

PREPARTUM NUTRITIONAL STRATEGIES TO MANAGE POSTPARTUM HYPOCALCEMIA

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INTRODUCTION

Dairy cows experience a negative calcium balance in the days immediately following calving as a result of the rapidly increased demand for calcium as the cow transitions from supporting growth of the fetus to supporting the needs of lactation. In order to maintain calcium homeostasis and support high levels of milk production, a coordinated hormonal response must increase the secretion of parathyroid hormone from the parathyroid gland, initiating increased activation of 1,25-dihydroxyvitamin-D₃. Together these hormones increase absorption of calcium in the intestine, increase osteoclastic activity at the bone to release stored calcium, and decrease the excretion of calcium at the kidney (Degaris and Lean, 2007). When this system is inadequate, hypocalcemia puts the animal at increased risk for several transition cow disorders including metritis, ketosis, displaced abomasum, and mastitis (Curtis et al., 1983, Chapinal et al, 2012, Martinez et al., 2012). Ultimately, cows with compromised blood calcium, despite having no clinical signs of paresis, have decreased reproductive performance and milk production (Chapinal et al., 2012). According to the 2002 Dairy NAHMS study, 47% of multiparous cows have subclinically low levels of blood calcium in the day after calving (Reinhardt et al., 2011).

Even cows that experience severe clinical hypocalcemia will respond with high PTH secretion; however, in late gestation cows, the ability of tissues to respond to PTH may be diminished (Goff et al., 1989). Goff et al. (2014) showed that feeding a diet with a negative dietary cation-anion difference [DCAD; (Na + K) – (Cl + S)] resulted in increased sensitivity of tissues to PTH and a more efficient response to decreased blood calcium. Feeding a prepartum ration with a low or negative DCAD is an accepted strategy for improving the ability of cows to recover from the initial drop in blood calcium postpartum (Charbonneau et al., 2006). With the implementation of these strategies, the rate of clinical hypocalcemia has been reduced to less than 5% in the U.S. (Reinhardt et al., 2011); however, the use of negative DCAD feeding strategies to minimize subclinical hypocalcemia incidence is still being investigated.

Low potassium rations are commonly used with or without the addition of anion supplements to reduce dietary DCAD and to control the incidence of hypocalcemia in transition cows. Improvements in blood calcium status and performance are evident when the DCAD of the ration is reduced to -10 to -15

mEq/100 g of diet dry matter (Moore et al., 2000, DeGroot et al., 2010). However, many nutritionists and producers will implement a partial supplementation strategy, either due to starting cation concentrations in the ration as a result of available forages, to control costs, or because of ease of implementation given variability of mineral content of forages. The relative efficacy of a partial versus full anion supplementation strategy, in comparison to a low potassium ration without anion supplementation, has not been fully evaluated. Previous work compared varying levels of anion supplementation by feeding diets containing decreasing levels of DCAD (15 mEq, 0 mEq and -15 mEq/100 g of diet DM) in the prepartum diet; however, dietary calcium concentrations were increased as DCAD decreased. Multiparous cows fed the lowest DCAD had the highest plasma ionized calcium concentrations at calving, and tended to have the lowest incidence of hypocalcemia compared to cows fed the +15 mEq and 0 mEq/100 g DM rations (Moore et al., 2000). Dietary calcium in this study ranged from 0.44 to 1.50% of diet dry matter and therefore the impact of increasing calcium and decreasing DCAD likely both contributed to the differences in calcium status.

The objective of this study was to determine the effect of increasing anion supplementation, while maintaining a high dietary calcium, on plasma mineral status, intake and performance of multiparous Holstein cows. We hypothesized that feeding decreasing DCAD would result in improved plasma mineral status and increased intake and milk production.

EXPERIMENTAL APPROACH

All animal procedures were approved prior to the commencement of the trial by the Cornell University Institutional Animal Care and Use Committee. Multiparous Holstein cows (n=89) were fed a control diet (Table 1) beginning at 31 to 38 d prior to expected calving. At 24 d prior to expected calving cows were assigned randomly to receive the low potassium control ration (CON; n=30), a low potassium ration with partial anion supplementation (MedDCAD; n=30) or a low potassium ration with full anion supplementation (LowDCAD; n=29). Randomization was restricted to balance for previous mature equivalent 305 d milk yield, parity, and body conditions score (BCS). After calving, all cows were milked three times daily and fed a common postpartum ration through 63 DIM.

