Effect of Prepartum DCAD Strategy and Level of Dietary Calcium on Postpartum Calcium Status and Performance of Multiparous Holstein Cows

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Introduction

The transition period, three weeks prior to and post calving, provides opportunity for disruptions of homeostasis. Commonly, the rapidly increased demand for calcium (**Ca**) for parturition, colostrum production, and milk production may leave the cow at risk for hypocalcemia. Low blood calcium, or hypocalcemia, is recognized as a notable detriment to health and productivity of dairy cows (Curtis et al., 1983). Subclinical hypocalcemia (**SCH**) can predispose cows to an increased risk of other health disorders, decreased milk production, and decreased reproductive performance (Kimura et al., 2006; Reinhardt et al., 2011; Caixeta et al., 2017; McArt and Neves, 2020). Circulating concentrations of Ca in cattle are generally well maintained by a complex homeostatic mechanism involving parathyroid hormone (**PTH**) and 1,25-dihydroxyvitamin D_3 to manage functions of Ca ruminal and intestinal absorption, renal excretion and retention, and Ca resorption of the bone (Goff, 2008). When Ca demand increases abruptly around parturition, the homeorhetic systems' ability to meet this demand, via these mechanisms coupled with Ca intake, will determine the presence and degree of hypocalcemia in the cow.

It is understood that there are dietary interventions to support the cow's homeostatic mechanisms and either prevent or reduce the severity of Ca imbalances at the time of calving. In the United States, a common method for managing hypocalcemia is by manipulating the dietary cation anion difference (**DCAD**) in the close-up period prior to calving (Ender et al., 1971; Goff et al.,1995). Feeding a negative DCAD diet creates a state of compensated metabolic acidosis within the cow, resulting in decreased urine pH and increased urinary Ca excretion, thereby improving tissue sensitivity to homeostatic signaling (Goff, 2008; Leno et al., 2017). While feeding a negative DCAD diet is a generally accepted method of reducing risk of SCH and milk fever, the level of anionic supplementation remains a point of debate (Santos et al., 2019). Recent meta-analyses observed that lower levels of DCAD reduced the risk of milk fever and overall disease while improving performance of parous cows (Lean et al., 2019; Santos et al., 2019).

Dietary Ca manipulation in conjunction with some level of DCAD can be used to improve plasma Ca status and cow health; however, the improvement in milk production has been debated. Ryan et al. (2020) and Glosson et al. (2020) reported that negative DCAD (prepartum urine pH 5.5-6.0) with supplemental dietary Ca had positive effects on cow health, production, and reproduction parameters. Goff and Koszewski (2018) recommend restricted or low dietary Ca with a negative DCAD (prepartum urine pH 6.5-7.0) to mitigate hypocalcemia and improve overall production. A meta analysis by Santos et al. (2019) included experiments with varying Ca supplementation, found that positive and negative DCAD programs targeting different urine pH levels with increased dietary Ca was associated with an increase in risk of milk fever. More work needs to be conducted to elucidate the level of DCAD and dietary Ca supplementation to optimize dairy cow health and performance.

The objective of this study was to compare the effects of two levels of prepartum DCAD, two levels of dietary Ca, and their interactions on the parameters of Ca metabolism, health, and milk performance of transition dairy cows. We hypothesized that cows fed a lower level of DCAD (evaluated by urine pH) and higher dietary level of Ca would have improved Ca status and greater overall performance than alternative experimental diets.

Materials and Methods

All procedures involving animals were approved by the Cornell University Institutional Animal Care and Use Committee prior to the beginning of the experiment. Multiparous cows (n = 98) were enrolled between October 2019 and July 2020 in a completely randomized design, restricted to balance for parity (entering 2nd lactation vs. 3rd and greater), body condition score (**BCS**), and previous 305-d mature equivalent milk production. Cows diagnosed with twins or entering their first parity were excluded from this study. Cows were housed in sawdust bedded, individual tie-stalls and fed with individual feed bins at the Cornell University Ruminant Center (Harford, NY). All cows were moved in weekly approximately 35 d prior to expected date of parturition and fed a standard far-off or control diet for a 7d covariate period. At 26 d prior to expected parturition, cows were assigned to one of four dietary treatments until parturition. All diets were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS, v 6.55, Cornell University, Ithaca, NY). Diets were identical except for the main effects of DCAD level (PART: -2.6 mEq/100 g DM or FULL: -10.3 mEq/100 g DM) and dietary Ca concentration (1.50% or 0.70% DM). After calving, all cows were fed the same fresh cow diet. Ingredients and analyzed composition of the diets are presented in Table 1 and 2.

