 7 d prior to expected calving, then daily through 7 DIM, with 2 samples collected within 24 h of parturition, and 3×/wk from 8 to 28 DIM. A subset of serum samples were analyzed for Ca, P, and Mg concentrations at the Cornell University Animal Health Diagnostic Center (Ithaca, NY). Samples were classified as normal Ca (Ca ≥ 2.12 mmol/L) or low Ca (Ca < 2.12 mmol/L) to determine subclinical hypocalcemia (**SCH**) prevalence (Goff, 2008). The 4 serum samples analyzed between calving and 3 DIM were evaluated to determine overall Ca status of the cow. Similar to Caixeta et al. (2017), cows were also retrospectively classified as having eucalcemia (**no SCH**; 0 samples with Ca < 2.12 mmol/L), SCH (1 to 3 samples with Ca < 2.12 mmol/L), or chronic SCH (**cSCH**; all 4 samples with Ca < 2.12 mmol/L) between calving and 3 DIM.

A subset of plasma samples were analyzed for oxidant status according to Abuelo et al. (2016). In order to assess oxidative stress during the transition period, the oxidant status index [**OSi**; OSi = (reactive nitrogen and oxygen species; **RONS**) / (antioxidant potential; **AOP**)] was evaluated as it reflects the changes in redox balance (Abuelo et al., 2013, 2016).

Prepartum and postpartum data were analyzed separately. All statistical analyses were conducted with SAS (version 9.4, SAS Institute Inc., Cary, NC). The prevalence of hypocalcemia between treatments by day was tested with a Fisher's exact test using PROC FREQ. Continuous measures not repeated over time were subjected to ANOVA using the MIXED procedure with fixed effects of treatment, parity group and the interaction between treatment and parity group. Data analyzed over time were subjected to repeated measures ANOVA using the MIXED procedure and the repeated statement for time. Fixed effects included in the model were treatment, time, parity group, and all two-way interactions with the random effect of cow within treatment. Pretreatment measures were included in the model when available as covariates. The effect of treatment and Ca status, both with the fixed effect of parity, on time to pregnancy by 150 DIM were analyzed by a Cox Proportional Hazards model using the PHREG procedure. Cows that were removed from the herd before becoming pregnant or were not pregnant by 150 DIM were right censored.

RESULTS

Prepartum and postpartum serum mineral concentrations and plasma OSi are presented in Table 2 and treatment by day effects are presented in Figure 1 for serum Ca, P, and Mg. Cows fed EXP during the prepartum period had higher serum Ca concentrations, most notably as parturition approached and in the immediate postpartum period. Serum P concentrations in the EXP-fed cows were approximately half the concentration of the CON-fed cows during the entire prepartum period. Serum P levels in EXP-fed cows were significantly lower than CON-fed cows during the immediate postpartum period yet increased through 14 DIM such that EXP-fed cows had significantly higher P concentrations at d 14. Magnesium concentrations steadily decreased during the prepartum period for EXP-fed cows and on average, were lower than the CON-fed cows. Serum Mg was markedly lower for EXP-fed cows within the first DIM but we did not observe treatment by day interactions thereafter. There were no significant treatment differences in the OSi during the prepartum or postpartum periods.

Table 2. Least squares means and SEM for prepartum and postpartum serum mineral concentrations and geometric means with back transformed 95% confidence intervals for oxidant status index (OSi).

	Treatment			*P*-value	
Variable	**CON**	**EXP**	**SEM**	**Trt**	**Trt × Day**
Prepartum					
Ca (mmol/L)	2.31	2.47	0.02	<0.001	0.06
P (mmol/L)	2.03	1.03	0.04	<0.001	0.04
Mg (mmol/L)	0.92	0.85	0.01	<0.01	<0.01
OSi[4]	1.33 (1.24–1.44)	1.34 (1.24–1.45)	—	0.93	0.60
Postpartum					
Ca (mmol/L)	2.15	2.33	0.02	<0.001	<0.001
P (mmol/L)	1.39	1.19	0.04	<0.001	<0.001
Mg (mmol/L)	0.92	0.91	0.01	0.51	<0.001
OSi	0.97 (0.90–1.05)	0.99 (0.91–1.07)	—	0.77	0.33