Diets were formulated using CNCPS version 6.1. The prepartum diets were based on brown mid-rib (BMR) corn silage, wheat straw, and a common grain mix (Table 1). Anion supplementation was administered with a small inclusion rate grain mix containing varying levels of anion supplement and distillers grains. Adjustments in inclusion rates of these mixes were made throughout the trial to maintain urine pH of the LowDCAD treatment between 5.5 and 6.0. Equal adjustments were made to all treatments to maintain equal nutrient composition (Table 1).

Table 1. Ingredient composition and analyzed nutrient composition of three prepartum diets with varying DCAD levels and the common postpartum diet.

	CON	MedDCAD	LowDCAD	Lactating
Ingredients (% of DM)				
BMR corn silage	44.8	44.8	44.8	37.3
Wheat straw	28.1	28.1	28.1	5.9
Alfalfa silage	-	-	-	9.8
Amino Plus ¹	8.1	8.1	8.1	7.1
Citrus pulp	3.3	3.3	3.3	3.9
Soybean hulls	2.3	2.3	2.3	-
Canola meal	2.2	2.2	2.2	5.9
Corn distillers grains	2.2	1.3	0.4	2.0
Corn gluten feed	-	-	-	3.9
Wheat midds	3.2	2.6	1.9	-
Ground corn grain	0.42	0.42	0.42	19.6
Molasses	0.67	0.67	0.67	-
LysAAMet ²	-	-	-	0.78
Megamine L ³	-	-	-	0.39
Alimet ⁴	-	-	-	0.06
Megalac R ³	-	-	-	0.39
Calcium diphosphate	0.46	0.46	0.46	-
Calcium carbonate	2.9	2.8	2.7	-
Magnesium oxide	0.56	0.42	0.25	-
Min-Ad ⁵	-	-	-	1.6
Animate ⁶	-	2.0	4.0	-
Urea	0.42	0.21	-	-
Salt	0.25	0.25	0.25	0.39
Sodium bicarbonate	-	-	-	0.78
Vitamin/mineral mix	0.14	0.14	0.14	0.22
Rumensin ⁷	0.01	0.01	0.01	0.06
Chemical Analysis				
(Mean ± SD)				
DM (%)	46.3 ± 1.6	46.5 ± 1.3	46.4 ± 1.1	45.7 ± 1.8
CP (% DM)	13.0 ± 0.3	13.2 ± 0.4	13.2 ± 0.5	15.7 ± 0.2
ADF (% DM)	30.2 ± 0.7	30.5 ± 1.3	30.1 ± 1.3	20.6 ± 0.8
NDF (% DM)	44.3 ± 1.2	44.0 ± 2.1	43.2 ± 1.8	31.1 ± 1.0
Starch (% DM)	17.0 ± 0.5	16.0 ± 0.8	16.3 ± 0.9	26.0 ± 0.7
NFC (% DM)	33.6 ± 0.9	34.3 ± 2.5	35.0 ± 1.9	45.8 ± 1.2
Fat (% DM)	1.1 ± 0.1	1.3 ± 0.2	1.1 ± 0.3	2.3 ± 0.2
Ca (% DM)	1.54 ± 0.12	1.57 ± 0.14	1.57 ± 0.07	0.95 ± 0.03
P (% DM)	0.44 ± 0.01	0.43 ± 0.01	0.41 ± 0.01	0.41 ± 0.02
Mg (% DM)	0.47 ± 0.01	0.48 ± 0.03	0.50 ± 0.03	0.44 ± 0.02
K (% DM)	1.28 ± 0.07	1.26 ± 0.06	1.24 ± 0.07	1.37 ± 0.05
S (% DM)	0.20 ± 0.01	0.30 ± 0.02	0.41 ± 0.02	0.29 ± 0.01
Na (% DM)	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.44 ± 0.02
Cl (% DM)	0.27 ± 0.03	0.47 ± 0.05	0.69 ± 0.04	0.40 ± 0.02
DCAD (mEq/100g DM)	18.3 ± 0.8	5.9 ± 3.4	-7.4 ± 3.6	25.0 ± 1.5
Predicted MP (g/kg DM)	93.8	93.23	92.26	116.56