Cows were fed daily, feed offered and refused amounts were recorded. Prepartum cows were fed between 0900 and 1100 h and postpartum cows were fed between 0700 and 0900 h. Weekly TMR samples and feed ingredients were collected to evaluate DM and calculate daily DMI. Forages and TMR samples were dried, ground, and composited at 4-week intervals over the course of the study. Composites were submitted to a commercial laboratory for wet chemistry analysis (Cumberland Valley Analytical Services, Waynesboro, PA). Body weights and BCS (Edmonson et al., 1989) were measured weekly from enrollment until 63 DIM.

Prepartum urine pH (**UpH**) was collected and recorded 3x/week using a portable glass electrode pH meter to monitor compensated metabolic acidosis through urine pH (PART: 6.5-7.0 pH or FULL: 5.5-6.0 pH). Free-catch, midstream urine samples were collected at -25, -14, -7, +1, 2, 3, 4, 5, 7, 14 d relative to parturition and stored at -20ºC prior to analysis of ammonium the Cornell University Animal Health and Diagnostic Center (Ithaca, NY). Urine sample collection occurred between 1800 and 1900 h.

Table 1. Ingredient composition of the prepartum diets and the common postpartum diet.

	Prepartum				
Ingredient, % of DM	**~1.5% Ca, FULL**	**~1.5% Ca, PART**	**~0.7% Ca, FULL**	**~0.7% Ca, PART**	**Postpartum**
Corn silage	43.33	43.33	43.33	43.33	44.96
Hay crop silage	—	—	—	—	16.89
Wheat straw	30.00	30.00	30.00	30.00	—
Corn grain, finely ground	2.00	2.00	2.00	2.00	16.20
Soybean meal	5.00	5.00	5.00	5.00	4.66
Amino Plus	8.00	8.00	6.97	6.97	3.70
Soybean Hulls, ground	0.67	0.67	4.67	4.67	3.64
Animate[1]	4.33	3.33	4.33	3.33	—
Blood meal	1.67	1.67	1.67	1.67	2.24
Dextrose	—	—	—	—	1.61
Calcium carbonate	3.33	3.33	0.43	0.43	1.53
Palmit 80[2]	—	—	—	—	0.98
Sodium sesquicarbonate	—	—	—	—	0.78
Salt	0.37	0.37	0.33	0.33	0.47
Selenium	0.03	0.03	0.03	0.03	—
Molasses	—	—	—	—	0.32
Bypass fat	—	—	—	—	0.32
Magnesium oxide	0.33	0.33	0.33	0.33	0.23
Calcium sulfate	—	—	—	—	0.22
Mono-Dicalcium phosphate	0.8	0.8	0.8	0.8	0.8
Smartamine[3]	—	—	—	—	0.04
Dairy ADE[4]	0.03	0.03	0.03	0.03	0.02
Vitamin E[5]	0.03	0.03	0.03	0.03	0.00
Rumensin[6]	0.01	0.01	0.01	0.01	0.01
Corn distillers, ethanol	—	—	—	—	0.98
Trace mineral premix	0.04	0.04	0.04	0.04	0.10
Mineral oil	—	—	—	—	0.02
Filler[7]	—	1.00	—	1.00	—

[1] Commercial dietary anion supplement, (Phibro Animal Health Corp., Quincy, IL).

[2] Commercial high palmitic acid fat; Global Agri-trade Corporation (Rancho Dominguez, CA).

[3] Met, physically protected with pH-sensitive coating; Adisseo (Antony, France).

[4] Vitamin mix; Cargill Animal Nutrition (Minneapolis, MN). Contains 30,073 kIU/kg vitamin A, 5,783 kIU/kg vitamin D, and 92,534 IU/kg vitamin E.

[5] Contains 510,750 IU/kg vitamin E.

[6] Premix contained 26,400 g/t of monensin; Elanco Animal Health (Greenfield, IN)

[7] Filler contained ground rice hulls (47.4%), corn distillers ethanol (35.5%), urea (7.1%), Mg oxide (7.0%), Ca carbonate (2.6%), and Na bicarbonate (0.4%).

