Effects of level of dietary cation-anion difference and duration of prepartum feeding on performance and metabolism of dairy cows

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ABSTRACT

The objectives were to evaluate the effects of feeding diets with 2 levels of negative dietary cation-anion differences (DCAD) during the last 42 or 21 d of gestation on performance and metabolism in dairy cows. The hypothesis was that extending feeding from 21 to 42 d and reducing the DCAD from -70 to -180 mEq/kg of dry matter (DM) would not be detrimental to performance. Holstein cows at 230 d of gestation were blocked by parity prepartum (48 entering their second lactation and 66 entering their third or greater lactation) and 305-d milk yield, and randomly assigned to 1 of 4 treatments arranged as a 2×2 factorial. The 2 levels of DCAD, -70 or -180 mEq/kg of DM, and 2 feeding durations, the last 21 d (short) or the last 42 d (long) prepartum resulted in 4 treatments, short -70 ($n = 29$), short -180 ($n = 29$), long -70 ($n = 28$) and long -180 ($n = 28$). Cows in the short treatments were fed a diet with DCAD of $+110$ mEq/kg of DM from -42 to -22 d relative to calving. After calving, cows were fed the same diet and production and disease incidence were evaluated for 42 d in milk, whereas reproduction and survival was evaluated for 305 d in milk. Blood was sampled pre- and postpartum for quantification of metabolites and minerals. Reducing the DCAD linearly decreased prepartum DM intake between -42 and -22 d relative to calving ($+110$ mEq/kg of DM = 11.5 vs. -70 mEq/kg of DM = 10.7 vs. -180 mEq/kg of DM = 10.2 ± 0.4), and a more acidogenic diet in the last 21 d of the dry period reduced intake by 1.1 kg/d (-70 mEq/kg of DM = 10.8 vs. -180 mEq/kg of DM = 9.7 ± 0.5 kg/d). Cows fed the -180 mEq/kg of DM diet had increased concentrations of ionized Ca in blood on the day of calving (-70 mEq/kg of DM = 1.063 vs. -180 mEq/kg of DM = 1.128 ± 0.020 mM). Extending

the duration of feeding the diets with negative DCAD from 21 to 42 d reduced gestation length by 2 d (short = 277.2 vs. long = 275.3 d), milk yield by 2.5 kg/d (short = 40.4 vs. long = 37.9 ± 1.0 kg/d) and tended to increase days open because of reduced pregnancy per artificial insemination (short = 35.0 vs. long = 22.6%). Results suggest that increasing the duration of feeding diets with negative DCAD from 21 to 42 d prepartum might influence milk yield and reproduction of cows in the subsequent lactation, although yields of 3.5% fat- and energy-corrected milk did not differ with treatments. Reducing the DCAD from -70 to -180 mEq/kg of DM induced a more severe metabolic acidosis, increased ionized Ca concentrations prepartum and on the day of calving, and decreased colostrum yield in the first milking, but had no effects on performance in the subsequent lactation. Collectively, these data suggest that extending the feeding of an acidogenic diet beyond 21 d is unnecessary and might be detrimental to dairy cows, and a reduction in the DCAD from -70 to -180 mEq/kg of DM is not needed.

Key words: acidogenic diets, dairy cow, DCAD, transition

INTRODUCTION

A large proportion of dairy cows undergo a period of disruption in Ca homeostasis with the onset of colostrogenesis and lactation. The demands for Ca for colostrum and milk synthesis induce a sudden drop in blood concentrations of ionized (iCa) and total Ca (tCa), resulting in some cows developing clinical hypocalcemia, also known as milk fever. Normal concentrations of tCa in blood usually range between 2.2 and 2.7 mM, but the onset of lactation results in sequestration of Ca in the mammary gland followed by loss via colostrum secretion, which can represent 7 to 10 times the estimated amount of tCa present in blood of a cow (Horst et al., 2005). The irreversible loss of tCa results in reductions in blood tCa to values lower

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than 2.2 mM (Reinhardt et al., 2011; Martinez et al., 2012, 2016). The inability of the cow to re-establish concentrations of iCa and tCa in blood is responsible for the development of hypocalcemia in the first days of lactation. Reinhardt et al. (2011) used the cutoff values of serum concentrations of tCa between 1.4 and 2.0 mM for subclinical hypocalcemia and below 1.4 mM for clinical hypocalcemia. Using those cutoff values, the authors reported prevalence of 1% of clinical hypocalcemia and 25% subclinical hypocalcemia for primiparous cows, and 7% clinical hypocalcemia and 47% subclinical hypocalcemia for multiparous cows in the first 48 h of calving. Cows that develop subclinical and clinical hypocalcemia have impaired subsequent health and reproduction (Curtis et al., 1985; Martinez et al., 2012).

Strategies have been developed to minimize the risk of sudden changes in blood Ca concentrations in dairy cows. Ender et al. (1971) demonstrated that feeding prepartum diets richer in strong anions relative to strong cations increased blood concentrations of tCa and prevented clinical hypocalcemia in dairy cows. These findings eventually led to the practice of feeding acidogenic diets and introduced the concept of DCAD for formulation of diets for prepartum dairy cows. One of the effects of acidogenic diets is a reduction in DMI prepartum (Charbonneau et al., 2006; Martinez et al., 2018), and the decline seems to follow a linear relationship with the decrease in DCAD (Charbonneau et al., 2006). Although numerous experiments have documented the benefits of acidogenic diets prepartum (Block, 1984), the ideal DCAD to optimize postpartum health and performance remains unclear. Excessive feeding of acidogenic diets might influence energy metabolism and impair insulin sensitivity (Bigner et al., 1996). Despite the calculated DCAD fed, acidogenic diets will vary in DCAD in part because of the variability in mineral composition in major feedstuffs, especially forages (St-Pierre and Weiss, 2017), which seems to be common in dairy herds (Stone and Mosley, 2017).

Under some feeding management systems, providing a single diet prepartum might facilitate implementation of acidogenic diets, particularly in small dairy farms or those in which facilities are limited. Grouping cohorts of dry cows with similar calving dates in the same pen minimizes pen movements and regrouping, which minimizes changes in behavior of prepartum cows (Schirmann et al., 2011). Nevertheless, only 2 experiments have evaluated the effects of altering the duration of feeding acidogenic diets prepartum (Weich et al., 2013; Wu et al., 2014). In those experiments, cows were fed diets with -150 (Weich et al., 2013) or -200 mEq/kg of DM (Wu et al., 2014) and no detrimental effects were observed on performance when feeding was extended from 21 to 42 d prepartum.

Most experiments have focused on the effects of feeding prepartum diets with different levels of DCAD on Ca metabolism and performance of dairy cows (Block, 1984; Leno et al., 2017), but limited research has been conducted on the effects of a prolonged duration of feeding diets with negative DCAD to transition cows. Based on the data available, we hypothesized that decreasing the negative DCAD from -70 to -180 mEq/kg of DM and extending the duration of feeding from 21 to 42 d would not affect performance and metabolism in dairy cows. Therefore, our objectives were to evaluate the effects of 2 durations of feeding acidogenic diets prepartum, the last 21 or 42 d of gestation, and at 2 levels of negative DCAD, -70 or -180 mEq/kg of DM, on acid-base status, colostrum yield and composition, lactation performance, health, and reproduction in Holstein dairy cows.

MATERIALS AND METHODS

The University of Florida Institutional Animal Care and Use Committee approved all procedures involving cows in the experiment under protocol number 201509133.

Cows and Housing

The experiment was conducted in the University of Florida Dairy Unit (Gainesville) from November 2015 to July 2016. One hundred fourteen apparently healthy, dry Holstein cows, pregnant to AI, were enrolled in the experiment, 48 that completed the first lactation and 66 cows that completed lactation 2 or greater. Details of cows according to treatment are presented in Supplemental Table S1 (<https://doi.org/10.3168/jds.2018-14580>).

Pregnant, nonlactating cows at 230 ± 3 d of gestation were moved to the experimental barn to acclimate to the facilities. Cows were trained for the first 2 d to use individual feeding gates (Calan Broadbent feeding system, American Calan Inc., Northwood, NH), and measurements started at 232 ± 3 d of gestation. The last 42 d of gestation were used for statistical analysis of prepartum data. Cows were housed together in a freestall barn with sand-bedded stalls, and each cow was randomly assigned to a feeding gate. Immediately after calving, all postpartum cows were moved to a second pen and housed together for the first 42 DIM. The experimental pens were equipped with 2 rows of fans (1 fan/6 m) placed above the beds; a water soaker line with nozzles was placed above the feedbunk for evaporative cooling of cows. Fans and water spraying were controlled by thermostats and activated when ambient temperature reached 18°C .

Feeding Management and Treatments

Cows were fed once daily, at 0730 h, prepartum and thrice daily postpartum, at approximately 0700, 1100, and 1500 h. Individual feed intake was measured daily only during the prepartum period. Postpartum cows were fed as a group. The amounts of feed offered to individual cows prepartum were adjusted daily to result in at least 5% refusals, which were weighed once daily, before the morning feeding.

