Prepartum dietary DCAD and calcium concentrations effect on blood metabolites and performance of multiparous cows

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ABSTRACT

Eighty-two multiparous Holstein cows were enrolled 28 d prior to calving and remained on trial through 63 days in milk (DIM) in a randomized block design experiment with a 2 X 2 factorial arrangement of treatments. Dietary treatments provided two dietary cation-anion concentrations (DCAD): -22 (NEG) or -3 mEq/100 g DM (NEU); and two dietary Ca concentrations: 1.3% or 1.8% of DM. Cows were individually fed and dry matter intake (DMI) recorded daily. Urine pH was lower for NEG compared with NEU and tended to be lower for 1.8% compared with 1.3% Ca. Prepartum concentrations of urine Ca were higher whereas urine creatinine and urine K were lower for NEG compared with NEU. Fractional excretion of Ca and Mg was greater for NEG than NEU. Plasma bicarbonate was lower and plasma total P was higher for NEG compared with NEU. Plasma total P and blood urea nitrogen (BUN) to creatinine ratio was higher for 1.3% compared with 1.8% Ca prepartum. From 0 to 3 DIM ketone and ratio of ionized Ca/ionized Mg were higher for NEG compared with NEU whereas total protein, albumin, total Mg, ionized Mg were higher for NEU compared with NEG. Interactions of DCAD and DIM were observed for plasma total Mg and ionized Mg, as concentrations were similar on 0 and 3 days in milk but were higher for NEU on d 1 and 2. An interaction of Ca and DIM was observed for plasma total Ca due to higher concentration at d 1 for 1.8%, whereas concentrations were not different at d 2 or 3. After calving, cows were milked 3 times daily, yield recorded at each milking and samples collected once weekly for analysis of components. No differences were observed in body weight or body condition score due to DCAD or Ca. Prepartum dry matter intake (DMI) was lower for NEG compared with NEU and lower for 1.8% compared with 1.3% Ca. Postpartum DMI was not different among treatments. An interaction was observed for DCAD and DIM due to higher milk yield after 45 DIM for NEG compared with NEU. No differences were observed in percentage or yield of milk components among treatments. These results suggest that feeding -22 mEq/100 g DM DCAD prepartum alters plasma and urine mineral concentrations, compared to feeding -3 mEq/100 g DM DCAD and also supports increased milk yield after 45 DIM. Feeding 1.8% Ca prepartum improved plasma Ca at 1 DIM. Feeding -22 mEq/100 g DM compared with -3 mEq/100 g DM DCAD, or 1.8% Ca compared to 1.3% Ca reduced DMI prepartum.
KEY WORDS: DCAD, Ca, milk yield

INTRODUCTION

During the transition period, cows are at highest risk for metabolic diseases and health complications. Hypocalcemia often arises from the increase Ca demands paired with the delay in Ca absorption and mobilization after calving, resulting in low blood Ca concentrations. Cow with hypocalcemia have lower milk production and increased risk of other health disorders like mastitis, displaced abomasum, and retained placenta (Curtis et. al. 1985). Block (1984) and Oetzel (1991) determined that feeding anions to create a negative DCAD diet prepartum effectively altered Ca homeostasis to decrease the incidence of milk fever and improve production and health compared to positive DCAD.

Proper Ca supplementation during the prepartum period aids in meeting requirements for the cow and calf and also prepares mechanisms needed to meet the extreme Ca demand immediately postpartum. Cows fed negative DCAD diets prepartum have a Ca requirement of 1.0-1.5% (NRC, 2001). However, inclusion within the NRC recommendation to further alter Ca homeostasis has been debated by researchers and consultants given the higher milk yield of modern dairy cows. Mixed results have been reported on intake and blood Ca concentration due on inclusion rates. Some have observed that inclusion rates above 1.0% decrease DMI (Miller, 1983) while others have fed rates up to 1.8% with no effects on DMI (Beede & Shearer, 1991). Chan et. al. (2006) reported no change in blood Ca concentration when diets were supplemented 0.99% compared with 1.5% calcium in diets with a -6 meq/100g DCAD. Oba et. al. (2011) demonstrated improved blood Ca concentration feeding 0.9% compared to 0.3%. Based on these results, 1.0% Ca prepartum with a negative DCAD diet seems sufficient for maintaining blood Ca during the immediate postpartum period. The objective of this trial was to determine the effect of feeding a neutral or fully acidified diet varying in Ca concentration during the close-up dry period on plasma Ca status, health and postpartum performance.

MATERIALS AND METHODS

All methods were reviewed and approved by the University of Georgia Animal Care and Use Committee prior to conducting the trial. Eighty-two multiparous Holstein cows were enrolled in a randomized block design experiment from January to October 2016. Cows were assigned to 1 of 4 dietary treatments in a randomized block design experiment with a 2 X 2 factorial arrangement of treatments. Enrollment was 28 ± 4 d prepartum and conclusion at 63 DIM. Cows were blocked on expected calving date and parity. Dietary treatments provided two dietary cation-anion concentrations (DCAD): -22 (NEG) or -3 mEq/100 g DM (NEU); and two dietary Ca concentrations: 1.3% or 1.8% of DM, providing a total of four experimental diets: NEU-1.3 (n=20), NEU-1.8 (n=21), NEG-1.3 (n=20) and NEG-1.8, referring to the level of DCAD and Ca supplementation.

Animals were trained to eat behind Calan gates (American Calan, Northwood, NH) prior to initiation of the trial. Cows were housed in a 4-row sand bedded freestall barn with equipped with fans and misters for evaporative cooling. Cows had with free access to a dry lot 24 h/d. Cows were fed once daily and had access to water at all times. Experimental diets (Table 1) and a common lactating diet were formulated to meet or exceed NRC requirements with 5% refusals. Base ingredients were initially mixed using a Khun mixer wagon to form a base mix. The base mix was then added to premix supplements and concentrate mix and individually fed using a DataRanger. Amounts offered and refused were measured and recorded daily. Feed was manually pushed up by hand two times daily.

Samples of individual feed ingredients, experimental diets, and the common lactating diet were collected 3 times each week. Dry matter was determined using a forced air drying oven set at

55°C for 48 h. Samples were ground to pass through a 6mm screen and then a 2mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and composited by week. Samples were analyzed for protein (LECO FP-5258 Nitrogen Analyzer, St. Joseph, MO), ADF (AOAC International, 2000), NDF adjusted for ash (Van Soest et al., 1991), ether extract (AOAC International, 2000), and minerals (AOAC International, 2000). Body weight measurements were recorded on 3 consecutive days at the beginning of the trial and averaged to determine initial BW. A BCS was assigned by one individual as described by Wildman et al. (1982) to maintain consistency throughout the trial. Body weight and BCS was measured again immediately post calving and at 21 and 63 DIM.

Blood samples were collected from the coccygeal vessel once per week at -3, -2, -1 wk, and -2 d prior to expected calving date, immediately after calving (D0), at 1 (D1), 2 (D2), and 3 (D3) DIM. Blood was analyzed for selected metabolites: total protein, albumin, globulin, urea N, creatinine, total bilirubin, glucose, AST, CK, GGT, Ca, Mg, Na, K, Cl, bicarbonate, anion gap, (Siemens Medical Solutions USA, Inc., Malvern, PA), iCa, and ionized Mg (iMg) (pHOx Ultra, Nova Biomedical, Waltham, MA). Serum was separated from the second sample for analysis of NEFA concentrations (Waco Chemicals USA Inc., Richmond, VA). Concentrations of beta-hydroxybutyrate (BHB) concentrations were determined using a Nova Max Ketone Strop and a Nova Max Plus reader (Nova Biomedical, Waltham, WA). Pre-partum urine samples were collected once per week prior to calving, -3 wk for pH analysis and -2 and -1 wk for pH analysis of minerals (Ca, Mg, Na, K, and Cl) and creatinine concentrations as described previously. Additionally, pH was assessed using urine strips at the beginning of the trial to ensure proper acidification level via DCAD was being met.
After calving, cows were milked 3 times per day at 0800, 1600, and 2400 h. Milk weights were electronically recorded each milking (Alpro, Deleval, Kansas City, MO) and summed daily. Milk samples were collected once each week at three consecutive milking. Samples were refrigerated and shipped for next day delivery to Dairy One Cooperative (Ithaca, NY) for analysis of fat, protein, lactose, MUN and SCC using a Foss 400 instrument (Foss North America, Eden Prairie, MN) as described by AOAC International (2000).

Data were subjected to repeated analysis of variance using PROC MIXED procedures of SAS (SAS Institute, Cary, N. C.). The model included block, DCAD treatment, Ca treatment, interaction of DCAD and Ca, week and the interactions of week and treatments. The first-order autoregressive covariance structure was used according to Littell et al. (1998). Previous lactation ME milk, fat and protein yield along with average lactation SCC were used as covariates in the analysis of production data. Significance was declared at $P < 0.05$ and a trend when $P > 0.05$ and < 0.1.

RESULTS and DISCUSSION

The chemical composition of the experimental diets is outlined in Table 2. The prepartum experimental diets were originally formulated to provide 1.0 and 1.3% Ca on a DM basis; however actual concentrations were 1.3 and 1.8%, respectively. The increase was due to higher concentrations of calcium in the concentrate and wet brewers grains. Diets were originally formulated to be 0 and -15% DCAD for NEU and NEG treatments, but additional Animate was required to achieve the desired urinary pH resulting in final DCAD concentrations of -3 and -21 mEq/100g DM for NEG and NEU respectively. Concentrations of other nutrients were similar across all treatments. The postpartum diet was in agreement with formulated values.

No interactions of DCAD and Ca or treatment and day's prepartum were observed for DMI, expressed as kg/d or % of BW. Prepartum DMI was lower for cows fed NEG (P = 0.0096) compared with NEU and averaged 14.2 and 16.0 kg/d for NEG and NEU, respectively (Table 3, Figure 1). Feeding diets supplemented with 1.8% compared with 1.3% Ca also decreased (P = 0.0038) DMI which average 16.2 and 14.0, respectively (Table 3, Figure 2). Similar changes were observed for DMI expressed as a percentage of BW (1.94 and 2.13% of BW for NEG and NEU [Figure 3], respectively and 2.18 and 1.89% of BW for 1.3 and 1.8% Ca [Figure 4], respectively).