Blood samples were collected via coccygeal venipuncture between 0600 and 0730 h once a week prior to treatment assignment, twice weekly until 1 week prior to expected parturition, and then daily until parturition. After calving, 4 samples were collected every

12 h and categorized as 0.5, 1, 1.5, and 2 d postpartum samples. Samples were also collected at 3, 5, 7, 14, 21, and 28 d postpartum. One aliquot was stored at -20ºC to be submitted for analysis of total Ca (**tCa**), P, and Mg. Ionized calcium (**iCa**) was measured in whole blood collected in lithium heparin vacutainers on d -25 (covariate), -7, -3, -1, +0.5, 1, 1.5, 2, 3, and 5, relative to parturition using iSTAT CG8+ cartridges (Abbott Laboratories, Lake Bluff, IL).

Within approximately 2 h of parturition, the first colostrum was weighed, evaluated for BRIX, and a sample was subsequently collected and frozen for immunoglobulin G (**IgG**) analysis (Cornell University Animal Health Diagnostic Center, Ithaca, NY). All cows were milked 3x/d until 60 DIM and daily milk weights were recorded. Weekly milk samples were taken at 3 consecutive milkings for the duration of the study and analyzed for milk composition in the Barbano lab at Cornell University using Fourier transform mid-infrared techniques (Barbano et al., 2014).

Table 2. Analyzed nutrient composition (mean ± SD, % of DM unless otherwise noted) for the prepartum diet and the common postpartum diet.

	Prepartum				
Nutrient	**~1.5% Ca, FULL**	**~1.5% Ca, PART**	**~0.7% Ca, FULL**	**~0.7% Ca, PART**	**Postpartum**
CP	12.9 ± 0.2	12.6 ± 0.2	12.6 ± 0.2	12.8 ± 0.2	14.5 ± 0.2
NDF	44.1 ± 0.6	44.0 ± 0.6	45.0 ± 0.6	45.9 ± 0.6	30.9 ± 0.4
Starch	18.1 ± 0.5	18.0 ± 0.5	17.7 ± 0.6	17.7 ± 0.5	29.2 ± 0.4
Sugar	4.5 ± 0.3	4.1 ± 0.3	4.1 ± 0.3	4.21 ± 0.3	3.9 ± 0.2
Ash	9.08 ± 0.29	9.58 ± 0.27	7.86 ± 0.29	7.69 ± 0.29	7.83 ± 0.20
Ca	1.44 ± 0.04	1.53 ± 0.04	0.74 ± 0.04	0.67 ± 0.04	1.03 ± 0.03
P	0.34 ± 0.01	0.35 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.31 ± 0.01
Mg	0.50 ± 0.02	0.49 ± 0.02	0.49 ± 0.02	0.47 ± 0.02	0.31 ± 0.01
K	1.06 ± 0.03	1.05 ± 0.03	1.06 ± 0.03	1.07 ± 0.03	0.29 ± 0.02
Cl	0.90 ± 0.03	0.71 ± 0.03	0.88 ± 0.03	0.70 ± 0.03	0.45 ± 0.02
DCAD, (mEq/100g DM)	-8.47 ± 1.80	-2.01 ± 1.68	-11.86 ± 1.79	-2.86 ± 1.79	29.04 ± 1.27

Statistical Analysis

Prepartum and postpartum samples were analyzed separately. Statistical analyses were conducted using SAS software (version 9.4, SAS Institute Inc., Cary, NC). Continuous measures that were not repeated over time underwent ANOVA using PROC MIXED with fixed effects of Ca level, DCAD level, parity, and all possible interactions. Data evaluated over time underwent repeated measures ANOVA using PROC MIXED and the repeated measures statement for time. Fixed effects included in the model were Ca level, DCAD level, time, parity, and interactions with the random effect of cow nested within Ca and DCAD level. Covariate measures were included in the model when pretreatments were available.

Results

A total of 98 cows were included in final analysis. Urine pH and ammonium results are reported in Table 3. Urine ammonium pre- and postpartum is illustrated in Figure 1.

Cows fed FULL had lower UpH and significantly higher rate of ammonium concentration excreted than cows fed PART (5.64 vs. 6.71 ± 0.10; *P* < 0.001 and 0.65 vs. 0.34 ± 0.09 mg/L; *P* < 0.001, respectively). Dietary Ca did not affect UpH ((*P* = 0.27); however, cows fed higher Ca tended (*P* = 0.10) to have lower concentrations of ammonium in urine. Pre- and postpartum DMI are reported in Table 4 and Figure 2. Cows fed FULL had lower prepartum DMI than cows fed PART (13.1 vs. 14.1 ± 0.3 kg/d; *P* = 0.04). Dietary Ca did not affect prepartum DMI (*P* = 0.21). Analysis of postpartum DMI from wk 1 to 9 showed that DMI tended to be increased for those cows fed ~1.5% Ca (21.8 vs. 20.9 ± 0.5 kg/d; *P* = 0.07); prepartum DCAD did not affect postpartum DMI (*P* = 0.70).