The experiment followed a randomized complete block design with cow as the experimental unit. Weekly cohorts of prepartum cows at 230 d of gestation were blocked by parity (1 vs. >1) and previous lactation 305-d milk yield and, within each block, assigned randomly to 1 of the 4 treatments. Treatments were arranged as a factorial with 2 levels of negative DCAD, -70 or -180 mEq/kg of DM fed for 2 durations, the last 21 d of gestation, designated as the short period of feeding, or the last 42 d of gestation, designated as the long period of feeding; therefore, the 4 treatments were **-70_{short}** , **-180_{short}** , **-70_{long}** , and **-180_{long}** . For cows in short treatments, a diet with a positive DCAD of $+110$ mEq/kg of DM was fed from 230 to 254 d of gestation. Cows assigned to the -70_{short} and -180_{short} treatments were switched to the respective negative DCAD diets, -70 and -180 mEq/kg of DM, at 255 d of gestation and fed those diets until calving. Prepartum diets were formulated to be isocaloric, isonitrogenous, and have the same forage content and mineral composition, except for concentrations of strong ions to manipulate the DCAD to achieve -70 or -180 mEq/kg of DM (Table 1).

Ingredient Sampling, Chemical Analyses, and Calculation of DMI

Forages and concentrate mixtures were collected weekly, dried at 55°C for 48 h with moisture loss recorded, and stored for later analyses as monthly composites. Dried samples were ground to pass a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ), and analyzed for DM (105°C for 12 h). Dried samples were composited monthly and then analyzed for ash (512°C for 8 h), for NDF (sequentially using a heat-stable α -amylase) and ADF per Van Soest et al. (1991), for N using an automated quantitative combustion digestion method (Leco FP628, Leco Corp. St. Joseph, MI), for starch after acid hydrolysis (Vidal et al., 2009), for total fatty acids (Sukhija and Palmquist, 1988), and for minerals by inductively-coupled plasma MS. The NE_L density of the diets was calculated using chemical analyses of dietary ingredients and adjusted for 12.0 and 22.0 kg of DMI for the pre- and postpartum peri-

ods, respectively, using the NRC (2001) model (Table 1). Prepartum intake for each cow was calculated daily for the last 42 d of gestation based on the weekly DM content measured at 105°C of the ingredients and the respective composition of diets.

BW and BCS

Cows were weighed and body condition scored using a 1 to 5 scale (Ferguson et al., 1994) as depicted in the Elanco BCS chart (Elanco Animal Health, 2009) on the day of enrollment and then once weekly prepartum, in the morning, until calving. The same person scored all cows. Postpartum, immediately after each milking, cows were weighed on a walk-through scale (AfiWeigh, S.A.E. Afimilk, Kibbutz Afikim, Israel) and body condition was scored once weekly, as described previously.

Blood Sampling and Processing

Starting at 256 d of gestation, blood was sampled from all cows every other day until calving, and then on d 0, 1, 2, 3, 4, 5, 7, 14, and 21 postpartum by puncture of the coccygeal blood vessels into evacuated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Tubes contained either lithium heparin as an anticoagulant agent for plasma separation and subsequent analyses of tCa, total P (**tP**), total Mg (**tMg**), and glucose, or K_2 EDTA as an anticoagulant for plasma separation and analyses of nonesterified fatty acids (**NEFA**) and BHB. Immediately upon sampling, tubes were placed in ice and transported to the laboratory within 1 h. Cold tubes were centrifuged for 15 min at $2,500 \times g$ at 7°C for plasma separation. Plasma samples were transferred into multiple aliquots of 1.0 or 2.0 mL and stored frozen at -20°C until analyses.

Prepartum samples collected on d -14 , -12 , -10 , -8 , -6 , -4 , and -2 relative to calving were assayed for concentrations of tCa, tP, tMg, glucose, NEFA, and BHB. Samples collected on d 0, 1, 2, 3, 4, 5, 7, 14, and 21 were assayed for concentrations of glucose, NEFA, and BHB, whereas those collected on d 0, 1, 2, 3, 4, 5, and 7 were assayed for concentrations of tCa, tP, and tMg.

Sampling Whole Blood and Measurements of iCa, Na, K, and Acid-Base Status

Whole blood was sampled by puncture of the jugular vein from all cows at 254 d of gestation, the day before switching cows assigned to the short treatments to their respective negative DCAD diets. Whole blood was sampled again from a subset of the first 21 blocks comprising 80 cows ($-70_{\text{short}} = 21$, $-180_{\text{short}} =$

20, $-70_{\text{long}} = 19$, and $-180_{\text{long}} = 21$) at 268 and 272 d of gestation, corresponding to -10 and -6 d relative to expected calving, and at 0, 1, 2, 3, and 4 d postpartum. Samples were analyzed within 1 to 2 min for concentrations of iCa, Na, K, pH, bicarbonate, base excess, total dissolved CO_2 (tCO_2), saturation of O_2

Table 1. Ingredient composition and nutrient content of diets fed during the prepartum and postpartum periods

Item	Prepartum diet ¹			Lactation
	+110	-70	-180	
Ingredient, % of DM				
Alfalfa hay	—	—	—	14.7
Corn silage	34.2	34.2	34.2	33.8
Triticale silage	20.3	20.3	20.3	—
Bermuda hay	6.7	6.7	6.7	—
Wheat straw	13.8	13.8	13.8	—
Citrus pulp	7.7	7.1	6.7	7.8
Soybean meal, solvent extract	13.1	8.5	5.8	12.1
Acidogenic supplement ²	—	5.2	8.3	—
Corn grain, finely ground	—	—	—	10.7
Brewer's grains, wet	—	—	—	9.3
Soybean hulls	—	—	—	4.4
Saturated free fatty acids ³	—	—	—	1.7
Prepartum mineral-vitamin mixture ⁴	4.2	4.2	4.2	—
Postpartum mineral-vitamin mixture ⁵	—	—	—	5.0
Mycotoxin binder ⁶	—	—	—	0.5
Nutrient content, mean $\pm$ SD ⁷				
NE _L , ⁸ Mcal/kg	1.46	1.45	1.45	1.66
OM, %	92.5 $\pm$ 0.5	92.3 $\pm$ 0.4	92.4 $\pm$ 0.3	91.0 $\pm$ 0.9
CP, %	14.9 $\pm$ 0.8	14.7 $\pm$ 0.4	14.6 $\pm$ 0.6	17.4 $\pm$ 0.5
ADF, %	29.4 $\pm$ 1.4	28.9 $\pm$ 1.2	29.1 $\pm$ 1.1	22.5 $\pm$ 2.4
NDF, %	43.1 $\pm$ 1.7	43.7 $\pm$ 1.5	43.8 $\pm$ 1.5	31.6 $\pm$ 1.9
Forage NDF, %	39.3 $\pm$ 1.7	39.3 $\pm$ 1.7	39.3 $\pm$ 1.7	20.0 $\pm$ 1.6
NFC, ⁹ %	31.7 $\pm$ 1.3	31.1 $\pm$ 1.6	31.1 $\pm$ 1.9	37.3 $\pm$ 3.3
Starch, %	12.3 $\pm$ 0.4	12.6 $\pm$ 0.5	12.9 $\pm$ 0.6	20.5 $\pm$ 2.6
Fatty acids, %	2.8 $\pm$ 0.2	2.8 $\pm$ 0.1	2.8 $\pm$ 0.1	4.0 $\pm$ 0.1
Ca, %	0.67 $\pm$ 0.07	0.64 $\pm$ 0.05	0.62 $\pm$ 0.05	1.00 $\pm$ 0.38
P, %	0.33 $\pm$ 0.01	0.33 $\pm$ 0.02	0.33 $\pm$ 0.03	0.38 $\pm$ 0.02
Mg, %	0.44 $\pm$ 0.06	0.47 $\pm$ 0.06	0.48 $\pm$ 0.03	0.40 $\pm$ 0.03
K, %	1.54 $\pm$ 0.10	1.49 $\pm$ 0.09	1.46 $\pm$ 0.09	1.65 $\pm$ 0.09
S, %	0.29 $\pm$ 0.03	0.40 $\pm$ 0.03	0.47 $\pm$ 0.03	0.21 $\pm$ 0.01
Na, %	0.08 $\pm$ 0.03	0.11 $\pm$ 0.03	0.13 $\pm$ 0.04	0.55 $\pm$ 0.03
Cl, %	0.50 $\pm$ 0.07	0.86 $\pm$ 0.07	1.11 $\pm$ 0.03	0.66 $\pm$ 0.05
DCAD, ¹⁰ mEq/kg of DM	+109 $\pm$ 35	-66 $\pm$ 17	-176 $\pm$ 20	346 $\pm$ 19

¹Cows were fed the following diets according to treatment: -70_{short} = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with -70 mEq/kg of DM from 255 d of gestation to calving; -180_{short} = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with -180 mEq/kg of DM from 255 d of gestation to calving; -70_{long} = diet containing -70 mEq/kg of DM from 232 d of gestation to calving; -180_{long} = diet containing -180 mEq/kg of DM from 232 d of gestation to calving.

²Bio-Chlor (fermentation product containing dried condensed extracted glutamic acid fermentation product, dried condensed corn fermentation solubles, processed grain by-products, and magnesium chloride; Arm & Hammer Animal Nutrition, Princeton, NJ).