No differences (P > 0.10) were observed in changes of BW or BCS throughout the trial (Figure 5 and 6, respectively). Prepartum BW and BCS averaged 754.8 kg and 3.36, respectively. Postpartum BW at calving, 21 and 63 DIM averaged 707.2, 654.9, and 638.3 kg, respectively. Corresponding BCS were 3.18, 3.03, and 2.92 at calving, 21 and 63 DIM, respectively. No differences (P > 0.10) were observed in postpartum DMI (Figure 7) or percentage or yield of milk components among treatments (Table 3). However, milk yield was higher (DCAD x DIM, P = 0.0125) for NEG compared with NEU after 30 DIM, but most noticeably after 45 DIM (Figure 8). Concentrations of MUN exhibited an interaction of Ca and week postpartum (P = 0.0412, Figure 9) due to lower concentrations at 5 wk and higher concentration at wk for cows fed 1.3% Ca compared with 1.8%.

The reduction in DMI prepartum is consistent with previous research in which negative DCAD diets (Charbonneau et. al., 2006; Otezel, 1993; and Moore et. al., 2000). Miller (1983) reported reduced DMI when greater than 1% Ca was fed; however, Beede and Shearer (1991) fed Ca at inclusion rates up to 1.8% without any effect on DMI. In our current trial, increasing dietary Ca from 1.3 to 1.8% increased total ash content by approximately 1.2% which may have resulted in decreased palatability and/or increased fill that reduced in decreased DMI. However, DMI was not reduced below that predicted by NRC (2001) for multiparous Holstein cows. No changes in BW or BCS would be expected unless the reduction in DMI persisted into lactation resulting in a greater negative energy balance than normal which was not the case in the current trial as postpartum DMI was not different among treatments. This in in contrast to the reports of DeGroot et. al. (2010) and Leno et. al. (2017) who observed improved DMI for cows fed negative DCAD diets prepartum. This may partially explain the differences in our current trial compared with Leno et al. (2017) who reported improved milk yield immediately postpartum whereas milk yield in the current trial did not improve until approximately 45 DIM. Another factor that could have affected the results observed in our current trial is that the majority of the cows calved during the summer and early fall which was characterized by higher than normal THI.

Concentrations of prepartum metabolites in blood are presented in Table 4. There was a tendency for lower Na (142.64 vs. 143.25 mEq/L, P = 0.0646) and higher Cl (104.77 vs. 102.10 mmol/L, P = 0.0519) resulting in lower bicarbonate concentrations (26.22 vs 27.59, P = 0.0011) for cows fed NEG compared with NEU, respectively. An interaction of day prepartum and DCAD was observed for creatine kinase (P = 0.0031, Figure 10). Cows fed diets supplemented with 1.3

compared with 1.8% Ca had higher concentration of BUN (22.3 vs. 19.8 mg/dL, P = 0.0056), BUN to Creatinine ratio (31.89 vs. 27.81, P = 0.015), and P (7.35 vs 6.76 mg/dL, P = 0.0013). An interaction of day prepartum and Ca was observed for albumin (P = 0.0297, Figure 11) and Na (P = 0.0363, Figure 12).

Urinary metabolite concentrations and fractional excretion rates are presented in Table 5. Cows fed the NEG had higher concentrations of Ca (42.31 vs. 29.22 mg/dL, P = 0.0207) and lower concentrations of creatinine (62.81 vs. 92.75 mg/dL, P = 0.0027) and K (131.01 vs. 167.20 mmol/L, P = 0.0096), and lower pH (6.03 vs. 7.05, P < 0.0001) compared with NEU. An interaction of days prepartum and Ca was observed for creatinine which was higher for 1.3 compared with 1.8 at -2 d prepartum (Figure 13, P = 0.0500). Fractional excretion rates were higher for cows fed NEG compared with NEU for Ca (0.19 vs. 0.13, P = 0.0156) and Cl (1.47 vs 1.03, P < 0.0001). Feeding NEG DCAD diets has been demonstrated to increase blood and urinary Ca concentrations because of greater absorption resulting in higher fractional excretion rates to maintain Ca homeostasis. Lower creatinine most likely reflects greater water intake and excretion. Cows fed diets supplemented with 1.3 compared with 1.8% Ca tended to had higher urinary pH (6.75 vs 6.33, P = 0.0628) and fractional excretion rates of Na (0.28 vs 0.18, P = 0.0262) and K (35.23 vs 30.42, P = 0.0375).

The decrease in urine pH and change in plasma metabolites observed for NEG are consistent with previous research in which increasing acidification of the diet creates a mild metabolic acidosis which decrease urinary pH and alters blood metabolite concentrations and excretion (Charbonneau et. al. 2006; Jardon, 1995; Moore et. al. 2000; Vagoni and Otzel, 1998). Cows fed NEG had higher urinary concentrations of Ca and greater fractional excretion of Ca and Cl which is consistent with feeding a fully acidified diet (Leno et. al., 2017).

Increasing dietary Ca to 1.8% decreased urinary pH compared with 1.3%. The reason for this change is not apparent nor has it been observed in previous research. The decrease in BUN and fractional excretion of Na and K observed for cows fed 1.8 compared with 1.3% Ca is most likely related to the lower DMI which would have reduced total protein Na and K intake.

Blood metabolite concentrations during 0-3 DIM are presented in Table 6. Beta-hydroxybutyrate concentrations were higher (P = 0.0548, 1.12 vs 0.95 mmol/ml), but total protein (P = 0.0065, 6.46 vs 6.79 g/dL), albumin, P = 0.0070, 3.44 vs 3.57 mg/dL), ionized Mg (P = 0.0196, 0.59 vs 0.62 mmol/L) and the ratio of iCa to iMg (P = 0.0464, 2.02 vs 1.91) were lower for NEG compared with NEU, respectively. An interaction of DCAD and Ca (P = 0.0165) was observed for Mg concentrations which were lower for NEG-1.3% Ca compared with the other treatments. An interaction of DCAD and DIM was observed for Mg (P = 0.0349, Figure 14) and iMg (P = 0.0021, Figure 15) which was lower at 1 and 2 DIM for NEG compared with NEU. An interaction of Ca and DIM was observed for total plasma Ca which was higher for 1.8 compared with 1.3 at 1 DIM (P = 0.0030, Figure 16). Plasma anion gap concentrations were lower for cows fed 1.3% Ca at 1 DIM compared with 1.8% Ca, but were not different at 0, 2 or 3 DIM (P = 0.0038, Figure 17).

The lower total protein and albumin concentrations observed for NEG compared with NEU at 0-3 DIM suggest lower inflammation postpartum. Feeding NEG slightly lowered Mg and iMg at 0-

3 DIM compared with NEU which is most likely due to slight differences in prepartum dietary Mg concentrations (0.51 and 0.54% of DM for NEG and NEU, respectively) and lower DMI observed for NEG. The trend for higher beta-hydroxybutyrate at 0-3 DIM is most likely due to the lower DMI prepartum for NEG compared with NEU.

No differences were observed among Ca treatments postpartum. Shire and Beede (2013) suggested that 1.0% Ca prepartum was sufficient to maintain blood Ca. Based on the results of our current trial, 1.3% Ca provided adequate Ca during the prepartum period.

CONCLUSIONS

Results of this trial indicate that feeding fully acidified diet (NEG) as defined by maintaining a urine pH of 5.5 to 6.0 alters plasma and urine mineral concentrations to successfully improve plasma Ca concentrations by 3 DIM and increased milk yield from 45 through 63 DIM, compared to NEU. Feeding 1.8% Ca prepartum improved total plasma Ca at 1 DIM only compared to 1.3% Ca, but reduced prepartum DMI. Postpartum DMI and milk composition was not different among Ca treatments.

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Table 1. Ingredient composition of diets differing in DCAD and calcium concentration prepartum and common postpartum diet (% of DM).

DCAD	NEU[1]		NEG		
Ca	1.3%	1.8%	1.3%	1.8%	Postpartum
Ingredient					
Tift 85	15.00	15.00	15.00	15.00	4.32
Corn silage	43.33	43.33	43.33	43.33	38.04
Brewers grains, wet	9.17	9.17	9.17	9.17	10.38
QLF-dairy 28					4.32
molasses	8.33	8.33	8.33	8.33	
Ground corn	2.50	2.50	2.50	2.50	12.54
Soybean hulls					5.19
Corn gluten feed					4.76
Citrus pulp					5.19
Cottonseed, whole					2.16
Cottonseed hulls[2]	4.22	2.98	1.83	0.60	
Soybean meal, 48%	5.00	5.00	5.00	5.00	1.30
AminoPlus[3]	5.00	5.00	5.00	5.00	2.59
Prolak[4]	3.33	3.33	3.33	3.33	3.03
Urea					0.17
Mepron[5]					0.05
AjiPro-L[6]					0.26
Megalac[7]					1.73
Omnigen AF[8]	0.38	0.38	0.38	0.38	0.20
Procreatin-7[9]	0.03	0.03	0.03	0.03	0.01
Rumensin[10]	0.33	0.33	0.33	0.33	0.26
Salt	0.13	0.13	0.13	0.13	0.35
Sodium sequinate					1.04
Calcium carbonate[2]	1.27	2.50	1.17	2.40	0.86
Magnesium oxide[2]	0.18	0.18			0.35
Animate[2,11]	1.50	1.50	4.17	4.17	
DCAD Plus[12]					0.52
Dynamate[13]					0.09
TM-Vitamin premix[14]	0.30	0.30	0.30	0.30	0.30

[1] NEG= negative DCAD, NEU= neutral DCAD
[2] Ingredients for treatment premix added individually to mixer prior to feeding
[3] Ruminally protected soybean meal, Ag Processing, Inc. Omaha, NE
[4] Marine-animal rumen undegradable protein supplement, H. J. Baker & Bros., Inc. Westport, CT
[5] Ruminally protected methionine, Envonik, Theodore, AL
[6] Ruminally protected lysine, Ajinomoto, Itasca, IL
[7] Rumen bypass fat, Arm & Hammer Animal Nutrition, Princeton, NJ
[8] Immune stimulant, Phibro Animal Health, Corp., Teaneck, NJ

[9] Yeast culture, Phibro Animal Health, Corp., Teaneck, NJ
[10] Monensin, Elanco, Greenfield, IN
[11] Anionic mineral supplement, Phibro Animal Health, Corp., Teaneck, NJ
[12] Potassium and magnesium sulfate, The Mosaic Company, Plymouth, MN
[13] Potassium carbonate, Arm & Hammer Animal Nutrition, Princeton, NJ
[14] Mineral-vitamin premix contained (DM basis): 29.5% Ca; 0.42% Mg; 0.31% S; 377 ppm Co; 3,472 ppm Cu; 530 ppm Fe; 388 ppm I; 23,882 ppm Mn; 110 ppm Se; 13,313 ppm Zn; 1,221,966 IU/kg Vitamin A; 129,456 IU/kg Vitamin D; 2,817 IU/kg Vitamin E.