Table 3. Least squares means and SEM of prepartum urine pH and ammonium (mg/L) concentration.

	Treatment					***P*-value**	
Variable	**~1.5% Ca, FULL**	**~1.5% Ca, PART**	**~0.7% Ca, FULL**	**~0.7% Ca, PART**	**SEM**	**Ca**	**DCAD**
Prepartum UpH	5.68	6.81	5.59	6.62	0.13	0.27	<0.001
Prepartum NH4+	0.61	0.30	0.69	0.39	0.13	0.10	<0.001

Pre- and postpartum serum mineral concentrations are reported in Table 5 and iCa is presented in Figure 3. Postpartum circulating iCa and P from d 0 to 3 tended to be increased for cows fed FULL compared to PART (iCa: 0.98 vs. 0.94 ± 0.02 mM; *P* = 0.07 and P: 1.51 vs.1.42 mM ± 0.04; *P* = 0.09). Cows fed ~1.5% Ca had lower postpartum circulating iCa and tCa from d 0 to 3 (iCa: 0.97 vs. 1.01 ± 0.02 mM; *P* = 0.02, tCa: 2.09 vs. 2.17 ± 0.04 mM; *P* = 0.04). There was no effect of the interaction between Ca and DCAD on cow mineral status pre- or postpartum.

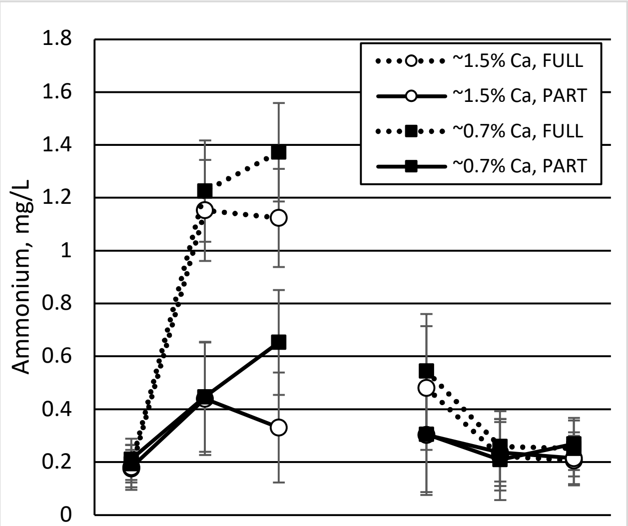

Figure 1. Urine ammonium excretion pre- and postpartum by treatment.

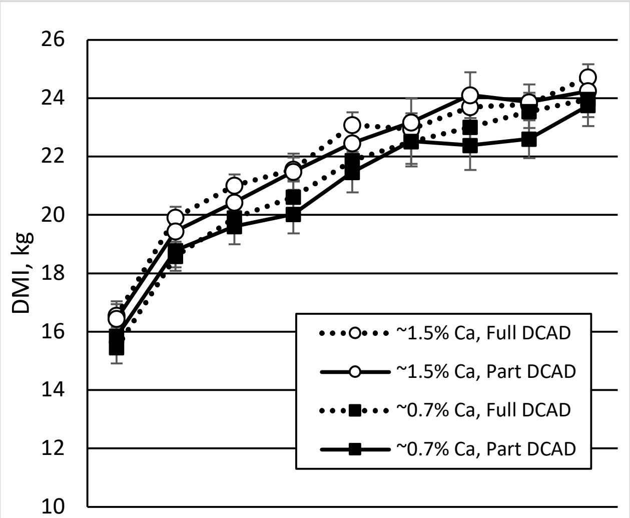

Figure 2. Weekly average postpartum DMI reported by treatment.