³Energy-Booster Mag (fat supplement containing 95.8% fatty acids and 2.3% Mg, Milk Specialties Global, Eden Prairie, MN).

⁴Contains (DM basis) 3.7% Ca, 0.9% P, 5.5% Mg, 0.9% K, 2.3% S, 1.1% Na, 1.6% Cl, and per kg of DM, 724 mg of Zn, 165 mg of Cu, 543 mg of Mn, 9 mg of Se, 16 mg of I, 4 mg of Co, 232,000 IU of vitamin A, 67,000 IU of vitamin D₃, 3,300 IU of vitamin E, 750 mg of monensin, and 54 g of choline ion in a rumen-protected form.

⁵A mixture containing 20% blood meal enriched with rumen-protected lysine and methionine (LysAAMet, Perdue Ag Solutions LLC, Salisbury, MD). Contains (DM basis) 19.1% CP, 6.3% Ca, 2.0% P, 3.4% Mg, 8.3% K, 0.3% S, 9.3% Na, 5.3% Cl, and per kg of DM, 500 mg of Zn, 115 mg of Cu, 421 mg of Mn, 6.4 mg of Se, 11.1 mg of I, 3.9 mg of Co, 110,000 IU of vitamin A, 27,000 IU of vitamin D₃, 1,300 IU of vitamin E, 380 mg of monensin (Rumensin 90, Elanco Animal Health, Eli Lilly and Co, Indianapolis, IN), 22 mg of biotin, and 16 g of choline ion in a rumen-protected form (Reashure, Balchem Co., New Hampton, NY).

⁶Novasil Plus (BASF Corp., Florham Park, NJ).

⁷Samples of ingredients were collected weekly and composited each month for chemical analyses. A total of 5 samples of each ingredient was analyzed for chemical composition.

⁸Estimated using NRC (2001) according to chemical analyses of dietary ingredients and adjusted for DMI of 12 kg/d prepartum and 22 kg/d postpartum.

⁹OM - (CP + fatty acids + NDF).

¹⁰Calculated as [(mEq of K) + (mEq of Na)] - [(mEq of Cl) + (mEq of S)].

(sO₂), and partial pressures of O₂ and CO₂ (pCO₂) using a hand-held biochemical analyzer (VetScan i-STAT, Abaxis, Union City, CA).

Blood Assays

All assays followed the initial randomization with blocks, such that samples from each block were analyzed in the same assay. Plasma concentrations of NEFA (NEFA-C kit; Wako Diagnostics Inc., Richmond, VA; according to Johnson and Peters, 1993) and BHB (Wako Autokit 3-HB; Wako Diagnostics, Inc.) were analyzed using colorimetric enzymatic assays. The intra- and interassay coefficients of variation (CV) were, respectively, 3.4 and 8.4% for NEFA and 1.0 and 4.0% for BHB. Concentrations of glucose in plasma were determined by colorimetric continuous flow analysis (Autoanalyzer II, Seal Analytical, Southampton, UK) using a modification of the method described by Gochman and Schmitz (1972). Plasma concentrations of tCa and tMg were analyzed by atomic absorption using a spectrophotometer equipped with Ca- and Mg-specific hollow cathode lamps (AAnalyst 200, Perkin-Elmer Inc., Waltham, MA) as described by Martinez et al. (2012). Intra- and interassay CV were, respectively, 1.8 and 5.1% for tCa and 1.6 and 4.4% for tMg. Concentrations of tP were quantified in plasma using the molybdenum blue method (Quinlan and DeSesa, 1955). The intra- and interassay CV were, respectively, 3.4 and 10.1%.

Urine Collection and Analysis

Urine was sampled twice weekly prepartum, on Wednesdays and Saturdays, by manually stimulating the perineal area until obtaining a clean and copious stream. Approximately 50 mL was sampled, placed in ice, and had pH measured within 10 min of collection using a pH meter (Accumet AR15 pH meter, Fisher Scientific International Inc., Hampton, NH). Samples collected in the last 2 wk of gestation were composited by week, and each composite sample was split into multiple aliquots and stored at -20°C until analyses. Concentrations of tCa, tMg, and creatinine were analyzed in duplicate for each sample using the colorimetric methods in a biochemical analyzer (kits no. CA3871, MG3880, and CR3814, Randox Laboratories Ltd., Crumlin, UK). The intra- and interassay CV were, respectively, 3.2 and 1.4% for tCa, 2.5 and 4.5% for tMg, and 3.3 and 5.8% for creatinine. All assays followed the initial randomization with blocks such that samples from each block were analyzed in the same assay. Creatinine was used as a marker to estimate daily urinary volume based on the constant excretion

of 29 mg of creatinine/kg of BW per day as urinary volume = BW × 29/creatinine concentration (mg/L) (Valadares et al., 1999). The estimate of daily urinary volume was calculated using the mean BW of each cow in the week the urine sample was taken. Daily urinary excretions of tCa and tMg were calculated as the product of urinary volume and the respective concentrations of those minerals in the urine samples.

Measurement and Analysis of Colostrum

Cows were milked for the first time within the first 6 h after calving and colostrum yield was measured and recorded for the first and second milkings (AfiFlo; S.A.E. Afikim, Israel). The second postpartum milking occurred 6 h later and yield of colostrum or transition milk was also measured. Duplicate samples were collected during the first and second milkings and each sample was diluted 1:1 with skim milk. Samples of skim milk and diluted colostrum samples were analyzed in duplicates for concentrations of fat, true protein, lactose, SNF, TS, and SCC at the DHI Southeast Milk laboratory in Belleview, Florida. Concentrations in the original colostrum samples were then back calculated. Concentrations of tCa and tMg in first and second milking colostrum were analyzed by atomic absorption as described by Vieira-Neto et al. (2017b). Intra- and interassay CV were, respectively, 0.9 and 1.4% for tCa and 0.3 and 0.5% for tMg.

A sample of colostrum from first milking was frozen for subsequent analysis of concentration of IgG by radial immunodiffusion assay (Triple J Farms, Bellingham, WA) per the manufacturer's protocol (<https://kentlabs.com/jjj/product/bovine-igg-test-kit-radial-immunodiffusion-test-kit/>). Briefly, colostrum was diluted 1:5 in 0.9% saline such that the concentration of IgG would fall within the linear range of the standard curve of the assay. The diluted samples were pipetted in duplicates into the bovine anti-bovine IgG antibody plate and incubated for 27 h on a flat surface protected from light. The diameter of the precipitin ring was measured using a 7× scale loupe (no. 1975, Peak Optics, GWJ Company, La Quinta, CA) and used to calculate the IgG concentrations; intra- and interassay CV were, respectively, 1.8 and 2.3%.

Measurements of Yields of Milk and Milk Components

Cows were milked twice daily, at 0600 and 1800 h, and yields of milk were recorded automatically (AfiFlo milk meters, Afimilk) for the first 42 DIM. Samples of milk were collected from 2 consecutive milkings, morning and afternoon, every Monday and Wednesday for

analyses of concentrations of fat, true protein, lactose, and SCC at the DHI Southeast Milk laboratory (Bellevue, FL). Milk yield from each sampling milking was used to calculate the final concentrations of milk components. For calculation of yields and content of milk components, 3.5% FCM, and ECM, the samples collected on Mondays represented the concentrations of milk components on Saturday, Sunday, and Monday, whereas the samples collected on Wednesday represented the concentrations on Tuesday, Wednesday, Thursday, and Friday of each week. Yields of milk corrected for 3.5% fat content and for energy and the NE content of milk were calculated as 3.5% FCM (kg/d) = $0.4324 \times \text{milk (kg)} + [16.218 \times \text{milk fat (kg)}]$; ECM (kg/d) = $(0.3246 \times \text{milk yield}) + (12.86 \times \text{fat yield}) + (7.04 \times \text{protein yield})$; NE_L (Mcal/kg) = $(0.0929 \times \text{fat \%}) + (0.0563 \times \text{protein \%}) + (0.0395 \times \text{lactose \%})$.

Measurement of Net Energy Balance Prepartum

Energy balance was calculated using the NE system according to NRC (2001). The needs for maintenance were calculated based on metabolic BW ($0.08 \times \text{BW}^{0.75}$). Net energy of lactation for pregnancy was calculated at 3.7 Mcal/d, considering a newborn calf weighing 43 kg. Net energy intake was calculated based on the NE_L content of the diets multiplied by each cow's DMI.

Characterization and Diagnosis of Health Problems

Milk fever was characterized by a down cow that responded to intravenous administration of a solution containing 10.8 g of Ca as Ca borogluconate (Cal-Dex CMPK injection, Agrilabs, St. Joseph, MO). A blood sample was collected immediately before treatment to confirm the diagnosis by measuring the concentration of iCa. Retained placenta was characterized by the retention of fetal membranes 12 h after calving. Metritis was evaluated by transrectal palpation on d 4, 7, and 12 postpartum and characterized by an enlarged uterus with fetid watery discharge. Rectal temperature of all cows was measured on d 4, 7, and 12 postpartum, and those with temperatures greater than 39.5°C were classified as having fever. Cows with fever concurrent with metritis were classified as having puerperal metritis. At every milking, cows were examined for signs of clinical mastitis by the herd personnel immediately before milking based on presence of abnormal milk in one or more quarters. Displaced abomasum was diagnosed by percussion and auscultation of the flanks and confirmed during surgical intervention for correction by omentopexy. Cows with more than 1 clinical disease event were classified as having multiple diseases. Mor-

bidity included milk fever, retained placenta, metritis, mastitis, or displaced abomasum recorded from calving to 42 DIM. Removal from the herd was determined for the first 305 DIM.