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All cows were fed for ad libitum intake once daily at approximately 0800 h and feed delivered and refused was recorded daily. Samples of rations and ingredients were collected weekly and a subsample was used for dry matter determination. Four week composites of all rations and ingredients were analyzed for nutrient composition by wet chemistry methods (Cumberland Valley Analytical Services, Hagerstown, MD). Milk weights were recorded daily and milk samples were collected at three consecutive milkings once per week and analyzed for fat, true protein, lactose, total solids, urea nitrogen, and somatic cell count (Dairy One Laboratories, Ithaca, NY). Body weights were measured weekly. Body condition scores were assigned by two scorers weekly using a 5-point scale (Wildman et al., 1982) and the average of the two scorers was used for analysis. Data were collected through 63 DIM.

To determine degree of compensated metabolic acidosis, clean midstream urine samples were collected on one day during the week before assignment to treatments and three times weekly during the prepartum period at 9 h post feeding and urine pH was measured by a calibrated, glass electrode pH meter (model UP-5 pH meter, Denver Instruments, Denver, CO). Samples of blood were obtained from each cow via coccygeal venipuncture into lithium heparin vacutainers at 0700 h on one day during the week prior to assignment to treatment, twice weekly from 24 d prior to expected calving until calving, twice during the first 24 h postcalving, daily through 5 DIM and three times per week thereafter through 56 DIM. Plasma was harvested, snap frozen in liquid nitrogen and frozen at -20°C until analysis. Plasma minerals (Ca, P, Mg, K, Na, Cl, bicarbonate, and anion gap) were determined on one sample per a week prepartum and on all samples collected through 14 DIM. Samples were sent to the Cornell Animal Health and Diagnostic Center (Ithaca, NY) for mineral panel analysis.

Repeated measures data were analyzed using the REPEATED statement in the MIXED procedure of SAS version 9.3 (SAS Institute Inc., Cary, NC). Prepartum and postpartum data were analyzed separately. Postpartum intake, milk production and milk composition were analyzed as wk 1 to 3 and wk 1 to 9 to determine effects that were manifested primarily in the early lactation period. The effects of treatment, time, parity (2nd vs. 3rd and greater), and all two way interactions were included in the model, when $P > 0.10$ two way interactions with parity were removed from the model. Covariate measurements were included for plasma variables, urine pH, intake, and milk production. For repeated measures variables, four covariance structures were tested (autoregressive, heterogeneous autoregressive, compound symmetry and heterogeneous compound symmetry) and the covariance structure with the Akaike's information criterion closest to zero was used.

The Kenward-Roger option was used in the model statement to estimate degrees of freedom. F-tests for differences between treatment groups at individual timepoints were determined using the slice option in the LSMEANS statement. Linear and quadratic effects of decreasing DCAD were tested using orthogonal

contrasts. Fisher's exact test was used to determine differences in frequency of hypocalcemia for parity groups by day after calving. Least squares means are reported throughout. Significance was declared at $P \leq 0.05$ and trends are discussed at $0.05 < P < 0.10$.

RESULTS

Feeding decreasing DCAD in the prepartum period resulted in a quadratic effect on urine pH ($P < 0.01$) as depicted in Figure 1. Mean urine pH during the treatment period was 8.20, 7.84 and 5.98, for CON, MedDCAD and LowDCAD, respectively. These results suggest that the dietary strategies employed were effective in modulating cow physiology consistent with the principles of dietary DCAD manipulation.