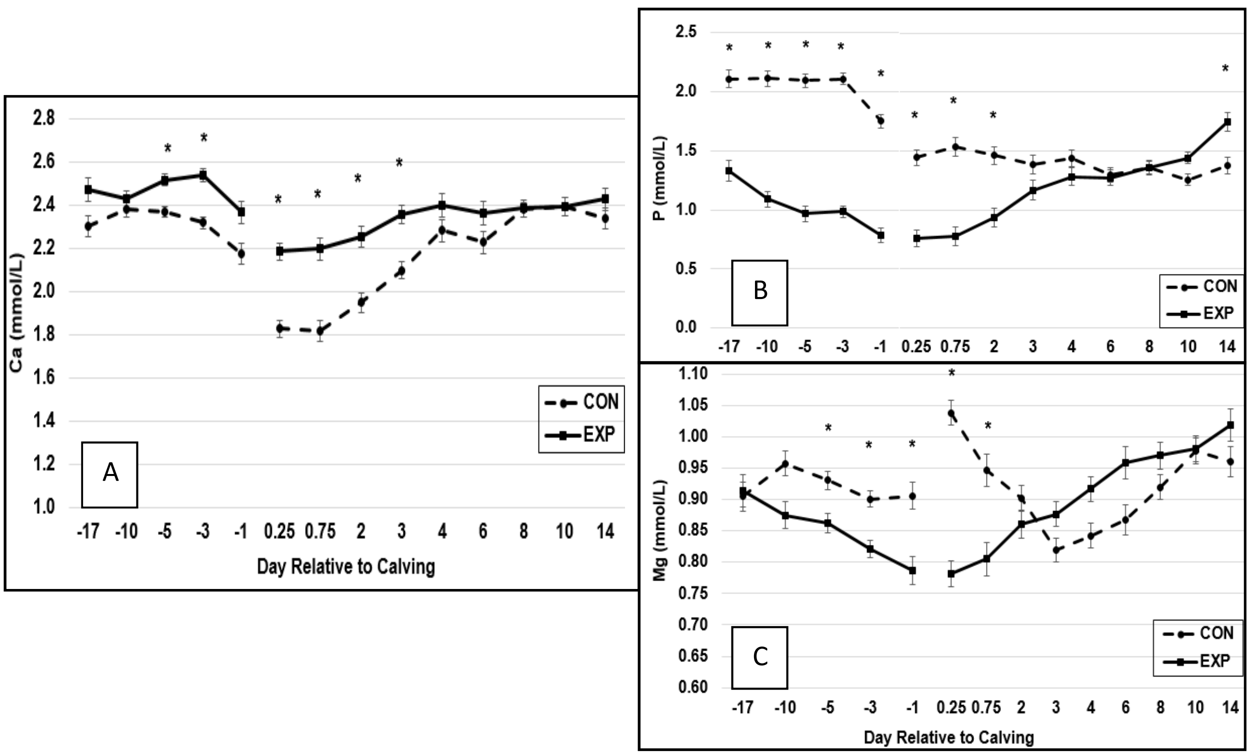

Figure 1. Serum concentrations of Ca (A), P (B), Mg (C), by day relative to calving with significant differences indicated by an asterisk (*; $P \leq 0.05$).

Subclinical hypocalcemia (**SCH**) prevalence for the two treatment groups at different time points are presented in Figure 2A. There were 3× as many CON-fed cows that had serum Ca concentration < 2.12 mmol/L within the first DIM compared to the EXP-fed cows. The highest prevalence of SCH occurred within d 1 postpartum for both treatment groups. The CON-fed cows had a greater prevalence of SCH from 3 days prior to calving through 3 DIM. Fourteen out of 55 cows were categorized as not having SCH from calving through 3 DIM; only one of these cows were in the CON-fed group. Fifty percent (n = 13) of EXP-fed cows and 62.1% (n = 18) of CON-fed cows were categorized as having SCH. We did not observe any EXP-fed cows with cSCH while 34.5% (n = 10) of CON-fed cows were categorized as having cSCH (Figure 2B).