Subclinical hypocalcemia was analyzed considering 3 distinct thresholds: whole blood iCa ≤ 1.0 mM (Oetzel, 1988), plasma tCa ≤ 2.0 mM (Reinhardt et al., 2011), or plasma tCa < 2.15 mM (Martinez et al., 2012). Incidence of subclinical hypocalcemia was based on at least 1 sample with concentration below the respective threshold on d 0, 1, 2, 3, or 4 postpartum. Daily prevalence was calculated using the same thresholds from 0 to 4 d postpartum.

Reproductive Management and Reproductive Responses

All cows were subjected to the double Ovsynch protocol for first AI (Souza et al., 2008). Briefly, cows received an i.m. injection of 100 μg of GnRH (Cystorelin, 50 $\mu\text{g}/\text{mL}$ of gonadorelin diacetate tetrahydrate, Merial, Duluth, GA) at 53 ± 3 DIM, followed by an i.m. injection of 25 mg of PGF_{2 α} (Lutalyse Sterile Solution, 5 mg/mL of dinoprost as tromethamine salt; Zoetis, Florham Park, NJ) at 60 ± 3 DIM and another injection of 100 μg of GnRH at 63 ± 3 DIM. Seven days later, at 70 ± 3 DIM, the same sequence of injections was repeated with the final GnRH administered in the afternoon of d 79 ± 3 postpartum, and timed AI was performed in the morning of d 80 ± 3 postpartum, approximately 14 to 16 h after the last GnRH treatment.

Pregnancy was diagnosed by transrectal ultrasonography on d 32 after each AI based on the presence of an amniotic vesicle with an embryo with heartbeat. Nonpregnant cows had the estrous cycle resynchronized for timed AI with the Ovsynch protocol to be reinseminated 10 d after the nonpregnancy diagnosis. Pregnant cows on d 32 were re-evaluated for pregnancy 35 d later, at 67 d after AI. For statistical analyses, the diagnosis on d 67 after AI was used to determine if a cow became pregnant to the first or subsequent AI. Interval to pregnancy up to 305 d postpartum was recorded. Cows that became "do not inseminate," were sold or died, or remained nonpregnant by 305 d postpartum were censored on the respective dates. Responses measured included proportion of cows receiving AI, days postpartum at first AI, pregnancy per AI (**P/AI**) at first AI, **P/AI** at all AI, and interval to pregnancy.

Statistical Analysis

The experiment followed a randomized complete block design with a 2×2 factorial arrangement of

treatments with 2 durations of feeding (21 vs. 42 d) and 2 levels of negative DCAD (−70 vs. −180 mEq/kg of DM) and cow as the experimental unit. Parturient cows at 230 ± 3 d of gestation were blocked by parity (lactation 1 vs. lactation >1) and previous lactation 305-d milk yield and, within each block, assigned randomly to 1 of the 4 treatments.

Normality of residuals and homogeneity of variance were examined for each continuous dependent variable analyzed after fitting the final model. Responses that violated the assumptions of normality were subjected to power transformation according to the Box-Cox procedure (Box and Cox, 1964) using the TRANSREG procedure of SAS (SAS ver. 9.4, SAS/STAT, SAS Institute Inc., Cary, NC). The variables transformed were parturient blood BHB transformed to the inverse, and postpartum blood NEFA, BHB, and glucose transformed to the logarithm. The least squares means and standard errors of the means were back transformed for presentation of results according to Jørgensen and Pedersen (1998).

During the parturient period, 2 statistical models were built, one for d −42 to −22 relative to calving and another for d −21 to −1 relative to calving. This was done because, from 232 to 254 d of gestation, cows assigned to the short treatments were fed a diet with +110 mEq/kg of DM based on the design of the experiment.

Data from −42 to −22 d relative to calving were analyzed by ANOVA with the MIXED procedure of SAS (SAS/STAT). The statistical models included the fixed effects of level of DCAD (+110 vs. −70 vs. −180 mEq/kg of DM), day of measurement, the interaction between treatment and day, parity group (primiparous vs. multiparous), and calf sex (female vs. male vs. twin), the random effects of block, and cow nested within treatment. Data with a single measurement per cow were analyzed with the fixed effects of treatment, parity group, and calf sex and the random effect of block. Orthogonal polynomial contrasts were used to determine linear and quadratic effects of DCAD. Data collected in the last 21 d of gestation were analyzed by ANOVA with the MIXED procedure of SAS (SAS/STAT). The statistical models included the fixed effects of duration of feeding (21 vs. 42 d), level of DCAD (−70 vs. −180 mEq/kg of DM), day of measurement, parity group (primiparous vs. multiparous), calf sex (female vs. male vs. twin), the interactions between duration and DCAD, duration and day, DCAD and day, and duration and DCAD and day, and the random effects of block and cow nested within duration and DCAD.

Postpartum data were analyzed separately from parturient data. Continuous variables were analyzed

by ANOVA with the MIXED procedure of SAS (SAS/STAT). The statistical models included the fixed effects of duration of feeding (21 vs. 42 d), level of DCAD (−70 vs. −180 mEq/kg of DM), day of measurement, parity group (primiparous vs. multiparous), calf sex (female vs. male vs. twin), the interactions between duration and DCAD, duration and day, DCAD and day, and duration and DCAD and day, and the random effects of block and cow nested within duration and DCAD. For yields of milk and milk components, the previous lactation yield was used as covariate. Colostrum composition and changes in BW and BCS were analyzed with models that included the fixed effects of duration of feeding, level of DCAD, parity group, calf sex, the interactions between duration and DCAD, and the random effect of block.

For all mixed models with continuous data, the Kenward-Roger method was used to approximate the denominator degrees of freedom for the *F* tests in the statistical models. Model fit was assessed based on the smallest corrected Akaike's information criterion. For repeated measures, the covariance structure was selected for each model based on spacing of measurements and the smallest corrected Akaike's information criterion. When an interaction was significant, pairwise comparisons were performed with the adjustment by Tukey.

Binary data were analyzed by logistic regression with the GLIMMIX procedure of SAS (SAS/STAT). The statistical models included the fixed effects of duration of feeding, level of DCAD, interaction between duration and DCAD, parity group, and calf sex, and the random effect of block. Binary data with repeated measures within cow, such as daily prevalence of subclinical hypocalcemia and hyperketonemia, the statistical models included the fixed effects of duration of feeding, level of DCAD, day, parity group, calf sex, and interaction between duration and DCAD, duration and day, DCAD and day, and duration and DCAD and day, and the random effects of block and cow nested within duration and DCAD.

Days to pregnancy or leaving the herd was analyzed with the Cox's proportional hazard regression using the PHREG procedure of SAS (SAS/STAT). The model included the effects of duration of feeding, level of DCAD, and interaction between duration and DCAD, parity group, and calf sex. When the interaction between duration and DCAD was nonsignificant ($P > 0.10$), it was dropped from the final model. The adjusted hazard ratio and the 95% confidence interval were calculated. The LIFETEST procedure of SAS (SAS/STAT) was used to generate the survival curves and compute the least squares means $\pm$ standard error of the means and

median days to event. Statistical significance was considered at $P \leq 0.05$, and tendency was considered at $0.05 < P \leq 0.10$.

RESULTS

All 114 cows enrolled in the experiment were included in all statistical analyses, unless the cow died before 42 d postpartum, in which case she contributed data until the day of death. One cow fed -180_{long} developed obturator nerve paralysis the day after delivery because of difficult calving; thus, health data for this cow included only diagnosis of retained placenta and milk fever and she was excluded from all other postpartum analyses. Number of days dry and days prepartum on the experiment did not differ among treatments (Supplemental Table S1; <https://doi.org/10.3168/jds.2018-14580>). Gestation length was 2 d shorter ($P = 0.03$) for cows fed the long compared with the short treatments, but no effect ($P = 0.15$) of DCAD or interaction between duration and DCAD ($P = 0.68$) was detected. As designed, cows fed the short treatments received the diets with negative DCAD for approximately 21 d, whereas those fed the long treatments received the diets with negative DCAD for a mean of 43 d.