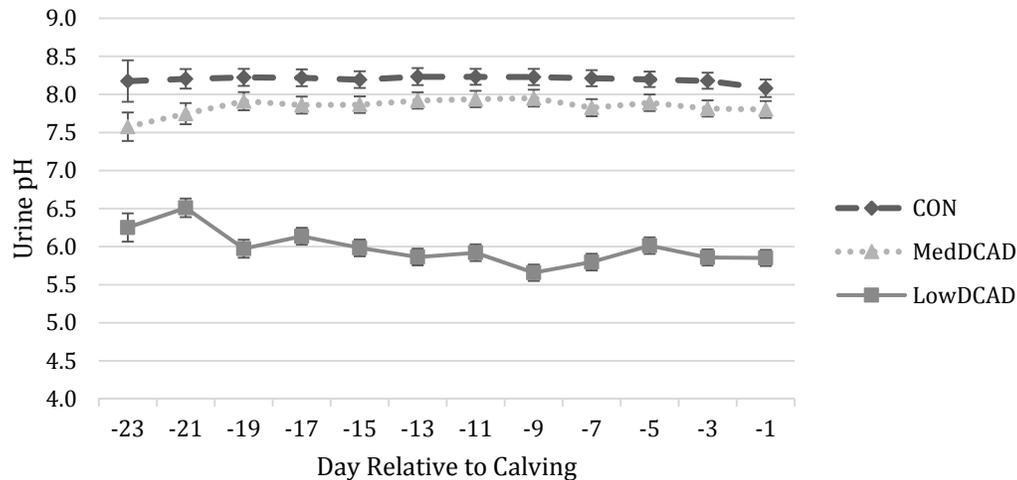


Figure 1. Urine pH least squares means and standard errors during the treatment period for cows fed one of three levels of DCAD prepartum.

Prepartum plasma mineral concentrations were not affected by DCAD treatment with the exception of a trend for a treatment by parity interaction for prepartum plasma magnesium. Older cows (3rd lactation and greater) fed the LowDCAD diet prepartum had lower plasma magnesium prepartum compared to older cows fed the CON and MedDCAD diets (1.89, 1.85 and 1.76 mg/dL for CON, MedDCAD and LowDCAD, respectively; $P = 0.07$). There was a linear effect on mean postpartum plasma calcium over the first 14 DIM such that decreasing prepartum DCAD resulted in increased postpartum plasma calcium concentrations (8.84, 8.89 and 9.19 for CON, MedDCAD and LowDCAD, respectively; $P < 0.01$). There was also a tendency for decreased postpartum plasma phosphorous with decreasing prepartum DCAD (4.74, 4.67 and 4.49 for CON, MedDCAD and LowDCAD, respectively; $P = 0.08$). A trend for a treatment by day interaction was observed for postpartum plasma calcium ($P = 0.08$) and a significant treatment by day interaction was observed for postpartum plasma magnesium ($P < 0.01$). Plasma calcium was higher, or tended to be higher, for 5 d postpartum for cows

fed a lower DCAD and plasma magnesium was lower for cows fed a lower DCAD for 2 d postpartum (Figure 2).