Table 2. Chemical composition of diets differing in DCAD and calcium concentration prepartum and common postpartum diet (Mean ± SD).

DCAD	NEU[1]		NEG		
Ca	1.3	1.8	1.3	1.8	Postpartum
DM, %	52.5 ± 4.4	52.7 ± 3.8	53.2 ± 3.9	53.8 ± 3.9	54.7 ± 3.7
	------------ % of DM ------------				
CP	21.4 ± 1.3	21.2 ± 1.5	21.8 ± 1.0	21.3 ± 1.3	17.4 ± 1.4
ADF	17.9 ± 0.6	17.8 ± 1.8	18.9 ± 1.5	18.1 ± 0.5	18.3 ± 2.3
NDF	38.1 ± 1.7	37.3 ± 1.2	39.9 ± 1.2	40.1 ± 1.2	36.5 ± 3.0
EE[2]	2.9 ± 0.6	2.3 ± 0.2	2.9 ± 0.5	2.7 ± 0.6	5.6 ± 1.6
Ash	8.5 ± 0.4	9.7 ± 0.4	8.7 ± 0.4	9.9 ± 0.4	7.5 ± 0.5
Ca	1.31 ± 0.07	1.81 ± 0.07	1.30 ± 0.07	1.80 ± 0.07	1.05 ± 0.08
P	0.41 ± 0.01	0.40 ± 0.01	0.42 ± 0.01	0.42 ± 0.01	0.42 ± 0.02
Mg	0.50 ± 0.06	0.51 ± 0.06	0.53 ± 0.01	0.54 ± 0.06	0.38 ± 0.02
K	1.60 ± 0.08	1.59 ± 0.08	1.60 ± 0.08	1.59 ± 0.08	1.46 ± 0.05
Na	0.11 ± 0.04	0.11 ± 0.04	0.12 ± 0.04	0.12 ± 0.04	0.42 ± 0.07
S	0.42 ± 0.03	0.42 ± 0.03	0.57 ± 0.12	0.57 ± 0.03	0.26 ± 0.01
Cl	0.77 ± 0.09	0.77 ± 0.09	1.11 ± 0.09	1.11 ± 0.09	0.41 ± 0.03
DCAD[3]	-2.30 ± 3.23	-2.45 ± 3.23	-21.11 ± 3.23	-21.26 ± 3.23	27.10 ± 2.34

[1] NEG= negative DCAD, NEU= neutral DCAD

[2] EE= crude fat

[3] Calculated as DCAD = (Na + K) – (Cl + S) mEq/100g DM

Table 3. Dry matter intake, milk yield and composition of cows fed diets differing in prepartum DCAD and Ca concentrations

DCAD	NEU		NEG			P		
Ca, %	1.3%	1.8%	1.3%	1.8%	SE	DCAD	Ca	DCAD x CA
Prepartum DMI, kg/d	17.3	14.8	15.1	13.0	0.7	0.0096	0.0038	0.6042
% of BW	2.06	1.83	2.30	1.96	0.09	0.0051	0.0046	0.5406
Postpartum DMI, kg/d	22.5	23.0	24.0	23.0	0.9	0.4288	0.7628	0.4435
% of BW	3.31	3.31	3.72	3.46	0.16	0.2544	0.8304	0.1486
Milk, kg/d	39.7	38.9	40.6	41.0	1.6	0.6040	0.8787	0.8777
Fat, %	4.32	4.22	4.25	4.04	0.14	0.3980	0.2765	0.7117
Fat, kg/d	1.72	1.64	1.73	1.66	0.06	0.5883	0.2303	0.8268
Protein, %	2.68	2.64	2.68	2.65	0.04	0.9028	0.4506	0.8741
Protein, kg/d	1.07	1.03	1.09	1.09	0.04	0.3197	0.6277	0.5640
Lactose, %	4.62	4.61	4.57	4.66	0.05	0.9675	0.4059	0.3609
Lactose, kg/d	1.84	1.79	1.86	1.91	0.08	0.4093	0.8926	0.4678
SNF, %	8.33	8.30	8.33	8.32	0.06	0.9084	0.7334	0.8455
SNF, kg/d	3.31	3.23	3.38	3.41	0.13	0.3502	0.8565	0.6221
ECM, kg/d	43.3	41.8	44.0	43.2	1.6	0.3347	0.4225	0.9592
MUN, mg/dL	10.06	9.56	8.90	9.56	0.37	0.1194	0.8308	0.1360
SCC, cells x 1,000/ml	383	356	389	250	120	0.6743	0.4922	0.6542
Efficiency, ECM/DMI	1.81	1.79	1.74	1.83	0.07	0.7985	0.5751	0.4536

Table 4. Prepartum (-28 through -2 d) blood metabolites of cows fed diets differing in prepartum DCAD and Ca concentrations

DCAD	NEU		NEG			P		
Ca	1.3%	1.8%	1.3%	1.8%	SE	DCAD	Ca	DCAD x Ca
Plasma								
Beta-hydroxybutyrate, mmol/ml	0.73	0.73	0.76	0.75	0.14	0.6223	0.8804	0.9134
Total protein, g/dL	7.02	6.97	6.86	6.78	0.12	0.1611	0.6228	0.9010
Albumin, g/dL	3.48	3.43	3.42	3.38	0.04	0.1102	0.2191	0.8641
Globulin, g/dL	3.53	3.54	3.44	3.41	0.12	0.3437	0.9091	0.8619
A/G ratio	1.05	1.00	1.02	1.01	0.03	0.8345	0.3490	0.5333
BUN, mg/dL	22.98	19.47	21.57	20.13	0.85	0.6623	0.0056	0.2317
Creatinine, mg/dL	0.70	0.73	0.70	0.75	0.03	0.6395	0.1191	0.6417
BUN/Creatinine	32.14	27.97	31.65	27.65	1.62	0.8023	0.0150	0.9603
Total Bilirubin, mg/dL	0.05	0.10	0.08	0.17	0.03	0.1859	0.0552	0.4851
Glucose, mg/dL	55.40	57.46	52.92	54.55	1.64	0.1017	0.2630	0.8968
Aspartate transaminase, U/L	69.01	74.99	70.18	77.79	4.16	0.6332	0.1093	0.8454
Creatinine Kinase, U/L	177.62	229.49	193.27	170.74	31.67	0.4942	0.6437	0.2456
Gamma glutamyl transferase, U/L	16.90	14.96	16.76	16.73	1.16	0.4850	0.4043	0.4189
Ca, mg/dL	9.24	8.98	10.78	9.00	1.28	0.5383	0.4293	0.5562
P, mg/dL	7.27	6.66	7.44	6.86	0.17	0.2938	0.0013	0.9402
Mg, mg/dL	2.41	2.35	2.37	2.41	0.04	0.7876	0.7938	0.1210
Na, mEq/L	143.55	142.95	142.90	142.37	0.33	0.0646	0.0896	0.9040
K, mEq/L	5.65	4.81	4.99	5.20	0.37	0.7110	0.3865	0.1562
Cl, mEq/L	100.58	103.62	104.39	105.15	1.36	0.0519	0.1668	0.4028
Bicarbonate, mmol/L	27.38	27.69	25.83	27.81	0.41	0.0011	0.1486	0.6797
Anion Gap, mmol/L	17.26	16.85	17.10	16.81	0.52	0.8428	0.5068	0.9117
iCa, mmol/L	1.27	1.24	1.26	1.26	0.01	0.7996	0.5791	0.2249
iMg, mmol/L	0.60	0.61	0.60	0.62	0.01	0.2585	0.1778	0.5931
iCa/iMg	2.07	2.08	2.09	2.05	0.03	0.8589	0.5998	0.4334

Table 5. Prepartum (-28 through -2 d) urine metabolites and fractional excretion of cows fed diets differing in prepartum DCAD and Ca concentrations

DCAD	NEU		NEG			P		
Ca	1.3%	1.8%	1.3%	1.8%	SE	DCAD	Ca	DCAD x Ca
Urine								
Ca, mg/dL	28.41	30.03	44.60	40.02	5.43	0.0207	0.7908	0.5862
Cl, mmol/L	124.99	118.21	131.65	115.26	13.24	0.8908	0.3941	0.7249
Creatinine, mg/dL	103.05	82.44	69.60	56.03	9.43	0.0027	0.0813	0.7187
K, mmol/L	176.34	158.05	141.55	120.47	13.40	0.0096	0.1542	0.9195
Mg, mg/dL	50.55	48.61	31.80	33.31	15.53	0.2840	0.9895	0.9160
Na, mmol/L	29.23	22.15	24.83	22.54	5.67	0.7287	0.4220	0.6915
pH	7.45	6.66	6.05	6.01	0.21	<0.0001	0.0628	0.0973
Fractional Excretion, %								
Ca	0.13	0.14	0.21	0.17	0.02	0.0156	0.7033	0.2757
Mg	15.06	12.68	13.78	13.69	0.89	0.8798	0.1757	0.2027
Na	0.27	0.14	0.30	0.21	0.05	0.3477	0.0262	0.6807
K	34.78	28.77	35.68	32.07	2.24	0.3460	0.0375	0.5937
Cl	1.05	1.01	1.52	1.42	0.09	<0.0001	0.4209	0.7244

Table 6. Postpartum blood metabolites (0 – 3 d) of cows fed diets differing in prepartum DCAD and Ca concentrations