Intake, Measures of Energy Status, and Acid-Base Balance in the Early Dry Period

From d -42 to -22 relative to calving, cows enrolled in the short treatments were fed a diet with $+110$ mEq/kg of DM and data were analyzed with 3 DCAD treatments, $+110$, -70 , and -180 mEq/kg of DM; results herein are presented in the same sequence. Reducing the DCAD from $+110$ to -180 mEq/kg of DM linearly reduced ($P = 0.02$) DMI (11.5 ± 0.3 vs. 10.7 ± 0.4 vs. 10.2 ± 0.4 kg/d), which, consequently, had a linear effect in reducing ($P < 0.02$) caloric intake (16.7 ± 0.5 vs. 15.5 ± 0.6 vs. 14.8 ± 0.7 Mcal of NE_L/d) and NE balance (1.2 ± 0.5 vs. -0.1 ± 0.6 vs. -0.5 ± 0.7 Mcal/d). An interaction ($P = 0.008$) between treatment and day was detected because the differences in DMI increased with days in the experiment (Figure 1A). The mean BW and BCS did not differ among treatments (Supplemental Table S2; <https://doi.org/10.3168/jds.2018-14580>). Blood pH did not differ with treatment, although blood of cows showed typical signs of a compensated metabolic acidosis with linear reductions in tCO_2 (27.8 ± 0.6 vs. 27.7 ± 0.8 vs. 24.6 ± 0.8 mM), pCO_2 (40.6 ± 1.0 vs. 43.4 ± 1.4 vs. 38.2 ± 1.4 mM), bicarbonate (26.6 ± 0.6 vs. 26.4 ± 0.8 vs. 23.6 ± 0.8 mM), base excess (2.12 ± 0.69 vs. 1.59 ± 0.95 vs. -1.17 ± 0.94 mM), and urine pH (8.05 ± 0.06 vs. 6.52 ± 0.07 vs. 5.48 ± 0.08). Urinary pH remained relatively

constant according to treatment throughout the first 21 d in the experiment (Figure 1C). Blood concentrations of iCa, Na, K, and glucose did not differ with treatment (Supplemental Table S2).

Intake and Measures of Energy Status in the Late Dry Period

Duration of feeding did not affect DMI in the last 21 d of gestation; however, reducing the DCAD from -70 to -180 mEq/kg of DM reduced ($P = 0.005$) DMI by 1.1 kg/d ($-70 = 10.8$ vs. $-180 = 9.7 \pm 0.4$ kg/d; Supplemental Table S3, <https://doi.org/10.3168/jds.2018-14580>), and the reduction was observed in cows fed short or long throughout the 21-d period (Figure 1B). The reduction in DMI resulted in less ($P = 0.007$) caloric intake in cows fed -180 rather than -70 mEq/kg of DM (15.6 vs. 13.9 ± 0.5 Mcal/d) as well as smaller ($P = 0.007$) NE balance (-0.3 vs. -2.0 ± 0.6 Mcal/d). An interaction ($P = 0.03$) between duration of feeding and DCAD was detected for BW in part because cows fed -180_{short} were heavier than cows fed -180_{long} ($-70_{\text{short}} = 754$ vs. $-180_{\text{short}} = 781$ vs. $-70_{\text{long}} = 772$ vs. $-180_{\text{long}} = 748 \pm 14$ kg). Also, an interaction ($P = 0.01$) between duration of feeding and DCAD was observed for BW change because cows fed -70_{short} gained more weight than cows fed -180_{short} or -70_{long} ($-70_{\text{short}} = 1.0$ vs. $-180_{\text{short}} = 0.4$ vs. $-70_{\text{long}} = 0.5$ vs. $-180_{\text{long}} = 0.7 \pm 0.2$ kg/d). The BCS did not differ with treatment, but the change from week -6 to -1 tended ($P = 0.06$) to differ with DCAD because cows fed -70 mEq/kg of DM lost less body condition than cows fed -180 mEq/kg of DM (-0.10 vs. -0.20 ± 0.05).

Acid-Base Balance and Urinary Excretion of Minerals Prepartum

Reducing the DCAD from -70 to -180 mEq/kg of DM in the last 21 d of gestation reduced ($P < 0.001$) blood pH, tCO_2 , bicarbonate, and base excess (Table 2). Duration of feeding acidogenic diets did not influence acid-base balance. An interaction between duration and DCAD tended ($P = 0.07$) to affect pCO_2 , and the value was smallest in cows fed -180_{short} .

Duration of feeding acidogenic diets prepartum did not influence urinary pH, volume, concentrations of Ca or Mg, and excretions of Ca and Mg (Table 2). On the other hand, reducing the DCAD from -70 to -180 mEq/kg of DM decreased ($P < 0.001$) urinary pH, but increased ($P < 0.01$) urinary volume and concentration and excretion of Ca in urine. Concentration of Mg in urine tended ($P = 0.07$) to be greater for cows fed -70 than -180 mEq/kg of DM, but urinary excretion did

Figure 1. Prepartum DMI and urine pH. (A) Dry matter intake from d -42 to -22 relative to calving when cows were fed diets with +110, -70, or -180 mEq/kg of DM; (B) DMI from d -21 to -1 relative to calving according to duration of feeding (Short, 21 d; Long, 42 d) and level of DCAD (-70 vs. -180 mEq/kg of DM); (C) urine pH from d -42 to -22 relative to calving when cows were fed diets with +110, -70, or -180 mEq/kg of DM; and (D) urine pH from d -21 to -1 relative to calving according to duration of feeding (Short, 21 d; Long, 42 d) and level of DCAD (-70 vs. -180 mEq/kg of DM). In (A) LSM and SEM were +110 = 11.5 ± 0.3 ; -70 = 10.7 ± 0.4 ; and -180 = 10.2 ± 0.4 kg/d; linear effect of level of DCAD ($P = 0.02$) and interaction between level of DCAD and day ($P = 0.008$). In (B) LSM and SEM were -70_short = 11.1 ± 0.5 ; -180_short = 9.7 ± 0.5 ; -70_long = 10.5 ± 0.5 ; and -180_long = 9.6 ± 0.5 kg/d; effects of duration of feeding ($P = 0.38$), level of DCAD ($P = 0.005$), and interactions between duration and DCAD ($P = 0.45$), duration and day ($P = 0.15$) and DCAD and day ($P = 0.008$). In (C) LSM and SEM were +110 = 8.05 ± 0.06 ; -70 = 6.52 ± 0.07 ; and -180 = 5.48 ± 0.08 ; linear effect of level of DCAD ($P < 0.001$) and interaction between level of DCAD and day ($P = 0.11$). In (D) LSM and SEM were -70_short = 6.46 ± 0.14 ; -180_short = 5.62 ± 0.14 ; -70_long = 6.48 ± 0.14 ; and -180_long = 5.56 ± 0.14 ; effects of duration of feeding ($P = 0.81$), level of DCAD ($P < 0.001$), and interactions between duration and DCAD ($P = 0.64$), duration and day ($P = 0.03$), DCAD and day ($P = 0.49$), and duration and DCAD and day ($P = 0.84$). Error bars represent the SEM; within a day, treatments with an asterisk differ ($P < 0.05$).

not differ with altering the level of DCAD. Once cows fed short were switched from a diet with +110 to either -70 or -180 mEq/kg of DM, urinary pH declined

within 24 h (data not shown) and pH remained stable according to treatment in the last 21 d of gestation, although an interaction ($P = 0.03$) between duration

and day was observed because of the changes in the first days after diet switch (Figure 1D).

Concentrations of Minerals and Metabolites in Blood Prepartum

Duration of feeding acidogenic diets prepartum did not affect concentrations of minerals or metabolites in whole blood or plasma in the last 21 d of gestation (Supplemental Table S4; <https://doi.org/10.3168/jds.2018-14580>). Reducing the DCAD from -70 to -180 mEq/kg of DM increased ($P = 0.002$) whole blood concentrations of iCa (1.233 vs. 1.272 ± 0.011 mM; Figure 2A). Nevertheless, DCAD did not affect concentrations of plasma tCa and tMg (Figure 2B and 2C) or whole blood concentrations of Na and K. An interaction ($P = 0.008$) between duration and DCAD was detected for plasma tP in part because, within cows receiving the -70 mEq/kg of DM diets, those fed long had greater ($P < 0.05$) concentrations of plasma tP than cows fed short ($-70_{\text{short}} = 1.74$ vs. $-180_{\text{short}} = 1.82$ vs. $-70_{\text{long}} = 1.83$ vs. $-180_{\text{long}} = 1.77 \pm 0.05$ mM; Figure 2D). Treatment did not affect concentrations of glucose and BHB in plasma prepartum (Figures 3A and

3C); however, an interaction ($P = 0.05$) between duration and DCAD affected the concentrations of NEFA in plasma, and cows fed -180_{short} had greater ($P < 0.05$) concentrations of plasma NEFA than cows fed -180_{long} ($-70_{\text{short}} = 0.260$ vs. $-180_{\text{short}} = 0.314$ vs. $-70_{\text{long}} = 0.275$ vs. $-180_{\text{long}} = 0.239 \pm 0.027$ mM; Figure 3B).