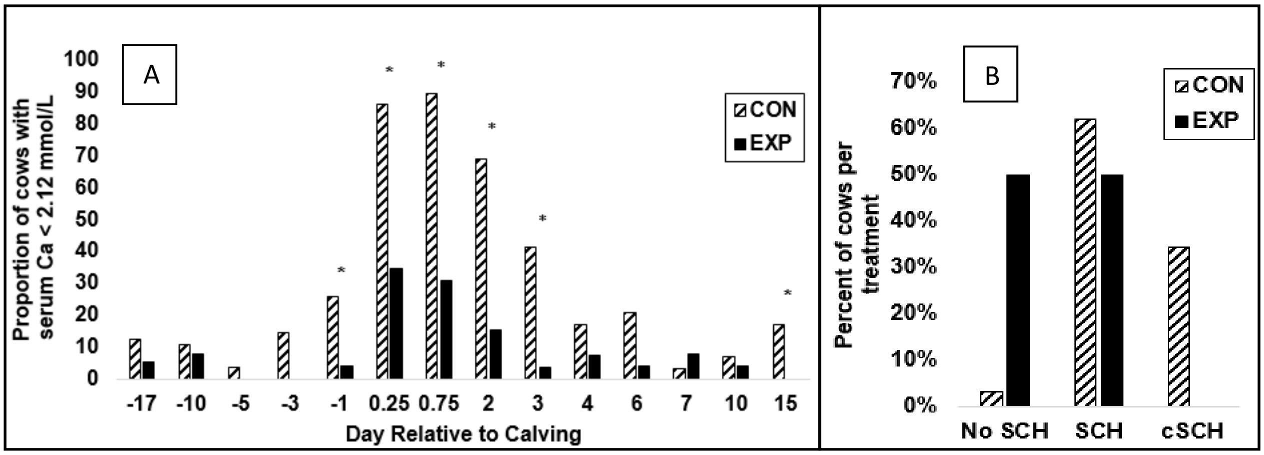

Figure 2. A) Prevalence of subclinical hypocalcemia (Ca < 2.12 mmol/L) by day relative to calving. Days at which treatments significantly differed are indicated with asterisks (*; $P \leq 0.05$). B) The proportion of cows classified based on the severity of hypocalcemia from calving through 3 DIM. Cows were eucalcemic (no SCH) if 0 samples were < 2.12 mmol/L, subclinically hypocalcemic (SCH) if 1 to 3 samples were < 2.12 mmol/L, and chronically subclinically hypocalcemic (cSCH) if all 4 samples were < 2.12 mmol/L.

Prepartum and postpartum DMI, EBAL, rumination, BW, change in BW, and BCS are presented in Table 3. We observed a treatment by week effect such that EXP-fed cows had similar DMI at 3 weeks prior to calving but had a greater reduction in DMI compared to CON-fed cows as parturition approached. There were no effects of treatment or interactions between treatment and week on postpartum DMI. While not significantly different at individual weeks, numerically DMI was 1 kg/d lower in the week before parturition and 1 kg/d higher in the first week postpartum for cows fed EXP compared to CON-fed cows. Cows fed the EXP diet ruminated for less time per day compared to CON-fed cows during the prepartum period with no effects of treatment during the postpartum period. Cows fed EXP gained less weight as calving approached compared to CON-fed cows. Prepartum BCS was lower in EXP-fed cows however, the absolute differences are small. We observed a treatment by week effect for BCS such that EXP-fed cows had a numerically lower BCS during wk 1 and 3 and was not different at wk 2 and 4.

Colostrum measurements, milk yield, and milk composition results are presented in Table 3. Despite improved Ca status during the periparturient period in cows fed the EXP diet, these cows did not have improved IgG concentration or IgG yield compared to cows fed the CON diet. We found no differences in milk yield between treatments nor did we observe a difference in ECM. Fat and protein yield also did not differ between treatments however, we observed a tendency for EXP-fed cows to have a higher protein and total solids concentration over wk 1 through 4.