DCAD	NEU		NEG			P		
Ca, %	1.3%	1.8%	1.3%	1.8%	SE	DCAD	Ca	DCAD x Ca
NEFA, mEq/L	1.13	1.04	0.95	0.99	0.22	0.1118	0.7546	0.3825
Beta-hydroxybutyrate, mmol/ml	0.92	0.99	1.06	1.20	0.10	0.0702	0.2531	0.7194
Total protein, g/dL	6.84	6.84	6.56	6.51	0.10	0.0040	0.7842	0.7942
Albumin, g/dL	3.59	3.55	3.53	3.40	0.05	0.0277	0.0904	0.3749
Globulin, g/dL	3.24	3.27	3.44	3.10	0.24	0.9367	0.5358	0.4468
A/G ratio	1.16	1.12	1.18	1.11	0.04	0.7514	0.1300	0.7134
BUN, mg/dL	19.89	16.16	15.94	16.45	1.16	0.1181	0.1737	0.0717
Creatinine, mg/dL	0.86[b]	0.75[a]	0.69[a]	0.78[ab]	0.03	0.0717	0.8311	0.0063
BUN/Creatinine	22.77	22.61	26.12	22.10	1.99	0.4765	0.3029	0.3375
Total Bilirubin, mg/dL	0.40	0.48	0.49	0.57	0.06	0.1100	0.1916	0.9670
Glucose, mg/dL	57.54	59.02	57.72	52.78	4.16	0.4678	0.6828	0.4426
Aspartate transaminase, U/L	105.05	130.47	125.83	146.27	29.06	0.5306	0.4381	0.9320
Creatinine Kinase, U/L	432.84	729.87	377.20	755.28	0.9207	0.9207	0.3195	0.8640
Gamma glutamyl transferase, U/L	21.15	13.90	14.20	16.72	2.60	0.4281	0.3720	0.0651
Ca, mg/dL	8.06	8.18	7.94	8.22	0.16	0.7835	0.1977	0.6114
P, mg/dL	5.83	5.90	5.72	5.82	0.26	0.7099	0.7407	0.9574
Mg, mg/dL	2.45	2.44	2.29	2.31	0.05	0.0089	0.97101	0.7525
Na, mEq/L	142.95	142.47	143.21	142.95	0.38	0.2587	0.2695	0.7500
K, mEq/L	5.75	5.92	6.00	5.76	0.22	0.8328	0.8799	0.3355
Cl, mEq/L	103.17	102.67	103.56	103.03	0.43	0.3866	0.2363	0.9742
Bicarbonate, mmol/L	29.61	30.13	29.49	29.35	0.40	0.2682	0.6320	0.4182
Anion Gap, mmol/L	15.87	15.66	16.17	16.36	0.41	0.2227	0.9813	0.6194
iCa, mmol/L	1.14	1.15	1.14	1.17	0.05	0.5722	0.1628	0.3666
iMg, mmol/L	0.62	0.61	0.58	0.59	0.01	0.0059	0.9665	0.4843
iCa/iMg	1.89	1.91	2.01	2.02	0.05	0.0210	0.8018	0.9867

Table 7. Postpartum blood metabolites (7 and 14 d) of cows fed diets differing in prepartum DCAD and Ca concentrations

DCAD	NEU		NEG			P		
Ca, %	1.3%	1.8%	1.3%	1.8%	SE	DCAD	Ca	DCAD x Ca
Beta-hydroxybutyrate, mmol/ml	1.67	1.33	1.44	1.74	0.22	0.9334	0.1395	0.7866
Total protein, g/dL	6.99	7.06	6.81	6.67	0.11	0.0146	0.7649	0.3773
Albumin, g/dL	3.50	3.39	3.38	3.27	0.06	0.0406	0.0745	0.0965
Globulin, g/dL	3.50	3.67	3.43	3.41	0.11	0.1581	.05051	0.4137
A/G ratio	1.04	0.95	1.32	0.97	0.16	0.3461	0.1780	0.4058
BUN, mg/dL	16.14	13.83	12.83	16.14	1.38	0.9430	0.4758	0.3392
Creatinine, mg/dL	0.91	.068	0.62	0.64	0.36	0.0757	0.2546	0.1543
BUN/Creatinine	19.86	20.49	21.49	19.26	1.68	0.9053	0.6328	0.3938
Total Bilirubin, mg/dL	0.41	0.38	0.42	0.42	0.07	0.7635	0.7981	0.8746
Glucose, mg/dL	46.80	42.74	41.94	41.69	4.63	0.5195	0.6425	0.6780
Aspartate transaminase, U/L	167.45	138.58	149.66	452.79	24.10	0.9401	0.5946	0.5037
Creatinine Kinase, U/L	627.61	279.46	337.12	320.22	243.17	0.6041	0.4553	0.4927
Gamma glutamyl transferase, U/L	23.07	21.62	16.36	18.90	2.97	0.1127	0.8562	0.4992
Ca, mg/dL	9.12	9.14	8.98	8.97	0.12	0.2031	0.9857	0.9081
P, mg/dL	5.30	5.62	5.65	5.53	0.22	0.5618	0.6531	0.3298
Mg, mg/dL	2.29	2.22	2.14	2.17	0.22	0.0719	0.6144	0.3824
Na, mEq/L	138.45	139.39	139.74	140.14	0.50	0.0453	0.1875	0.5835
K, mEq/L	5.60	6.50	6.41	5.18	0.64	0.7020	0.3012	0.3779
Cl, mEq/L	97.32	97.9	96.23	97.29	0.82	0.2996	0.3209	0.7710
Bicarbonate, mmol/L	29.77	30.22	31.44	30.89	0.61	0.0571	0.9388	0.4105
Anion Gap, mmol/L	17.05	16.78	17.33	17.08	0.61	0.6250	.09866	0.0618
iCa, mmol/L	1.25	1.25	1.23	1.24	0.01	0.1265	1.8568	0.4022
iMg, mmol/L	0.58	0.56	0.56	.056	0.02	0.0294	0.5902	0.4266
iCa/iMg	2.12	2.18	2.22	2.23	0.05	0.1247	0.5097	0.5816

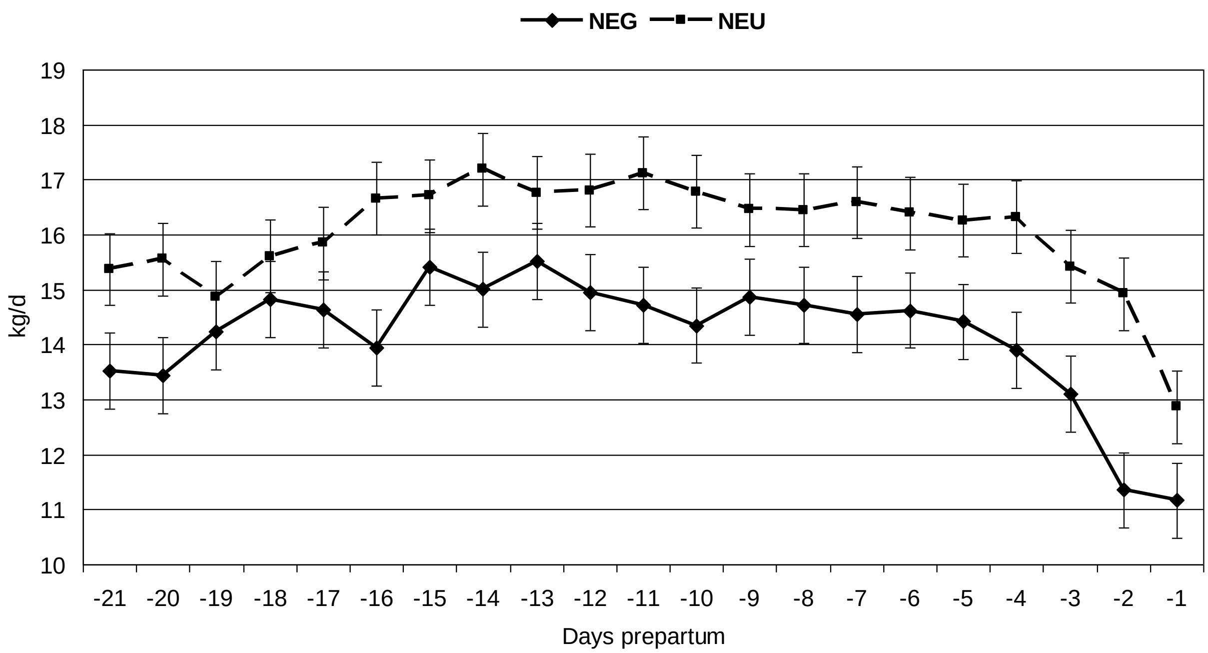

Figure 1. Prepartum DMI of cows fed diets with -21 (NEG) or -2 mEq/100g DM (NEU) DCAD (*P* = 0.0096).

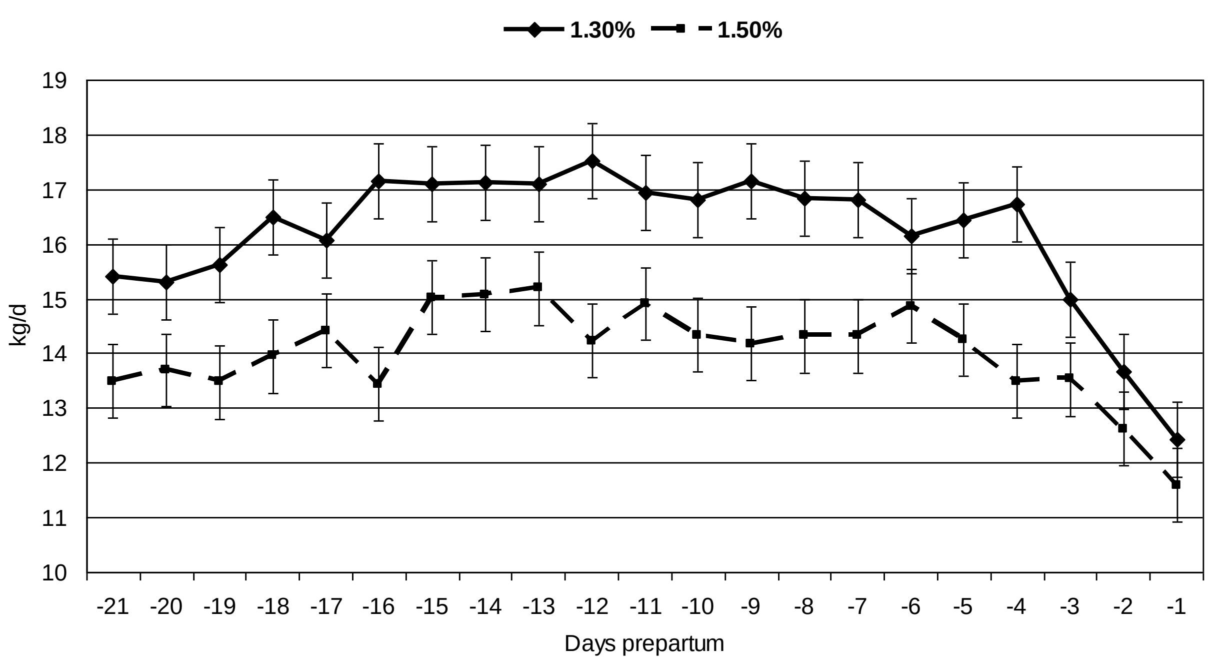

Figure 2. Prepartum DMI of cows fed diets supplemented with 1.3 or 1.8% Ca (*P* = 0.0038).