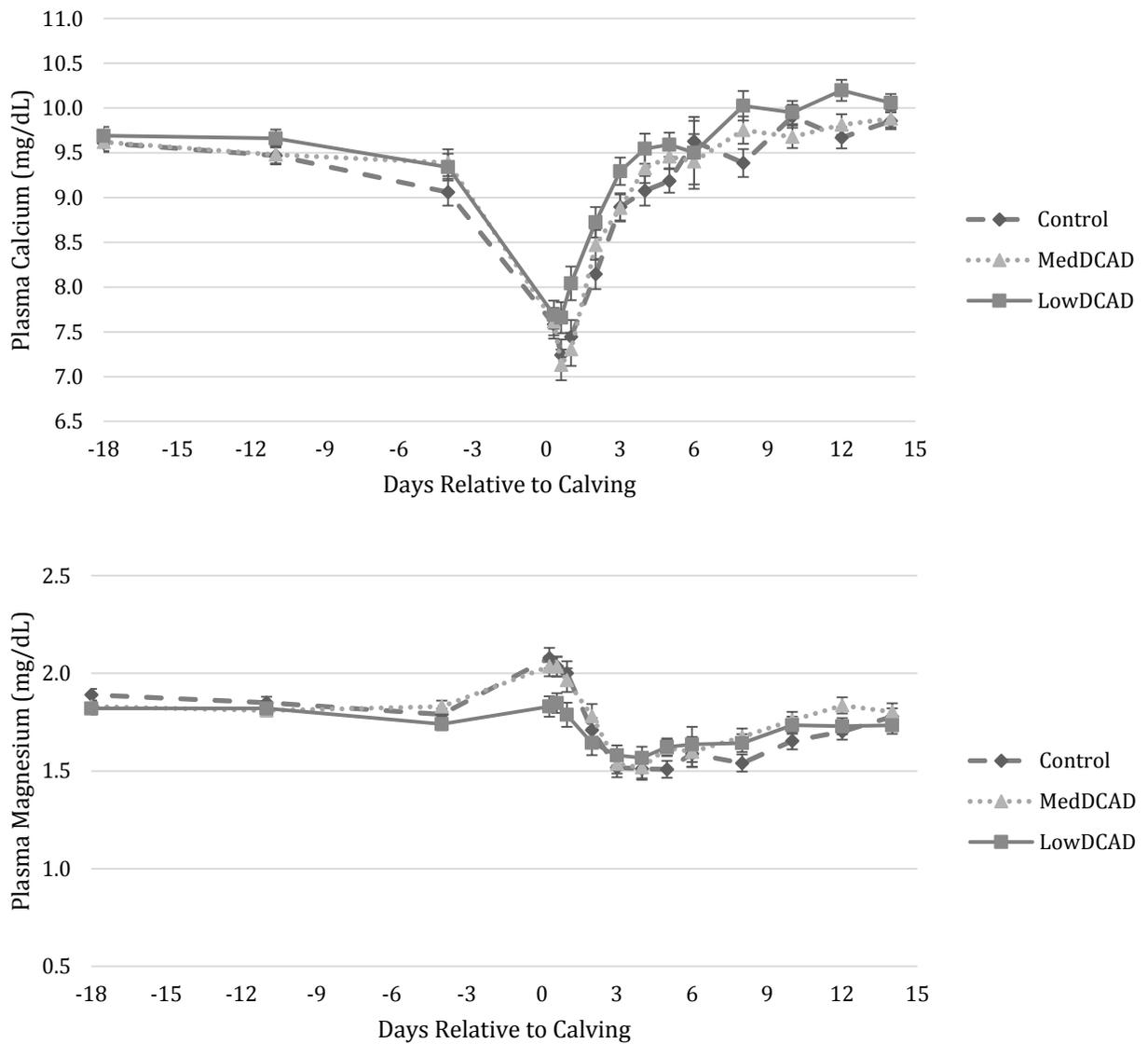


Figure 2. Least squares means and standard errors of plasma calcium (panel A) and plasma magnesium (panel B) in the period around calving for cows fed one of three levels of DCAD in the prepartum period.

A tendency for an interaction between treatment and parity was seen for postpartum plasma calcium such that older cows (3rd lactation and greater) had greater responses in postpartum plasma calcium concentration when fed the LowDCAD diet prepartum compared to the second lactation cows (Figure 3; P=0.06).

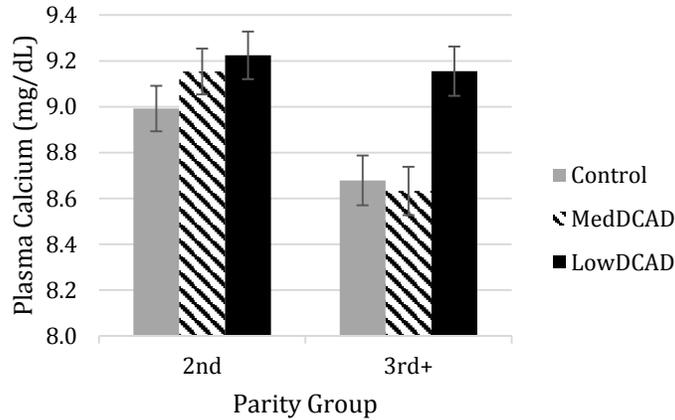


Figure 3. The interaction between parity group and prepartum DCAD level for mean plasma calcium over the first 14 DIM.