Table 3. Least squares means and SEM for prepartum and postpartum DMI, energy balance (EBAL), rumination, BW, BCS, colostrum measurements, and milk composition over the first 4 wk of lactation.

	Treatment			*P*-value	
Variable	**CON**	**EXP**	**SEM**	**Trt**	**Trt × Time**
Prepartum					
DMI (kg/d)	14.6	14.0	0.2	0.07	0.04
DMI (% of BW)	1.82	1.79	0.03	0.44	0.10
EBAL (Mcal/d)	5.4	4.4	0.4	0.05	0.11
Rumination (min/d)	521	500	7	0.03	0.77
BW (kg)	808	790	4	<0.001	0.02
BW change[1] (kg)	17	6	4	0.04	—
BCS	3.37	3.29	0.02	0.008	0.32
Postpartum					
DMI (kg/d)	21.7	22.2	0.4	0.51	0.16
DMI (% of BW)	3.09	3.16	0.06	0.36	0.20
EBAL (Mcal/d)	-11.8	-11.9	0.7	0.91	0.66
Rumination (min/d)	512	523	11	0.61	0.34
BW (kg)	713	708	5	0.42	0.82
BW change[1] (kg)	-36	-36	7	0.96	—
BCS	3.14	3.11	0.03	0.34	0.01
Colostrum weight (kg)	7.3	5.8	0.7	0.16	—
Colostrum IgG (mg/dL)	7628	8342	469	0.29	—
Colostrum IgG yield (g)	494	441	40	0.35	—
Milk yield (kg/d)	48.0	47.5	0.7	0.58	0.99
Fat (%)	4.17	4.32	0.08	0.17	0.05
Fat (kg/d)	1.98	2.03	0.04	0.35	0.26
3.5% FCM[2] (kg/d)	52.9	53.6	0.9	0.59	0.45
Protein (%)	3.19	3.30	0.05	0.09	0.24
Protein (kg/d)	1.51	1.55	0.03	0.33	0.88
Lactose (%)	4.58	4.59	0.02	0.78	0.59
Lactose (kg/d)	2.22	2.20	0.04	0.67	0.54
TS (%)	13.03	13.32	0.11	0.07	0.18
TS (kg/d)	6.23	6.30	0.10	0.65	0.66
ECM[3] (kg/d)	53.0	53.8	0.9	0.50	0.57
ECM/DMI	2.47	2.47	0.05	0.95	0.47
MUN (mg/dL)	12.46	10.90	0.43	0.01	0.87
SCS	1.07	1.08	0.25	0.98	0.72

[1] Change in BW from wk -3 to -1 (prepartum) and wk 1 to 4 (postpartum), relative to calving

[2] 3.5% FCM = (0.432 × kg of weekly average milk yield) + (16.216 × kg of fat).

[3] ECM = (0.327 × kg of weekly average milk yield) + (12.95 × kg of fat) + (7.65 × kg of true protein).

For the reproductive analysis, 11 out of 52 animals (CON = 8 cows, EXP = 3 cows) were right censored due to being culled before becoming pregnant (n = 1) or not becoming pregnant during the first 150 DIM (n = 10).The median time to pregnancy during the first 150 days postpartum for animals fed the EXP diet was 70 DIM while the CON-fed cows had a median time of 89 DIM (Figure 3A). Treatment [hazard ratio (95% CI) for

EXP vs. CON = 1.76 (0.93 – 3.33); P = 0.08] tended to be associated with time to pregnancy such that EXP-fed cows had a higher hazard of becoming pregnant by 150 DIM.

In relation to Ca status, 0/12 eucalcemic cows, 5/30 SCH cows, and 6/10 cSCH cows were right censored. The median time to pregnancy during the first 150 days for eucalcemic cows was 69 DIM compared to 73 DIM for cows categorized as having SCH. Only 40% of cows with cSCH became pregnant by 150 DIM (Figure 3B). Calcium status was found to be associated with time to pregnancy (P = 0.01). Compared to eucalcemic cows cSCH cows had a significantly lower hazard [0.18 (95% CI = 0.06 – 0.57); P < 0.01] of becoming pregnant within 150 DIM while cows with SCH did not differ from eucalcemic cows (P = 0.29).