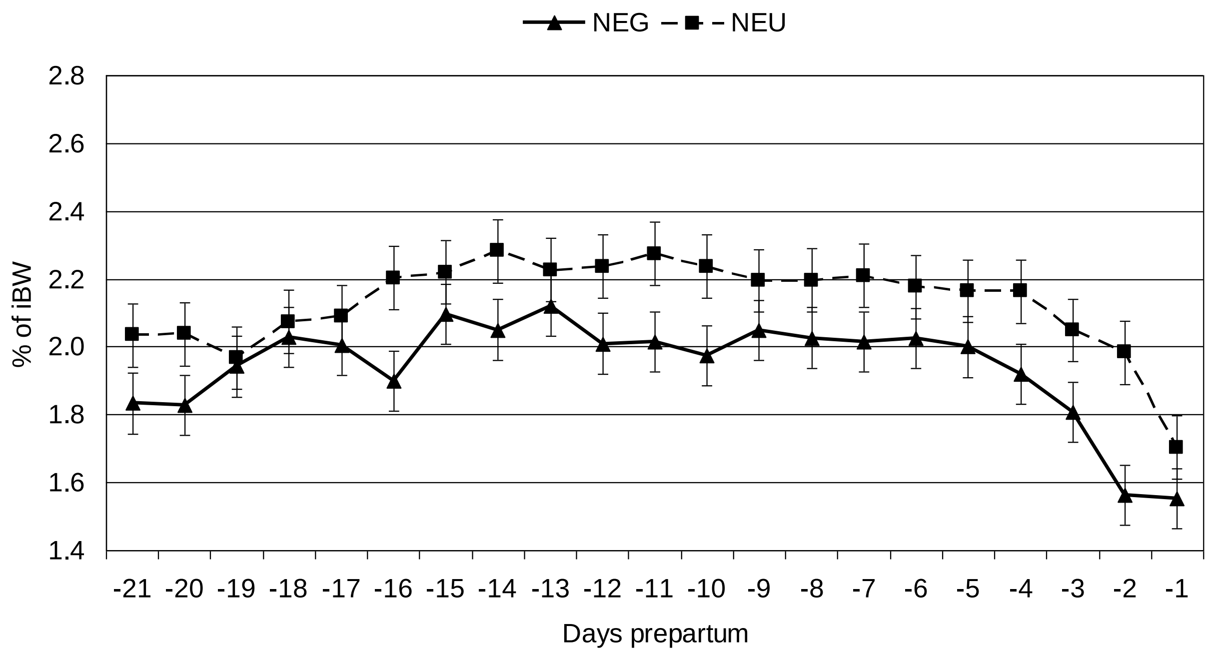

Figure 3. Prepartum DMI (% of initial BW) of cows fed diets with -21 (NEG) or -2 mEq/100g DM (NEU) DCAD (*P* = 0.0051).

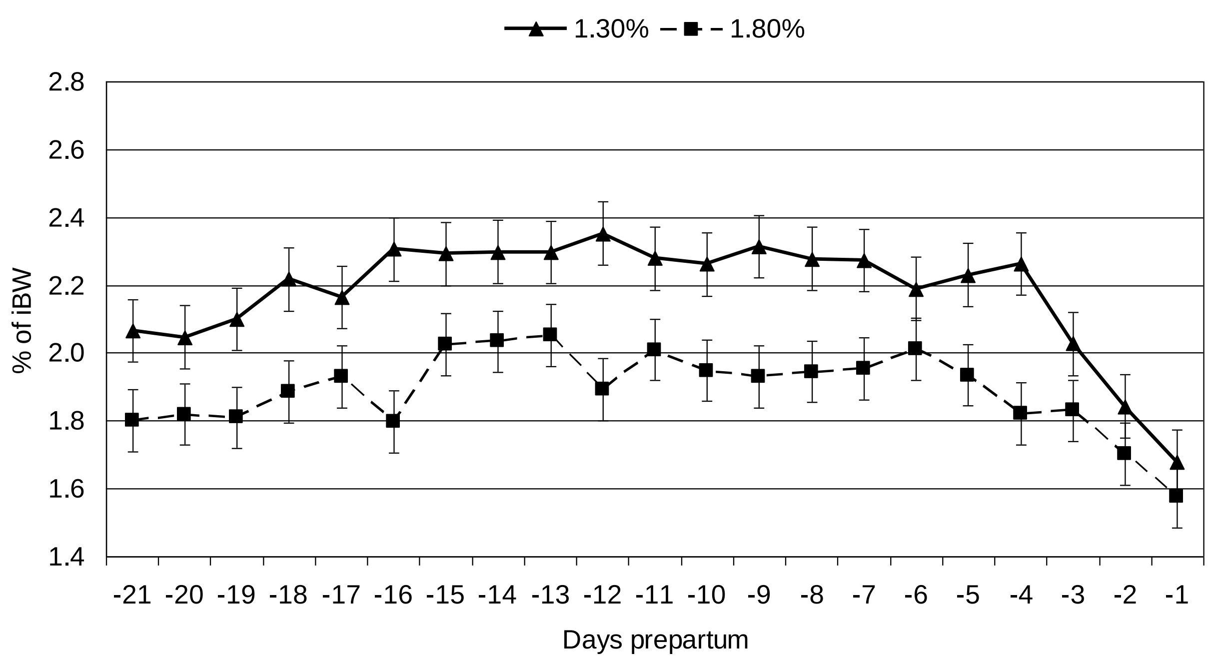

Figure 4. DMI (% of initial BW) of cows fed diets supplemented with 1.3 or 1.8% Ca (*P* = 0.0046).

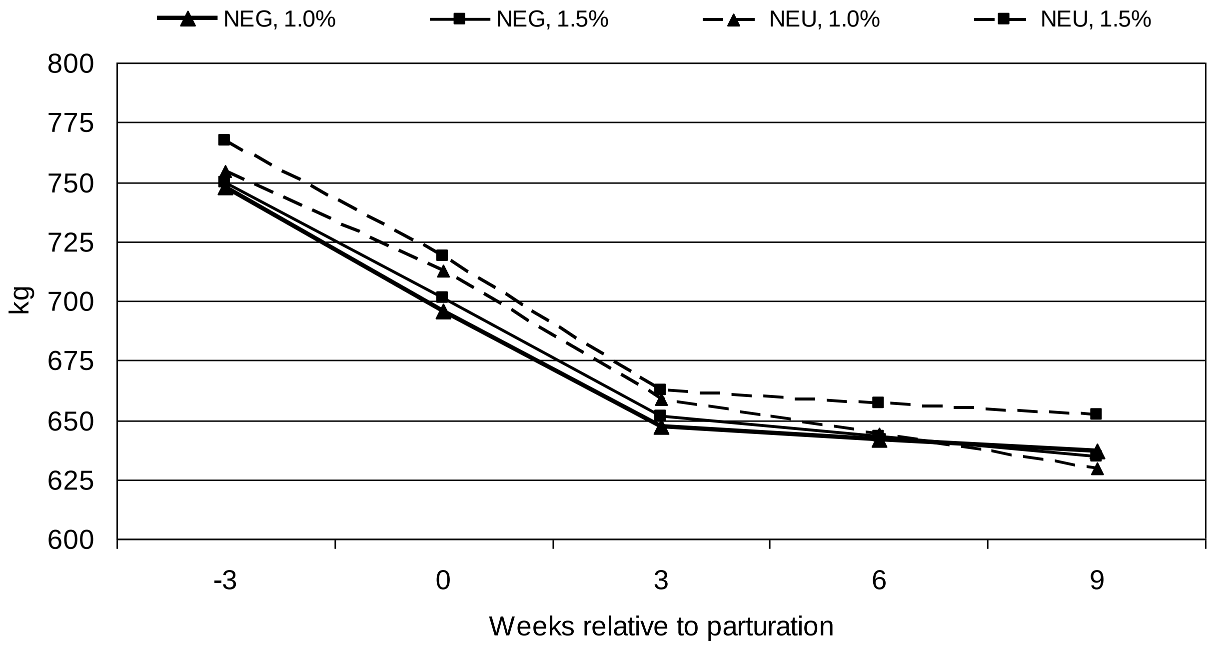

Figure 5. Body weight of cows fed diets with -21 (NEG) or -2 mEq/100g DM (NEU) DCAD (*P* = 0.4704) and supplemented with 1.3 or 1.5% Ca (*P* = 0.6653).

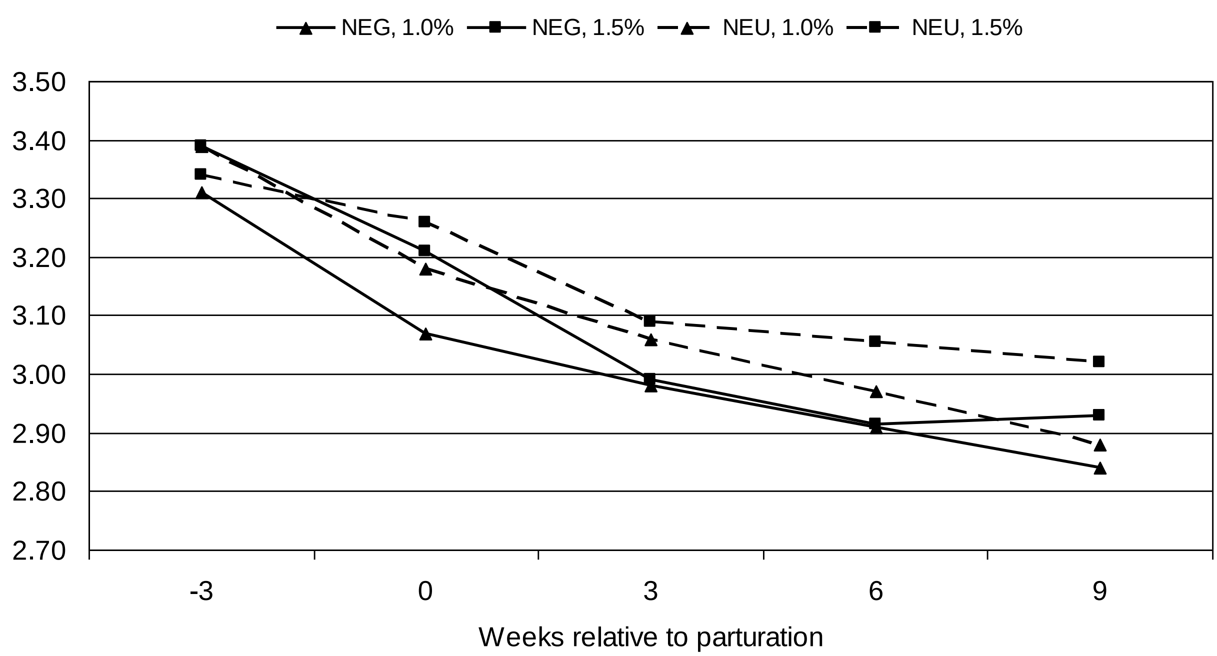

Figure 6. Body condition score of cows fed diets with -21 (NEG) or -2 mEq/100g DM (NEU) DCAD (*P* = 0.1538) and supplemented with 1.3 or 1.5% Ca (*P* = 0.1294).