Colostrum Yield and Composition

Yield of first milking colostrum did not differ with duration of feeding, but it was greater ($P = 0.04$) for cows fed the -70 than the -180 mEq/kg of DM diets (Table 3). Treatment did not affect concentrations of fat, lactose, TS, NE, Ca, Mg, IgG, and SCS. Tendencies ($P = 0.09$) for the effect of duration were observed for concentrations of true protein and SNF in first milking colostrum, although no statistical differences were observed among individual treatments after Tukey-protected pairwise comparisons. Duration of feeding did not affect yields of any of the components measured in colostrum; however, cows fed the -70 mEq/kg of DM diet tended ($P \leq 0.09$) to produce more true protein and SNF in colostrum, and produced more ($P = 0.03$)

Table 2. Effects of duration (DUR) of prepartum feeding and level of DCAD on measures of blood acid-base balance, and urinary pH and excretion of minerals in Holstein cows prepartum¹

Item	Short		Long		SEM	<i>P</i> -value ²		
	−70	−180	−70	−180		DUR	DCAD	DUR × DCAD
Blood ³								
pH	7.424	7.385	7.417	7.381	0.009	0.47	<0.001	0.86
Total CO ₂ , mM	27.2	24.0	26.6	25.0	0.7	0.72	<0.001	0.14
Saturation of O ₂ , %	58.1	59.4	59.5	57.7	2.5	0.94	0.89	0.47
pCO ₂ , mm Hg	40.1	37.8	39.5	39.9	0.9	0.36	0.22	0.07
pO ₂ , mm Hg	30.1	31.8	32.0	31.3	1.2	0.50	0.64	0.22
Bicarbonate, mM	26.2	22.6	25.9	23.8	0.7	0.47	<0.001	0.18
Base excess, ⁴ mM	1.75	−2.26	1.07	−1.43	0.78	0.91	<0.001	0.23
Urine								
pH ⁵	6.46	5.62	6.48	5.56	0.14	0.81	<0.001	0.64
Creatinine, ⁶ mg/L	642	519	673	508	66	0.84	<0.001	0.62
L/d ⁶	33.5	42.8	32.6	43.0	4.3	0.88	<0.001	0.82
Ca, ⁶ mg/L	233	319	259	318	31	0.64	0.007	0.63
Ca, ⁶ g/d	6.48	11.06	6.86	11.06	0.91	0.81	<0.001	0.81
Mg, ⁶ mg/L	193	175	211	165	31	0.85	0.07	0.39
Mg, ⁶ g/d	6.24	7.58	6.69	6.32	0.84	0.58	0.50	0.24

¹Cows were fed the following diets according to treatment: -70_{short} = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with -70 mEq/kg of DM from 255 d of gestation to calving; -180_{short} = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with -180 mEq/kg of DM from 255 d of gestation to calving; -70_{long} = diet containing -70 mEq/kg of DM from 232 d of gestation to calving; -180_{long} = diet containing -180 mEq/kg of DM from 232 d of gestation to calving.

²DUR = effect of feeding duration (42 vs. 21 d); DCAD = effect of level of DCAD (-70 vs. -180); DUR $\times$ DCAD = effect of interaction between DUR and DCAD (-70_{short} plus -180_{long} vs. -180_{short} plus -70_{long}).

³Jugular whole blood sampled and analyzed at 268 and 272 d of gestation, corresponding to -10 and -6 d relative to calving. pO₂ = partial pressure of O₂; pCO₂ = partial pressure of CO₂.

⁴Base excess calculated using the formula: Base excess mM = $[\text{HCO}_3^- - 24.8 + 16.2 (\text{blood pH} - 7.4)]$ (CLSI, 2001).

⁵Urine sampled and analyzed from 256 d of gestation to calving.

⁶Urine sampled and analyzed in the last 2 wk of gestation. Urinary volume was estimated based on creatinine concentration (Valadares et al., 1999).

Figure 2. Concentrations of whole blood ionized Ca (iCa, A), plasma total Ca (tCa, B), plasma total Mg (tMg, C), and plasma total P (tP, D) in dairy cows fed prepartum acidogenic diets of short (21 d) or long (42 d) durations of feeding and with -70 or -180 mEq/kg of DM (-70_{short} , -180_{short} , -70_{long} , and -180_{long}). (A) Prepartum interactions between duration and day ($P = 0.95$), DCAD and day ($P = 0.10$), and duration and DCAD and day ($P = 0.90$); postpartum interactions between duration and day ($P = 0.14$), DCAD and day (*, $P = 0.04$), and duration and DCAD and day ($P = 0.46$). (B) Prepartum interactions between duration and day ($P = 0.38$), DCAD and day ($P = 0.31$), and duration and DCAD and day ($P = 0.004$); postpartum interactions between duration and day ($P = 0.31$), DCAD and day ($P = 0.22$), and duration and DCAD and day ($P = 0.24$). (C) Prepartum interactions between duration and day ($P = 0.10$), DCAD and day ($P = 0.11$), and duration and DCAD and day ($P = 0.006$); postpartum interactions between duration and day ($P = 0.94$), DCAD and day ($P = 0.30$), and duration and DCAD and day ($P = 0.67$). (D) Prepartum interactions between duration and day ($P = 0.61$), DCAD and day ($P = 0.58$), and duration and DCAD and day ($P = 0.22$); postpartum interactions between duration and day ($P = 0.72$), DCAD and day ($P = 0.53$), and duration and DCAD and day ($P = 0.76$). Error bars represent SEM.

Figure 3. Concentrations of glucose (A), nonesterified fatty acids (NEFA) (B), and BHB (C) in plasma of dairy cows fed prepartum acidogenic diets of short (21 d) or long (42 d) durations of feeding and with -70 or -180 mEq/kg of DM (-70_short , -180_short , -70_long , and -180_long). (A) Prepartum interactions between duration and day ($P = 0.65$), DCAD and day ($P = 0.88$), and duration and DCAD and day ($P = 0.65$); postpartum interactions between duration and day ($P = 0.95$), DCAD and day ($P = 0.98$), and duration and DCAD and day ($P = 0.42$). (B) Prepartum interactions between duration and day ($P = 0.88$), DCAD and day ($P = 0.56$), and duration and DCAD and day ($P = 0.47$); postpartum interactions between duration and day ($P = 0.08$), DCAD and day ($P = 0.49$), and duration and DCAD and day ($P = 0.77$). (C) Prepartum interactions between duration and day ($P = 0.21$), DCAD and day ($P = 0.59$), and duration and DCAD and day ($P = 0.98$); postpartum interactions between duration and day ($P = 0.43$), DCAD and day ($P = 0.93$), and duration and DCAD and day ($P = 0.45$). Error bars represent the SEM.

Table 3. Effects of duration (DUR) of prepartum feeding and level of DCAD on colostrum yield and composition in Holstein cows¹

Item	Short		Long		SEM	<i>P</i> -value ²		
	–70	–180	–70	–180		DUR	DCAD	DUR × DCAD
First milking								
Yield, kg	6.79	4.88	6.30	5.41	0.88	0.98	0.04	0.45
Content								
Fat, %	4.97	5.53	5.06	5.18	0.47	0.76	0.41	0.59
True protein, %	13.0	13.9	14.3	14.1	0.5	0.09	0.44	0.22
Lactose, %	3.42	3.39	3.37	3.34	0.08	0.49	0.67	0.99
SNF, %	17.8	18.6	19.1	18.8	0.5	0.09	0.51	0.19
TS, %	22.8	24.2	24.2	24.0	0.8	0.35	0.35	0.23
Net energy, Mcal/kg	1.33	1.43	1.41	1.41	0.05	0.57	0.31	0.30
Ca, g/L	2.29	2.27	2.33	2.36	0.10	0.48	0.95	0.76
Mg, g/L	0.398	0.395	0.381	0.406	0.017	0.85	0.41	0.32
IgG, g/L	76.6	81.2	86.6	87.2	7.3	0.19	0.67	0.75
SCS	6.23	6.37	6.67	6.60	0.33	0.24	0.89	0.71
Yield								
Fat, kg	0.37	0.34	0.34	0.31	0.07	0.59	0.57	0.99
True protein, kg	0.84	0.68	0.92	0.76	0.12	0.39	0.09	0.98
Lactose, kg	0.24	0.17	0.23	0.18	0.03	0.96	0.03	0.66
SNF, kg	1.17	0.92	1.24	1.02	0.16	0.50	0.06	0.91
TS, kg	1.54	1.26	1.59	1.33	0.22	0.75	0.12	0.93
Net energy, Mcal	9.11	7.66	9.26	7.88	1.41	0.86	0.18	0.98
Ca, g	14.2	10.6	14.8	11.6	2.1	0.78	0.03	0.76
Mg, g	2.57	1.81	2.42	2.03	0.33	0.89	0.04	0.51
Second milking								
Yield, kg	3.03	2.74	2.89	2.78	0.42	0.90	0.58	0.82
Content								
Fat, %	5.14	5.20	4.70	4.97	0.51	0.45	0.71	0.82
True protein, %	10.2	11.4	11.3	11.7	0.6	0.17	0.11	0.38
Lactose, %	3.65	3.55	3.61	3.44	0.11	0.45	0.16	0.70
SNF, %	15.1	16.3	16.2	16.4	0.6	0.24	0.17	0.35
TS, %	20.2	21.5	20.9	21.4	0.8	0.73	0.25	0.61
Net energy, Mcal/kg	1.20	1.27	1.22	1.26	0.06	0.95	0.30	0.77
Ca, g/L	2.21	2.10	2.28	2.42	0.09	0.01	0.82	0.11
Mg, g/L	0.349 ^{ab}	0.329 ^b	0.341 ^{ab}	0.375 ^a	0.016	0.18	0.61	0.06
SCS	5.99	6.22	6.37	6.54	0.32	0.22	0.47	0.91
Yield								
Fat, kg	0.16	0.15	0.14	0.13	0.03	0.44	0.73	0.95
True protein, kg	0.28	0.30	0.32	0.31	0.05	0.62	0.97	0.67
Lactose, kg	0.11	0.10	0.11	0.10	0.02	0.82	0.45	0.94
SNF, kg	0.43	0.44	0.47	0.44	0.07	0.77	0.84	0.76
TS, kg	0.59	0.58	0.60	0.56	0.10	0.99	0.80	0.83
Net energy, Mcal	3.47	3.45	3.50	3.29	0.57	0.90	0.82	0.85
Ca, g	6.73	5.93	6.55	6.62	1.03	0.78	0.68	0.63
Mg, g	1.04	0.91	0.97	1.02	0.16	0.86	0.75	0.54

^{a,b}Superscripts in the same row differ ($P < 0.05$) after adjustment by the method of Tukey.