Colostrum measurements, milk yield, and milk composition are presented in Table 6 and Figure 4. We observed an effect of DCAD level on colostrum production (kg) such that cows fed FULL produced more colostrum than cows fed PART (7.70 kg vs. 5.43 kg ± 0.11; P = 0.02). There was also a trend for an interaction favoring ~1.5% Ca, FULL. There was no effect of Ca or DCAD on IgG concentrations. When evaluating milk yields for wk 1 to 9, a trend (P = 0.10) for an interaction of DCAD, dietary Ca, and week existed such that cows fed the PART and ~0.7% Ca had the lowest milk yield and cows fed the FULL and ~1.5% Ca had the highest milk yield. Cows fed ~1.5% Ca generally had higher milk yields in wk 1 to 3 postpartum (40.2 vs. 38.7 ± 0.9 kg/d; P = 0.02). There was no evidence that there was a difference in components between Ca, DCAD, or the interaction between Ca and DCAD for wk 1 to 9 or wk 1 to 3. There was a slight increase in lactose percentage for cows fed ~1.5% Ca prepartum (4.66% vs. 4.60% ± 0.03; P = 0.05).

Table 4. Least squares means and SEM for pre- and postpartum DMI, BW, and BCS.

	Prepartum					***P*-value**		
Variable	**~1.5% Ca, FULL**	**~1.5% Ca, PART**	**~0.7% Ca, FULL**	**~0.7% Ca, PART**	**SEM**	**Ca**	**DCAD**	**Ca*DCAD**
Prepartum								
DMI (kg/d)	13.5	14.2	12.6	14.0	0.50	0.21	0.04	0.47
BW (kg)	759	782	765	764	6.91	0.37	0.10	0.07
BW Change (kg)	17.43	22.57	9.97	6.35	0.48	0.03	0.81	0.38
BCS	3.58	3.61	3.58	3.58	0.03	0.49	0.65	0.67
BCS Change	0.20	0.19	0.24	0.17	0.10	0.85	0.39	0.48
Postpartum								
DMI (kg/d)	21.92	21.74	21.06	20.78	0.50	0.07	0.60	0.92
BW (kg)	685	678	685	680	12	0.93	0.59	0.92
BW Change (kg)	-31.90	-65.74	-24.92	-58.19	12.07	0.61	0.02	0.98
BCS	3.10	3.13	3.15	3.12	0.05	0.69	0.88	0.49
BCS Change	-0.59	-0.59	-0.31	-0.60	0.10	0.13	0.10	0.11

Table 5. Least squares means and SEM for iCa, tCa, P, and Mg pre- and postpartum (d 0 to 3).

	Treatment					***P*-value**			
Variable	**~1.5% Ca, FULL**	**~1.5% Ca, PART**	**~0.7% Ca, FULL**	**~0.7% Ca, PART**	**SEM**	**Ca**	**DCAD**	**Ca* DCAD**	**Ca* DCAD* Parity**
Prepartum									
iCa (mmol/L)	1.14	1.11	1.16	1.15	0.03	0.32	0.49	0.15	0.47
tCa (mmol/L)	2.43	2.45	2.42	2.48	0.02	0.62	0.05	0.49	0.13
P (mmol/L)	6.21	6.18	6.19	6.08	0.09	0.44	0.39	0.65	0.89
Mg (mmol/L)	2.27	2.31	2.32	2.33	0.04	0.36	0.52	0.49	0.25
Postpartum									
iCa (mmol/L)	0.96	0.91	1.01	0.98	0.02	0.02	0.07	0.44	0.30
tCa (mmol/L)	2.11	2.07	2.18	2.15	0.04	0.04	0.40	0.88	0.05
P (mmol/L)	1.48	1.42	1.54	1.42	0.06	0.56	0.09	0.58	0.89
Mg (mmol/L)	0.97	1.01	0.98	0.97	0.02	0.55	0.42	0.28	0.11

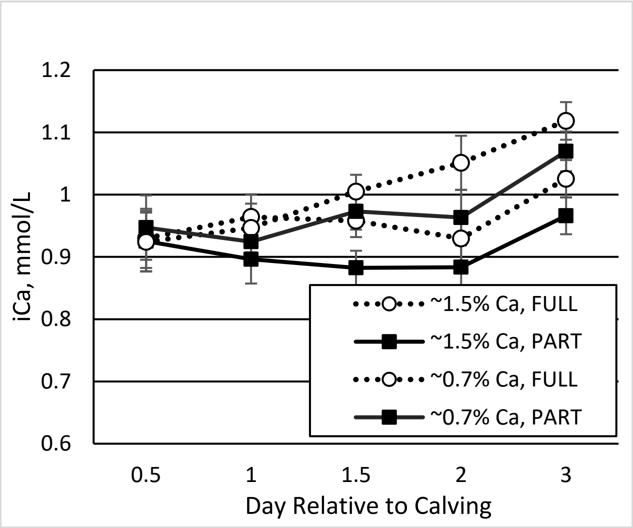

Figure 3. Average iCa concentration by treatment for d 0.5, 1,1.5, 2, and 3.0.