The effect of prepartum DCAD level on postpartum hypocalcemia incidence by day was also determined by testing the difference in the proportion of cows classified as hypocalcemic (plasma calcium <8.5 mg/dL) between treatments at each sampling point through 5 d postpartum. For second lactation cows, no difference in hypocalcemia incidence was observed between treatment groups at any of the sampling points in the 5 d postpartum. Incidence of hypocalcemia for older cows in each treatment group by day is shown in Figure 4. For older cows, there tended to be a difference in hypocalcemia incidence at the second sampling on d 0 (approximately 17.5 h postpartum), with significant differences in hypocalcemia incidence at d 1 (85%, 100% and 57% for CON, MedDCAD, and LowDCAD, respectively; $P=0.01$) and d 2 (69%, 57% and 14% for CON, MedDCAD and LowDCAD, respectively; $P<0.01$).

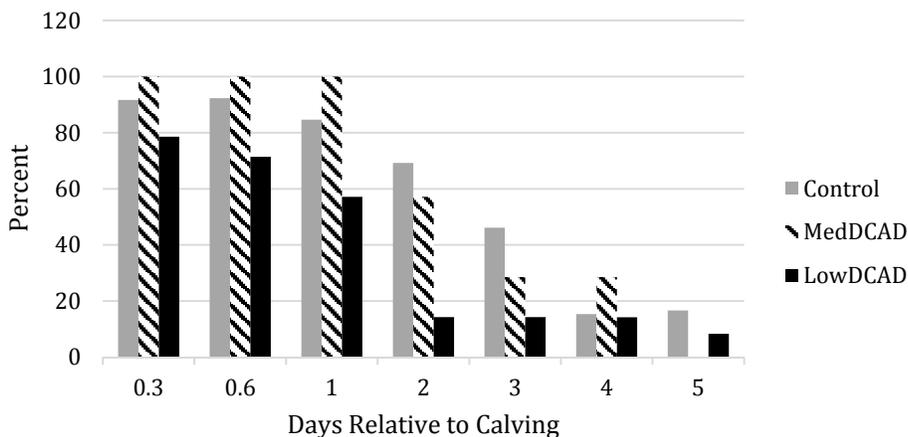


Figure 4. Proportion of cows in each treatment group entering their third lactation or greater with plasma calcium <8.5 mg/dL for each sampling point in the 5 days postpartum.

The effect of decreasing prepartum DCAD on DMI in the prepartum and postpartum periods is described in Table 2. A quadratic effect was seen such that cows fed MedDCAD in the prepartum period had the highest prepartum DMI ($P<0.01$); however, DMI were similar and within desired ranges for all groups during the prepartum period (14.6, 15.1, and 14.1 kg/d for CON, MedDCAD and LowDCAD, respectively). Individual F-tests indicated that intakes differed only at wk -4 and -3 relative to calving, with no difference in DMI detected between treatment groups at wk -2 and -1. A linear effect on postpartum DMI as a percent of body weight was seen such that cows fed decreasing DCAD prepartum had increasing postpartum intake as a percent of body weight in wk 1 to 3 ($P=0.03$), this effect tended to carry out through wk 9 of lactation ($P=0.07$). No effect of prepartum DCAD level on calculated energy balance was seen in the prepartum or postpartum periods.

Table 2. Least squares means of dry matter intake in the prepartum and postpartum periods for cows fed one of three prepartum DCAD levels.

Variable	Prepartum Diet			SEM	P-values		
	CON	MedDCAD	LowDCAD		Linear Contrast	Quadratic Contrast	Trt×Wk
Prepartum							
DMI, kg/d	14.6	15.1	14.1	0.2	0.15	0.01	0.45
DMI, % of BW	1.87	1.89	1.80	0.03	0.16	0.16	0.34
Energy Balance, Mcal/d	5.90	6.41	5.68	0.35	0.66	0.14	0.57
Postpartum (wk 1 to 3)							
DMI, kg/d	21.0	21.7	22.3	0.5	0.07	0.88	0.24
DMI, % of BW	2.94	3.04	3.15	0.07	0.03	0.99	0.37
Energy Balance, Mcal/d	-8.44	-8.51	-8.54	0.92	0.94	0.98	0.60
Postpartum (wk 1 to 9)							
DMI, kg/d	24.7	25.7	25.3	0.4	0.28	0.14	0.49
DMI, % of BW	3.48	3.61	3.61	0.05	0.07	0.23	0.48
Energy Balance, Mcal/d	-3.97	-3.39	-3.96	0.53	0.99	0.36	0.58