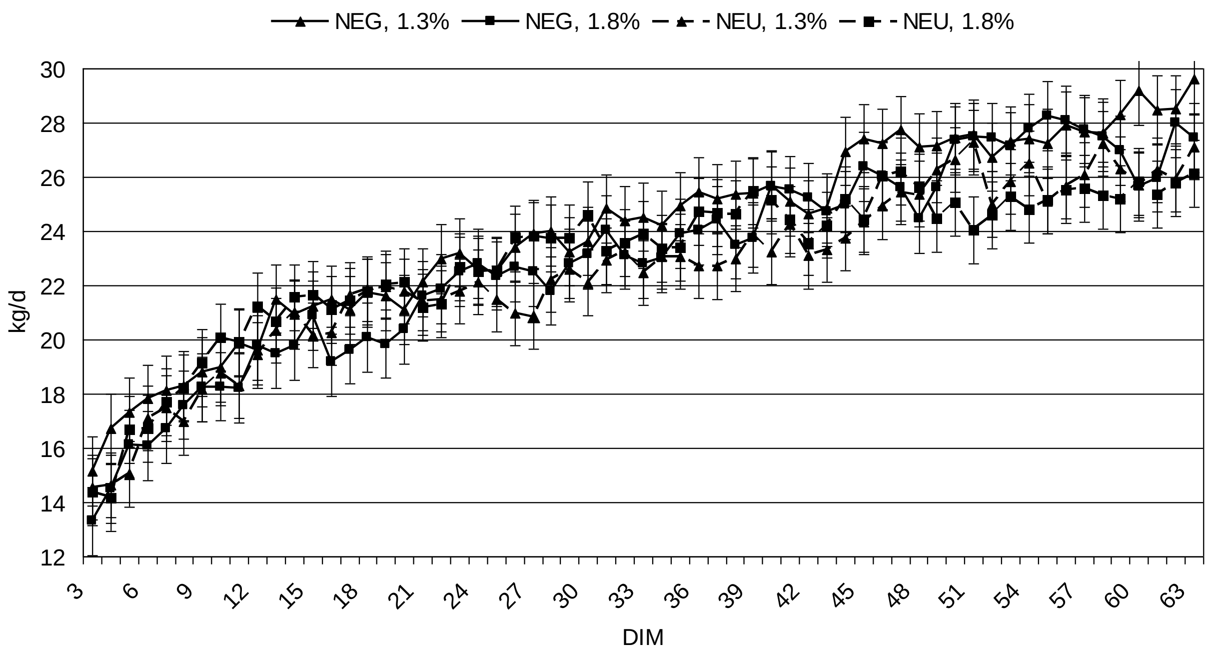

Figure 7. Postpartum DMI of cows fed prepartum diets with -21 (NEG) or -2 mEq/100g DM (NEU) DCAD (P = 0.4288) and supplemented with 1.3 or 1.8% Ca (P = 0.7628).

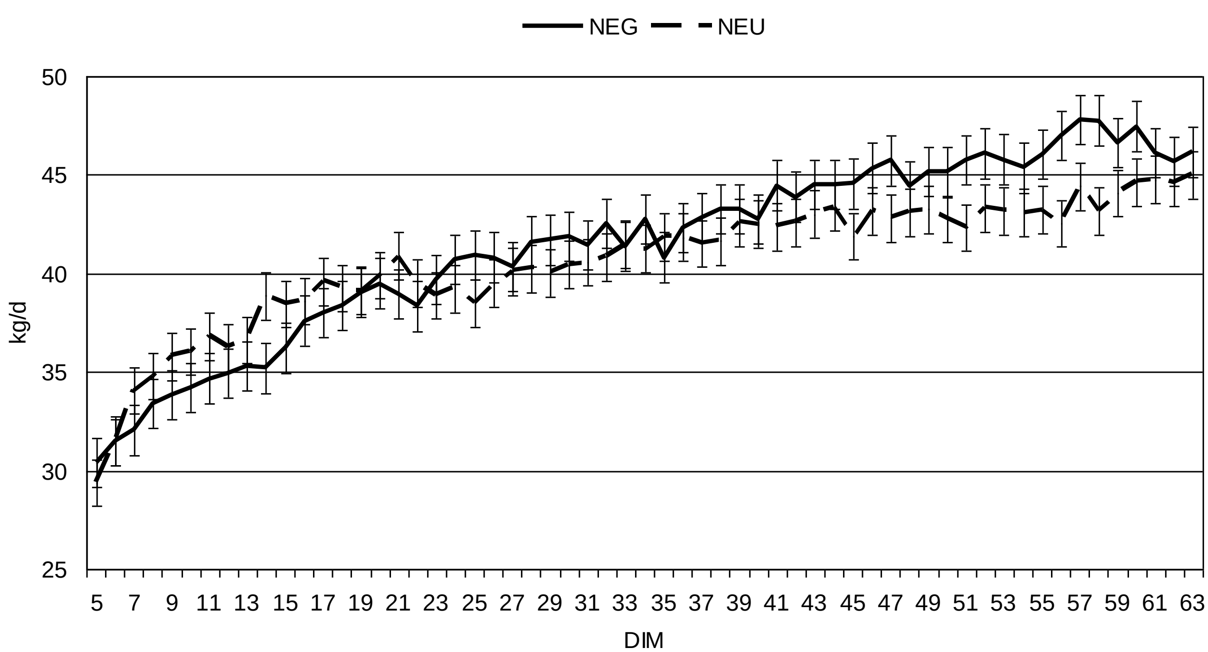

Figure 8. Daily milk yield of cows fed prepartum diets with -21 (NEG) or -2 mEq/100g DM (NEU) DCAD (DCAD x DIM, P = 0.0125).

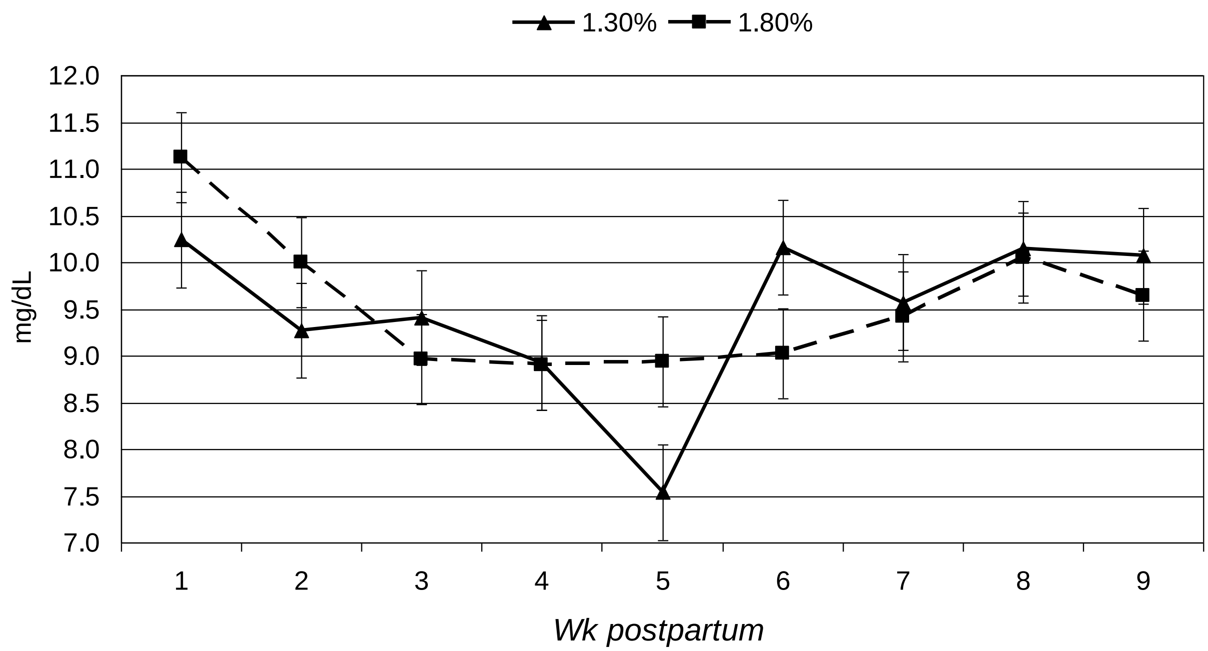

Figure 9. Concentrations of MUN for cows fed prepartum diets supplemented with 1.3 or 1.8% Ca (Ca X wk postpartum, *P* = 0.0412).