¹Cows were fed the following diets according to treatment: –70_short = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with –70 mEq/kg of DM from 255 d of gestation to calving; –180_short = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with –180 mEq/kg of DM from 255 d of gestation to calving; –70_long = diet containing –70 mEq/kg of DM from 232 d of gestation to calving; –180_long = diet containing –180 mEq/kg of DM from 232 d of gestation to calving.

²DUR = effect of feeding duration (42 vs. 21 d); DCAD = effect of level of DCAD (–70 vs. –180); DUR × DCAD = effect of interaction between DUR and DCAD (–70_short plus –180_long vs. –180_short plus –70_long).

lactose and secreted more ($P < 0.05$) Ca and Mg than cows fed the –180 mEq/kg of DM diet.

Dietary treatments had minor effects on second milking colostrum yield and composition (Table 3). Neither duration of feeding nor level of DCAD affected concentrations of fat, true protein, lactose, SNF, TS, and NE_L in second milking colostrum. The concentration of Ca in second milking colostrum was greater ($P = 0.01$) in cows fed long than short, whereas that of Mg

was greater ($P = 0.05$) only in cows fed –180_long compared with –180_short. Yields of second milking colostrum and components in second milking colostrum did not differ with treatment.

Lactation Performance

Increasing the duration of feeding acidogenic diets prepartum from 21 to 42 d reduced ($P = 0.03$) milk

Table 4. Effects of duration (DUR) of prepartum feeding and level of DCAD on productive performance in the first 42 d postpartum in Holstein cows¹

Item	Short		Long		SEM	<i>P</i> -value ²		
	–70	–180	–70	–180		DUR	DCAD	DUR × DCAD
Milk, kg/d	41.1	39.6	37.5	38.6	1.3	0.03	0.93	0.25
3.5% FCM, kg/d	44.3	43.7	41.8	42.6	1.4	0.15	0.91	0.58
ECM, kg/d	42.8	42.2	40.4	41.2	1.4	0.14	0.91	0.56
Fat								
%	4.11	4.27	4.33	4.26	0.11	0.24	0.60	0.22
Yield, kg	1.64	1.64	1.58	1.60	0.06	0.38	0.83	0.86
True protein								
%	2.97	3.01	3.05	3.05	0.05	0.17	0.56	0.70
Yield, kg	1.20	1.18	1.12	1.15	0.04	0.14	0.92	0.48
Lactose								
%	4.58	4.60	4.60	4.59	0.03	0.91	0.93	0.73
Yield, kg	1.90	1.84	1.74	1.79	0.06	0.04	0.94	0.32
SNF								
%	8.41	8.47	8.51	8.51	0.06	0.23	0.65	0.66
Yield, kg	3.45	3.36	3.18	3.27	0.11	0.06	0.99	0.37
SCS	2.14	1.79	2.19	2.08	0.39	0.59	0.48	0.72
BW								
kg	630.9	649.4	640.7	629.1	12.3	0.61	0.73	0.15
Change wk 1 to 6, kg/d	–1.56	–1.90	–1.72	–2.02	0.30	0.55	0.16	0.94
Body condition								
Score, 1 to 5	3.27	3.36	3.35	3.26	0.06	0.84	0.99	0.14
Change, wk 1 to 6	–0.248	–0.228	–0.257	–0.291	0.075	0.78	0.87	0.49

¹Cows were fed the following diets according to treatment: –70_short = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with –70 mEq/kg of DM from 255 d of gestation to calving; –180_short = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with –180 mEq/kg of DM from 255 d of gestation to calving; –70_long = diet containing –70 mEq/kg of DM from 232 d of gestation to calving; –180_long = diet containing –180 mEq/kg of DM from 232 d of gestation to calving.

²DUR = effect of feeding duration (42 vs. 21 d); DCAD = effect of level of DCAD (–70 vs. –180); DUR × DCAD = effect of interaction between DUR and DCAD (–70_short plus –180_long vs. –180_short plus –70_long).

yield by 2.5 kg/d (short = 40.4 vs. long = 37.9 ± 1.0 kg/d; Table 4; Figure 4A). Nevertheless, yields of 3.5% FCM and ECM did not differ with treatment (Table 4; Figure 4B). Altering the level of DCAD did not affect yields of milk, 3.5% FCM, or ECM. Treatment did not influence concentrations and yields of fat and true protein in milk; however, the reduced milk yield in cows receiving the long compared with the short treatments resulted in less ($P = 0.04$) yield of lactose and a tendency ($P = 0.06$) for less yield of SNF for those cows. The SCS did not differ with treatment. As expected, cows lost BW (Figure 4C) and body condition (Figure 4D), but the mean BW and BCS and the losses in the first 42 d postpartum did not differ among treatments (Table 4).

Acid-Base Balance Postpartum

Duration of prepartum feeding of acidogenic diets did not influence any of the measures of acid-base status in blood postpartum (Supplemental Table S5; <https://doi.org/10.3168/jds.2018-14580>). Blood pH postpartum tended ($P = 0.09$) to be less for cows fed the –70 than those fed the –180 mEq/kg of DM diets prepartum (7.449 vs. 7.455 ± 0.003). Feeding –70 mEq/kg of DM of DCAD prepartum caused a slight reduction ($P <$

0.05) in tCO₂ (30.10 vs. 30.88 ± 0.31 mM), bicarbonate (28.9 vs. 29.7 ± 0.3 mM), and base excess (4.87 vs. 5.77 ± 0.32 mM), although the reduction in bicarbonate was only evident in –70_long cows (–70_short = 29.2 vs. –180_short = 29.3 vs. –70_long = 28.6 vs. –180_long = 30.0 ± 0.4 mM; Supplemental Table S5).

Blood Concentrations of Minerals and Metabolites Postpartum

Concentrations of iCa, tCa, and tP sharply declined ($P < 0.001$) on the day of calving, whereas those of tMg increased with the onset of lactation (Figure 2A–D). Treatments did not influence concentrations of iCa, tCa, tP, and Na in the first days postpartum (Table 5), although an interaction ($P = 0.04$) between DCAD and day postpartum was observed for iCa because cows fed –180 mEq/kg of DM of DCAD had greater iCa concentrations on the day of calving than those fed –70 mEq/kg of DM of DCAD prepartum (Figure 2A). Cows fed –70_long and –180_long had greater ($P = 0.05$) concentrations of tMg than those fed –70_short and –180_short. An interaction ($P = 0.008$) between duration and DCAD affected blood K in the first 4 DIM because cows fed –180_short had a smaller ($P <$

Figure 4. Yields of milk (A) and ECM (B), BW (C), and BCS (D) of dairy cows fed prepartum acidogenic diets of short (21 d) or long (42 d) durations and with -70 or -180 mEq/kg of DM (-70_{short} , -180_{short} , -70_{long} , and -180_{long}). (A) Interactions between duration and day ($P = 0.001$), DCAD and day ($P = 0.96$), and duration and DCAD and day ($P = 0.96$). (B) Interactions between duration and day ($P < 0.001$), DCAD and day ($P = 0.55$), and duration and DCAD and day ($P = 0.71$). (C) Prepartum interactions between duration and week ($P = 0.66$), DCAD and week ($P = 0.65$), and duration and DCAD and week ($P = 0.42$); postpartum interactions between duration and week ($P = 0.35$), DCAD and week ($P = 0.19$), and duration and DCAD and week ($P = 0.72$). (D) Prepartum interactions between duration and day ($P = 0.08$), DCAD and week ($P = 0.61$), and duration and DCAD and week ($P = 0.61$); postpartum interactions between duration and week ($P = 0.10$), DCAD and week ($P = 0.66$), and duration and DCAD and week ($P = 0.78$). Error bars represent SEM.

0.05) concentration of K than those fed -70_{short} or -180_{long} .