There was a linear effect of feeding a decreasing DCAD in the prepartum period on milk yield in wk 1 to 3 of lactation ($P=0.03$) such that cows fed decreasing prepartum DCAD had increasing postpartum milk yield (40.5, 42.1, and 43.8 kg/d for CON, MedDCAD and LowDCAD, respectively). A trend for a linear increase in fat-corrected milk yield ($P=0.07$) was seen for cows fed decreasing DCAD. Lactose yield ($P=0.02$) and total solids percent ($P=0.01$) linearly increased with decreasing DCAD, resulting in a trend for a linear increase in total solids yield ($P=0.06$) and energy corrected milk yield ($P=0.08$). A linear decrease in protein percent was observed with decreasing DCAD in wk 1 to 3 postpartum ($P<0.01$); however, there was no difference in protein yield. Milk urea nitrogen linearly decreased with decreasing DCAD ($P=0.04$). Results for milk production and composition in wk 1 to 3 are shown in Table 3. For data collected in wk 1 to 9, a linear effect of lower protein percent for cows fed a lower DCAD

remained (2.99, 2.98, and 2.84% for cows fed CON, MedDCAD, and LowDCAD, respectively; P=0.02) as well as a linear decrease in total solids percent (12.51, 12.51, and 12.28% for cows fed CON, MedDCAD, and LowDCAD, respectively; P=0.02). In wk 1 to 9, numerical differences in milk yield (47.1, 48.5, and 48.7 kg/d for cows fed CON, MedDCAD, and LowDCAD, respectively) and fat-corrected milk yield (48.6, 49.8, and 50.1 kg/d for cows fed CON, MedDCAD, and LowDCAD, respectively) were observed but there were no significant differences.

Table 3. Least square means for milk yield and milk composition during wk 1 to 3 for cows fed one of three levels of DCAD prepartum.

Variable	Prepartum Diet			SEM	P-values		
	CON	MedDCAD	LowDCAD		Linear Contrast	Quadratic Contrast	Trt×Wk
Milk yield, kg/d	40.5	42.1	43.8	1.1	0.03	0.97	0.35
Fat, %	4.38	4.36	4.24	0.08	0.21	0.63	0.10
Fat, kg	1.74	1.81	1.87	0.06	0.13	0.99	0.58
3.5 % FCM, kg/d	45.6	47.5	49.3	1.4	0.07	0.98	0.52
True protein, %	3.54	3.49	3.27	0.07	0.01	0.33	0.36
True protein, kg	1.36	1.42	1.42	0.34	0.21	0.57	0.09
Lactose, %	4.64	4.67	4.69	0.03	0.25	0.94	0.38
Lactose, kg	1.89	1.98	2.09	0.06	0.02	0.84	0.02
Total Solids, %	13.63	13.61	13.27	0.10	0.01	0.20	0.10
Total Solids, kg	5.42	5.65	5.86	0.17	0.06	0.96	0.13
ECM, kg/d	46.1	48.0	49.5	1.4	0.08	0.89	0.39
ECM/DMI	2.22	2.25	2.28	0.07	0.55	0.99	0.71
MUN, mg/dL	10.32	9.72	9.44	0.30	0.04	0.67	0.17
SCS	2.62	3.26	2.73	0.25	0.75	0.06	0.27

CONCLUSIONS AND IMPLICATIONS

Full anion supplementation to a low potassium ration in the prepartum period, with targeted urine pH values of 5.5 to 6.0, resulted in improved postpartum calcium status when compared to a low potassium ration with either zero or partial anion supplementation. Cows entering their third lactation or greater benefited the most from full anion supplementation and had significantly decreased incidence of hypocalcemia. Dry matter intake and milk production both linearly increased postpartum as prepartum DCAD decreased, particularly in the first 3 weeks of lactation. While some benefits are seen with partial anion supplementation, this study would suggest that full anion supplementation is necessary for improved calcium status as well as improved performance.

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