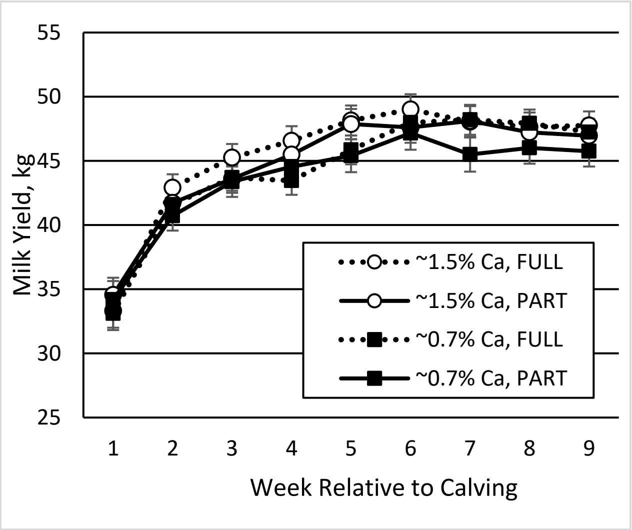

Figure 4. Average weekly milk yields and SE for wk 1 to 9 postpartum.

Table 6. Least squares means and SEM for colostrum measurements, milk yield, and milk composition from wk 1 to 9 of lactation.

	Treatment					***P*-value**			
Variable	**~1.5% Ca, FULL**	**~1.5% Ca, PART**	**~0.7% Ca, FULL**	**~0.7% Ca, PART**	**SEM**	**Ca**	**DCAD**	**Ca* DCAD**	**Ca* DCAD *Wk**
Colostrum (kg)	9.62	5.29	6.17	5.56	1.16	0.19	0.02	0.10	-
Colostrum IgG (mmol/L)	375	393	357	378	1.08	0.56	0.48	0.94	-
Milk Yield (kg/d)	45.4	44.8	44.3	43.6	1.02	0.24	0.48	0.96	0.10
Fat (%)	4.41	4.38	4.47	4.59	0.14	0.25	0.73	0.52	0.75
Fat (kg/d)	0.70	0.66	0.66	0.71	0.04	0.90	0.91	0.18	0.53
3.5% FCM (kg/d)	53.8	51.6	51.2	53.7	1.7	0.82	0.92	0.10	0.72
Protein (%)	2.75	2.73	2.76	2.74	0.04	0.66	0.62	0.98	0.82
Protein (kg/d)	1.29	1.36	1.22	1.28	0.10	0.43	0.47	0.93	0.93
Lactose (%)	4.66	4.66	4.64	4.57	0.03	0.05	0.15	0.14	0.65
Lactose (kg/d)	2.19	2.11	2.06	2.09	0.06	0.13	0.55	0.26	0.68
TS (%)	12.94	12.86	12.98	13.01	0.16	0.47	0.88	0.66	0.82
TS (kg/d)	6.08	5.82	5.74	5.95	0.17	0.47	0.84	0.10	0.71
ECM (kg/d)	51.9	49.8	49.5	51.6	1.6	0.82	0.99	0.10	0.61
MUN (mg/dL)	6.14	6.92	6.98	7.36	0.50	0.15	0.20	0.95	0.16
SCS	0.99	0.84	1.08	1.32	0.38	0.49	0.92	0.63	0.42

Conclusions and Implications

Cows fed the lower Ca diet did recover their normal iCa levels more quickly but didn't meet the production levels of their ~1.5% Ca, FULL counterparts. Cows fed ~0.7% Ca, PART were slower to return to normal iCa concentration post calving without marked increases in milk yields over time. This may warrant more investigation. Overall, feeding a higher Ca diet in conjunction with a more negative DCAD (5.5 ≤ UpH ≤ 6.0) reduced the blood calcium level of cows postpartum but numerically improved production of cows over the first 3 wk of lactation.

Acknowledgments

This project could not be possible without the help of Lisa Furman and the staff at the CURC for care of the animals, and undergraduate research assistants for their invaluable work executing the research.

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