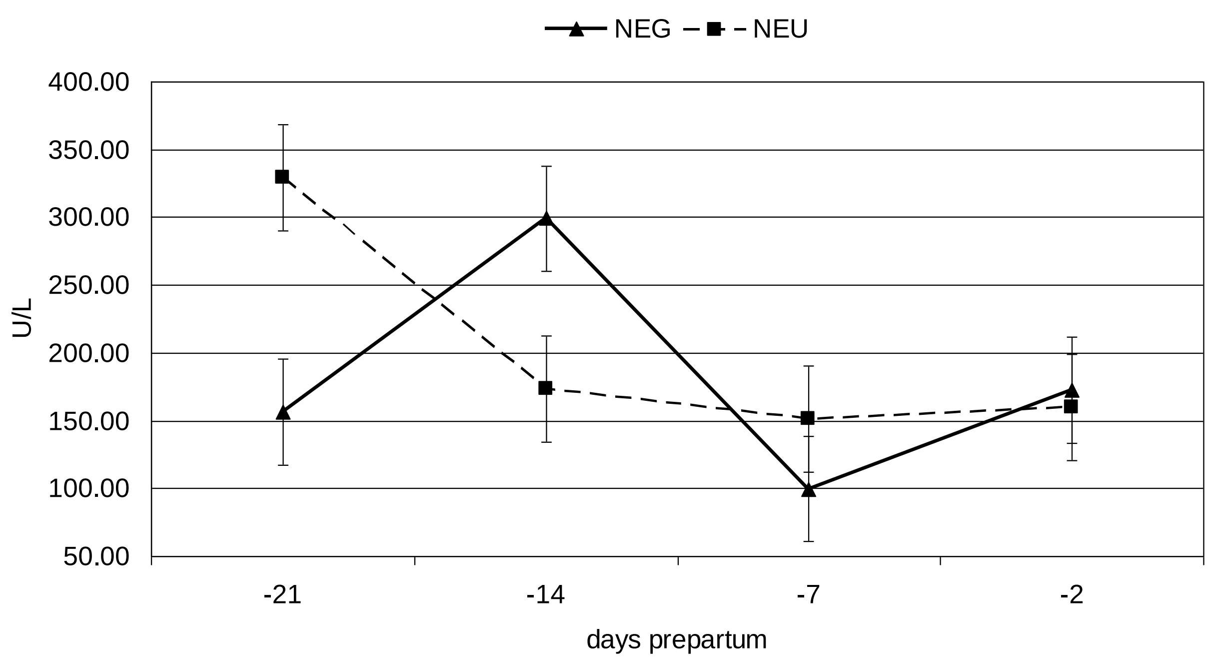

Figure 10. Prepartum creatinine kinase concentrations of cows fed prepartum diets with -21 (NEG) or -2 mEq/100g DM (NEU) DCAD (DCAD * days prepartum, *P* = 0.0031).

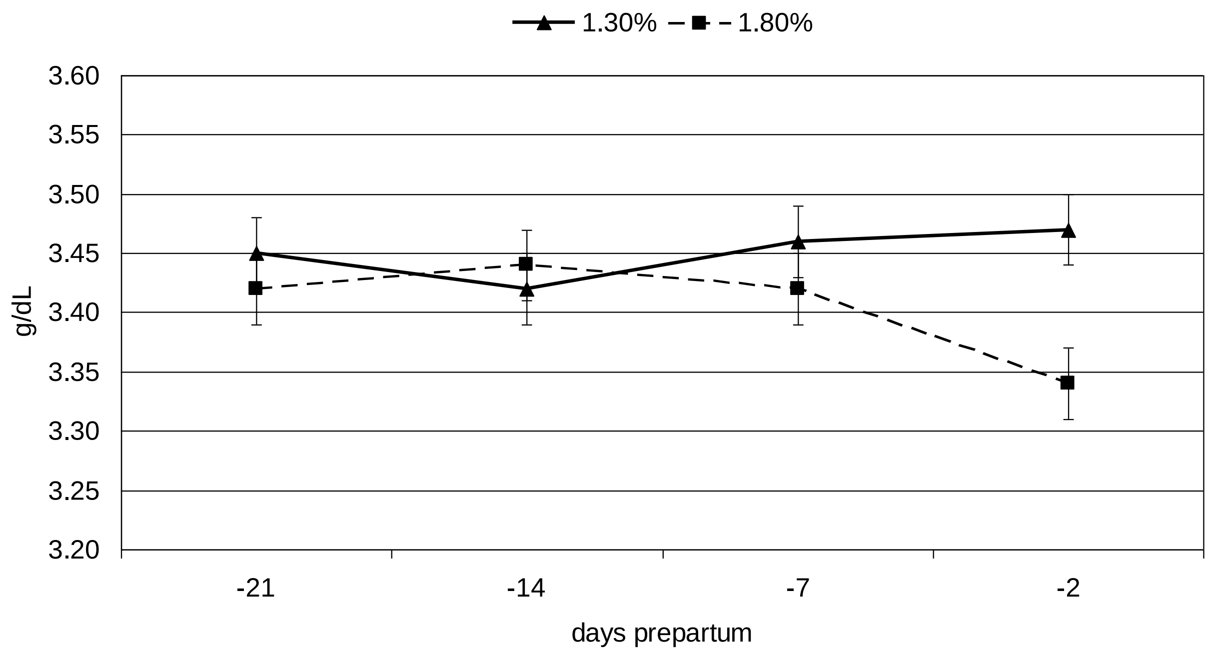

Figure 11. Prepartum albumin concentrations of cows fed prepartum diets supplemented with 1.3 or 1.8% Ca (Ca x days prepartum, $P = 0.0297$).

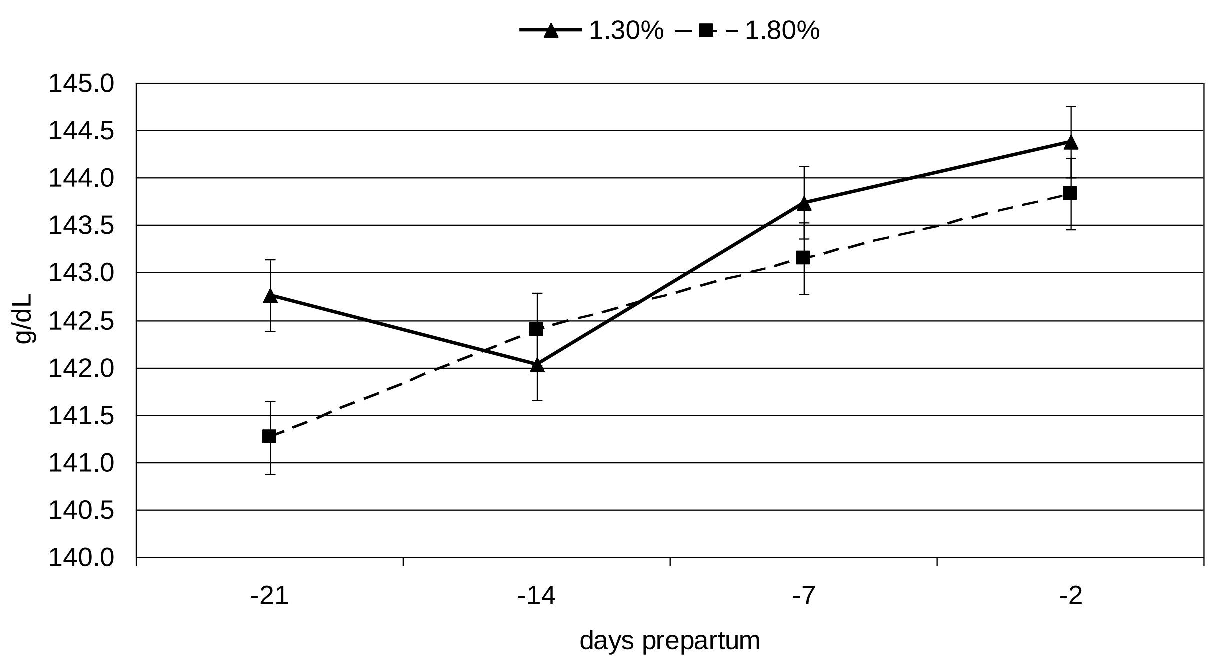

Figure 12. Prepartum sodium concentrations of cows fed prepartum diets supplemented with 1.3 or 1.8% Ca (Ca X days prepartum, $P = 0.0363$).

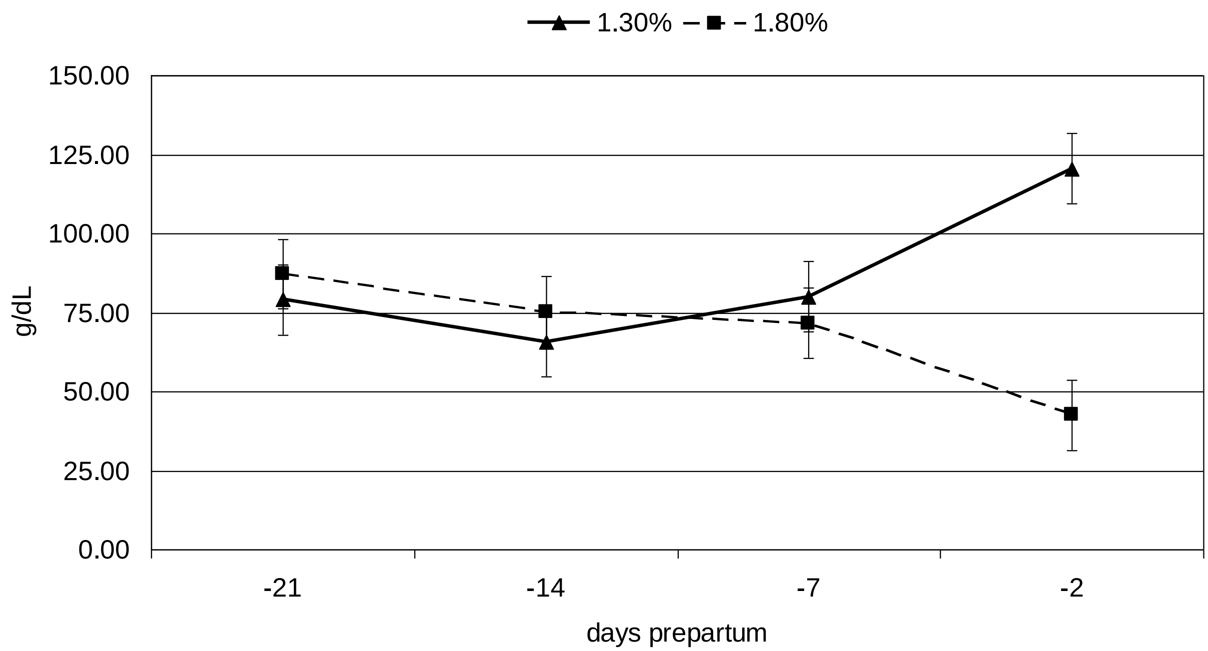

Figure 13. Prepartum urinary creatinine concentrations of cows fed prepartum diets supplemented with 1.3 or 1.8% Ca (Ca X days prepartum, *P* = 0.0500).