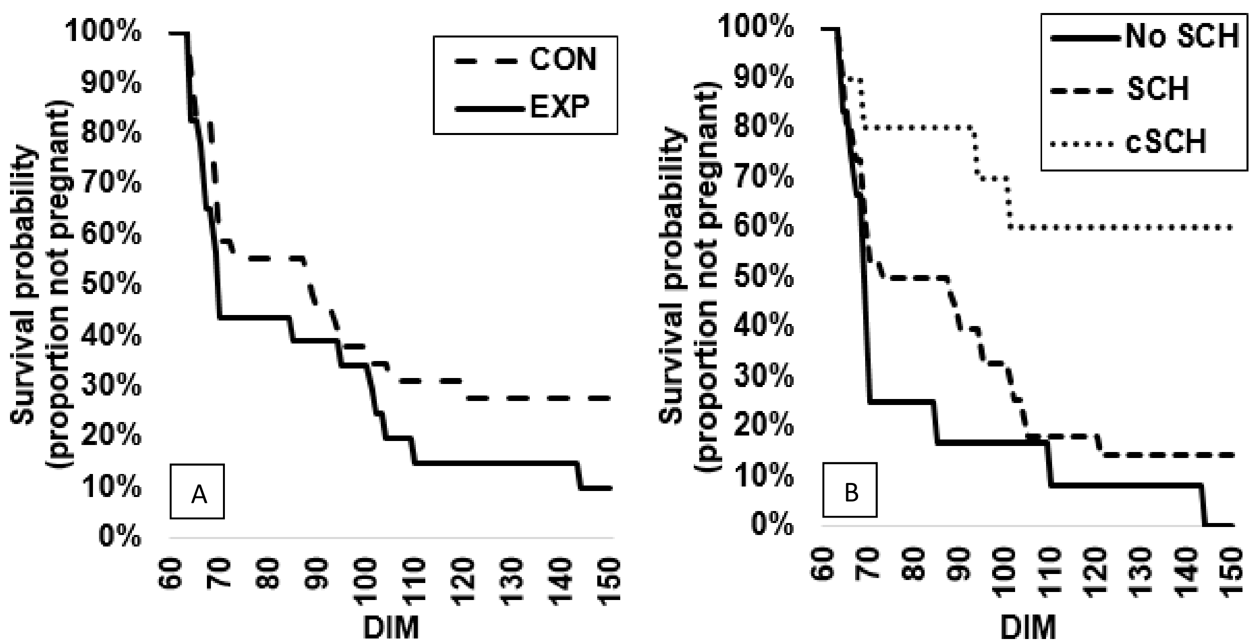

Figure 3. A) Survival curves estimating the time to pregnancy by treatment group B) Survival curves estimating the time to pregnancy for cows categorized as being eucalcemic (**no SCH**; 0 samples with Ca < 2.12 mmol/L), subclinical hypocalcemic (**SCH**; 1 to 3 samples with Ca < 2.12 mmol/L), or chronic subclinical hypocalcemic (**cSCH**; all 4 samples with Ca < 2.12 mmol/L) between calving and 3 DIM.

To our knowledge, this is the first study evaluating the effect of synthetic zeolite A on reproductive measures. Evaluating reproductive performance was not a primary objective of this study and the study was not powered to evaluate differences between treatments. These results should be interpreted as preliminary findings in need of further research involving more cows to validate the improved time to pregnancy findings of this study.

CONCLUSIONS AND IMPLICATIONS

Supplementing cows with synthetic zeolite A for three weeks before parturition improved Ca status during the periparturient period however, postpartum performance was similar between the two treatment groups. Numerically, cows fed zeolite A had improved time to pregnancy, though further work needs to be done to validate these findings. We also did not observe a treatment effect on oxidative metabolism as reflected in the lack of change of OSi.

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