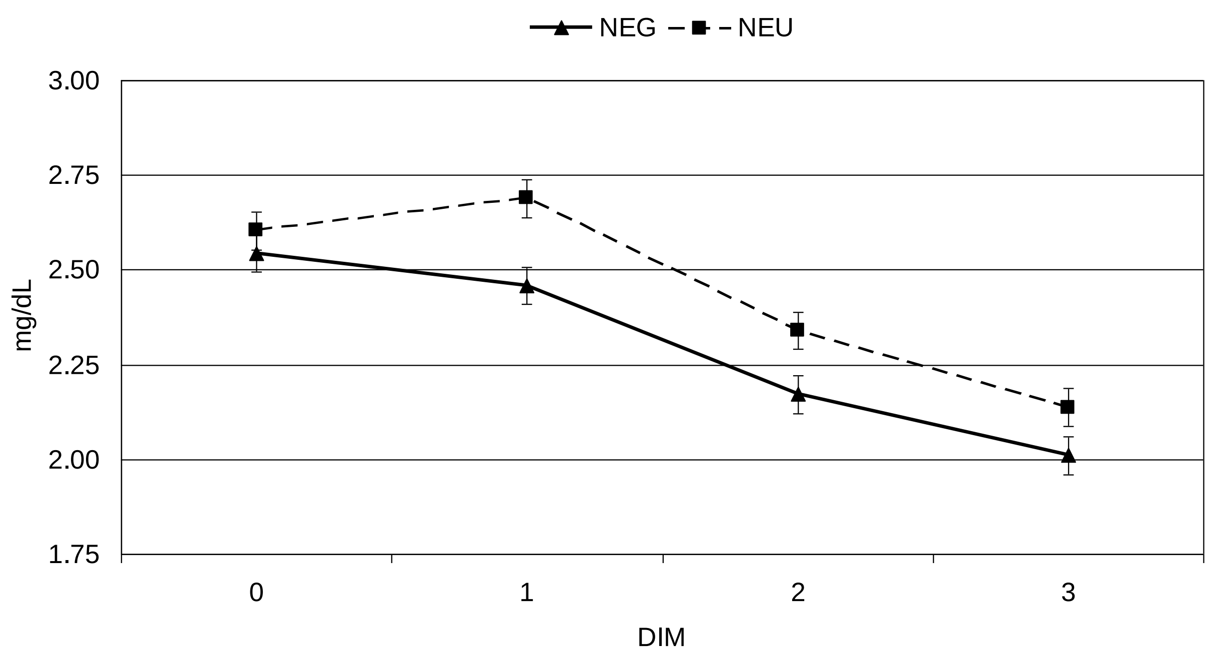

Figure 14. Plasma Mg concentrations on 0 to 3 DIM of cows fed prepartum diets with -21 (NEG) or -2 mEq/100g DM (NEU) DCAD (DCAD X DIM, *P* = 0.0349).

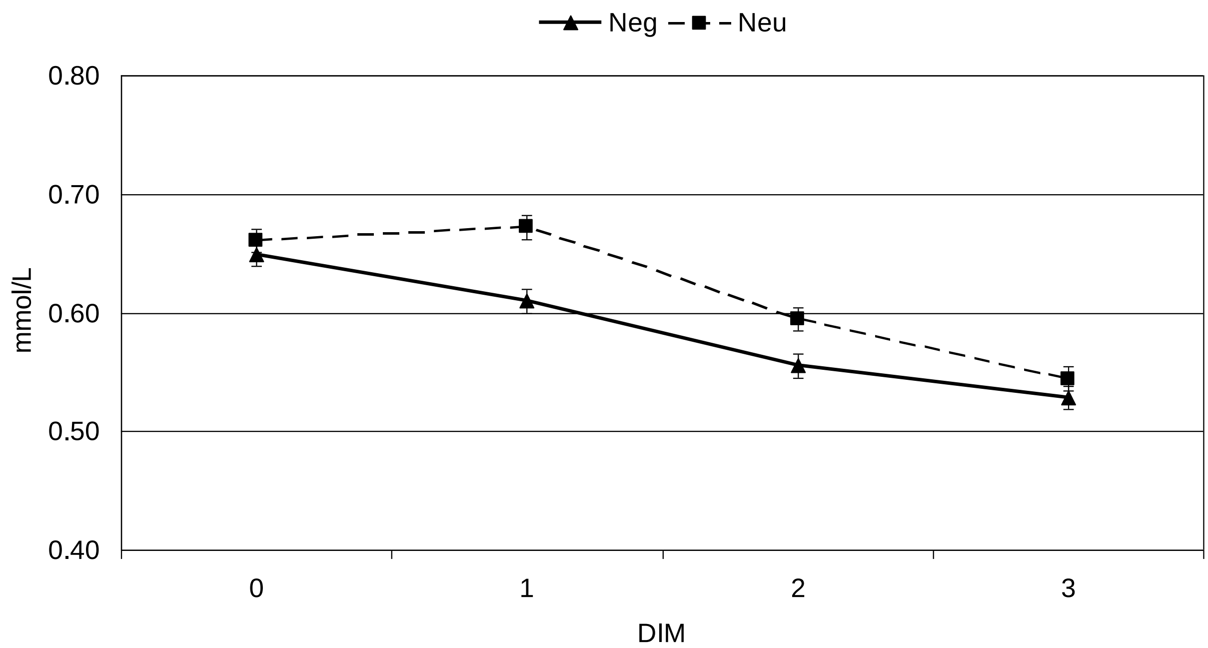

Figure 15. Plasma iMg concentrations on 0 to 3 DIM of cows fed prepartum diets with -21 (NEG) or -3 mEq/100g DM (NEU) DCAD (DCAD X DIM, *P* = 0.0021).

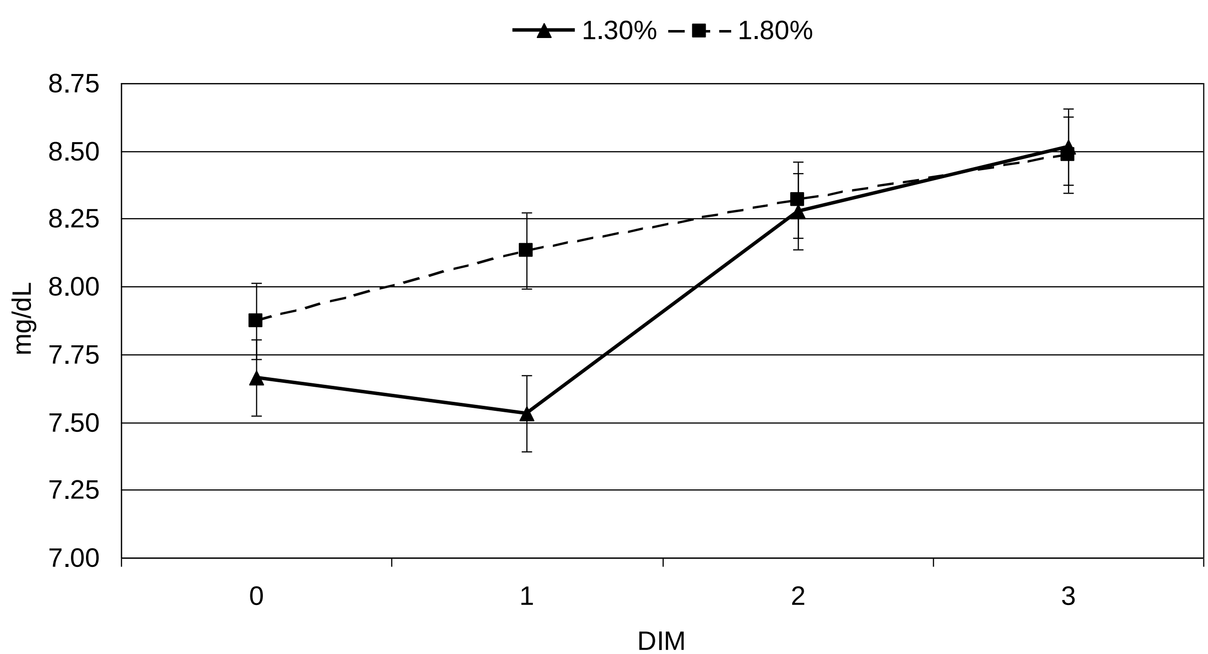

Figure 16. Plasma Ca concentrations on 0 to 3 DIM of cows fed prepartum diets supplemented with 1.3 or 1.8% Ca (Ca X DIM, *P* = 0.0038).

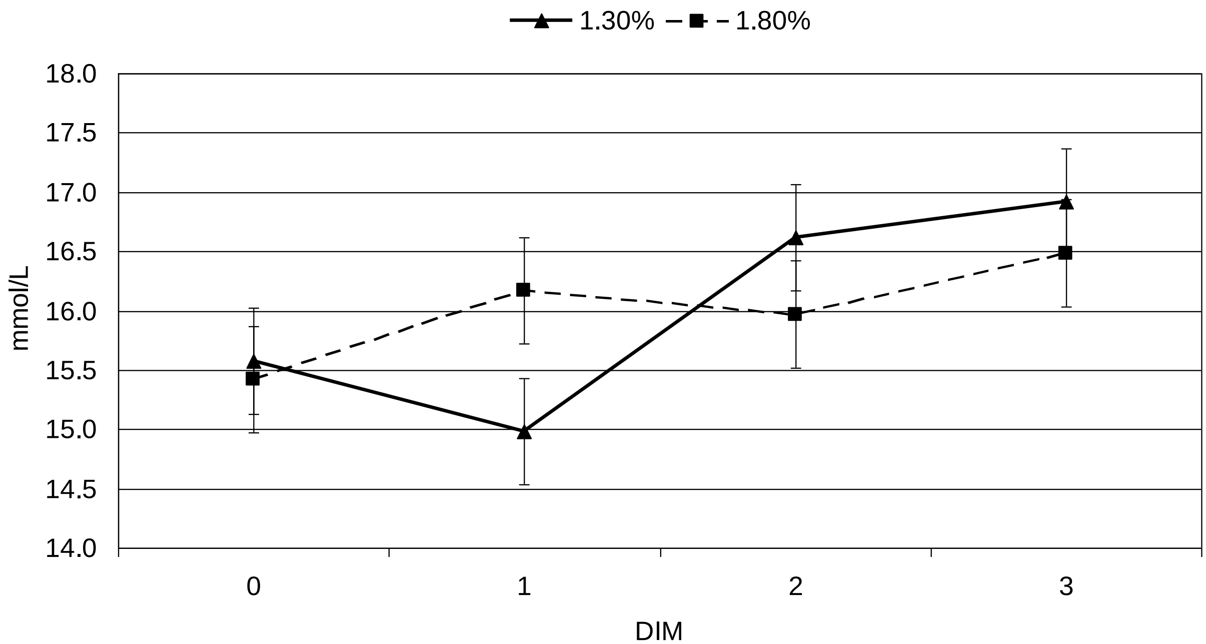

Figure 17. Plasma anion gap concentrations on 0 to 3 DIM of cows fed prepartum diets supplemented with 1.3 or 1.8% Ca (Ca X DIM, *P* = 0.0038).