Concentrations of glucose in plasma sharply increased ($P < 0.001$) on the day of calving to 5.18 ± 0.22 mM, and then declined to approximately 2.95 ± 0.13 mM by 3 d postpartum (Figure 3A). Increases in plasma NEFA and BHB concentrations with the onset of lactation were also observed, but they were not as acute, and their concentrations remained elevated until 21 d postpartum (Figure 3B and 3C). An interaction ($P = 0.05$) between duration of feeding and DCAD affected mean plasma glucose postpartum because concentrations were greater for cows fed -70_{short} and -180_{long} than -180_{short} and -70_{long} , although pairwise comparisons did not detect differences among individual treatments (Table 5). An interaction ($P = 0.04$) between duration and DCAD also affected plasma BHB concentrations, and cows fed -70_{short} and -180_{long} had less BHB than cows fed -180_{short} and -70_{long} . Similar to plasma glucose, pairwise comparisons did not detect differences among individual treatments for plasma BHB concentrations. Cows fed short tended ($P = 0.09$) to have greater NEFA concentrations in plasma than those fed long.

Health and Survival

Two cows had milk fever, 1 fed -70_{short} and 1 fed -180_{short} . One cow fed -70_{short} developed milk fever approximately 12 h before calving, and the blood

iCa concentration immediately before treatment with i.v. Ca solution was 0.58 mM. Twenty-four hours later, whole blood iCa was 1.16 mM. The second cow, fed -180_{short} , was diagnosed with milk fever hours after milking colostrum and blood iCa was 0.61 mM; she also recovered after i.v. Ca solution.

Treatment did not affect the incidence of retained placenta, metritis, puerperal metritis, mastitis, or displaced abomasum (Table 6). Morbidity affected 32.7% of the experimental cows and 15.1% of them were diagnosed with more than 1 clinical disease in the first 42 DIM.

The incidence of subclinical hypocalcemia changed according to threshold selected. It was lowest when based on whole blood iCa ≤ 1.0 mM, intermediate when based on tCa ≤ 2.0 mM, and highest when based on tCa < 2.15 mM (Table 6). Nevertheless, despite the threshold used, treatments did not influence the incidence of subclinical hypocalcemia postpartum. Similarly, the daily prevalence of subclinical hypocalcemia changed with threshold selected and followed the same pattern as that of incidence of subclinical hypocalcemia (Figure 5). The daily prevalence of subclinical hypocalcemia declined ($P < 0.001$) with day postpartum regardless of threshold used, but neither treatment nor interaction between treatment and day affected the daily prevalence of subclinical hypocalcemia.

By 42 DIM, 7.1% of the cows enrolled in the experiment had been sold or died, and this number increased to 21.2% by 305 d postpartum (Table 6). Duration of

Table 5. Effects of duration (DUR) of prepartum feeding and level of DCAD on blood concentrations of minerals and metabolites in Holstein cows postpartum¹

Item	Short		Long		SEM	P-value ²		
	-70	-180	-70	-180		DUR	DCAD	DUR $\times$ DCAD
Blood iCa, ³ mM	1.122	1.131	1.130	1.122	0.014	0.99	0.94	0.53
Plasma tCa, ⁴ mM	2.21	2.20	2.20	2.23	0.03	0.65	0.61	0.57
Plasma tMg, ⁴ mM	0.725	0.710	0.744	0.745	0.019	0.05	0.57	0.55
Plasma tP, ⁴ mM	1.33	1.30	1.37	1.34	0.06	0.44	0.59	0.94
Blood Na, ³ mM	143.0	143.0	143.2	143.1	0.4	0.62	0.89	0.90
Blood K, ³ mM	4.12 ^a	3.97 ^b	4.05 ^{ab}	4.14 ^a	0.08	0.21	0.50	0.008
Plasma glucose, ⁵ mM	3.26	3.16	3.19	3.36	0.09	0.30	0.57	0.05
Plasma NEFA, ⁵ mM	0.769	0.788	0.704	0.659	0.067	0.09	0.79	0.56
Plasma BHB, ⁵ mM	1.50	1.73	1.72	1.41	0.15	0.67	0.72	0.04

^{a,b}Superscripts in the same row differ ($P < 0.05$) after adjustment by the method of Tukey.

¹Cows were fed the following diets according to treatment: -70_{short} = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with -70 mEq/kg of DM from 255 d of gestation to calving; -180_{short} = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with -180 mEq/kg of DM from 255 d of gestation to calving; -70_{long} = diet containing -70 mEq/kg of DM from 232 d of gestation to calving; -180_{long} = diet containing -180 mEq/kg of DM from 232 d of gestation to calving.

²DUR = effect of feeding duration (42 vs. 21 d); DCAD = effect of level of DCAD (-70 vs. -180); DUR $\times$ DCAD = effect of interaction between DUR and DCAD (-70_{short} plus -180_{long} vs. -180_{short} plus -70_{long}).

³Jugular blood sampled on d 0, 1, 2, 3, and 4 postpartum and whole blood analyzed for concentrations of ionized Ca (iCa), Na, and K.

⁴Blood sampled by puncture of coccygeal vessels on d 0, 1, 2, 3, 4, 5, and 7 postpartum and plasma analyzed for concentrations of total (t) Ca, tMg, and tP.

⁵Blood sampled by puncture of coccygeal vessels on d 0, 1, 2, 3, 4, 5, 7, 14, and 21 and plasma analyzed for concentrations of glucose, nonesterified fatty acids (NEFA), and BHB.

feeding or level of prepartum DCAD did not affect the risk of cows leaving the herd.

Reproduction

Of the 113 cows considered for analyses of data for reproductive responses, 101 (89.3%) received at least 1 AI postpartum. Treatment did not influence the proportion of cows receiving AI (Table 7). Because of timed AI, days to first insemination did not differ among treatments. Pregnancy at first AI did not differ with treatment, but cows fed short had greater ($P = 0.03$) P/AI after all AI than cows fed long (short = 35.0 vs. long = 22.6%), which tended ($P = 0.08$) to increase the proportion of cows pregnant by 305 d postpartum (short = 76.0 vs. long = 60.0%). In fact, the rate of pregnancy was 55% greater ($P = 0.06$) in cows fed short than those fed long (Figure 6A), which reduced the mean and median days to pregnancy. Level of DCAD did not influence reproduction in dairy cows (Figure 6B).

DISCUSSION

According to the USDA (2008, 2016), at least 30% of the dairy farms in the United States use acidogenic diets to prevent hypocalcemia, and 60% separate prepartum cows into far-off and close-up groups during

the dry period. The recommended range of DCAD in diets for prepartum cows has been suggested as -50 to -150 mEq/kg of DM (NRC, 2001), although the ideal DCAD has not been established to optimize health and performance. Furthermore, some producers might opt to group prepartum cows together during the entire dry period, and some experiments suggested that feeding acidogenic diets longer than 21 d was not detrimental to early postpartum performance (Weich et al., 2013; Wu et al., 2014).

Increasing the duration of feeding acidogenic diets prepartum from 21 to 42 d reduced gestation length and milk yield and tended to compromise reproduction regardless of the level of DCAD fed. Concurrently, reducing the level of DCAD from -70 to -180 mEq/kg of DM decreased prepartum DMI and produced a more pronounced metabolic acidosis. The more acidogenic diet attenuated the decrease of blood iCa concentration at calving and reduced colostrum yield at the first milking, but had no subsequent effect on production, health, or reproduction in dairy cows.

Acidogenic diets are well known to minimize the risk of hypocalcemia in dairy cows (Ender et al., 1971; Block, 1984), but they can affect intake prepartum. Charbonneau et al. (2006) demonstrated a linear decrease in DMI with a reduction in DCAD, which corroborates the findings from the present experiment. A potential palatability issue with acidogenic salts has been sug-

Table 6. Effects of duration (DUR) of prepartum feeding and level of DCAD on health in Holstein cows¹

Item ³	Incidence (no./no.)	Short		Long		<i>P</i> -value ²		
		-70	-180	-70	-180	DUR	DCAD	DUR × DCAD
Milk fever, %	1.8 (2/114)	3.5	3.5	0	0	—	—	—
Retained placenta, %	14.0 (16/114)	13.8	13.8	17.9	10.7	0.92	0.87	0.80
Metritis, %	15.9 (18/113)	20.7	10.3	14.3	18.5	0.75	0.92	0.14
Puerperal metritis, %	8.0 (9/113)	6.9	6.9	7.1	11.1	0.73	0.66	0.66
Mastitis, %	12.4 (14/113)	13.8	10.3	14.3	11.1	0.94	0.73	0.95
Displaced abomasum, %	6.2 (7/113)	3.5	6.9	3.6	11.1	0.64	0.15	0.40
Morbidity, %	32.7 (37/113)	34.5	34.5	25.0	37.0	0.54	0.23	0.35
Multiple diseases, %	15.1 (14/113)	17.2	10.3	21.4	11.1	0.66	0.35	0.89
Subclinical hypocalcemia ⁴								
Blood iCa ≤1.0 mM, %	36.3 (29/80)	52.4	30.0	26.3	35.0	0.37	0.59	0.17
Plasma tCa ≤2.0 mM, %	57.9 (66/114)	62.1	58.6	64.3	46.4	0.61	0.27	0.45
Plasma tCa <2.15 mM, %	85.1 (97/114)	86.2	79.3	92.9	82.1	0.43	0.19	0.63
Sold and dead, %								
42 d postpartum	7.1 (8/113)	2.7	5.5	8.4	6.1	0.45	0.82	0.54
305 d postpartum	21.2 (24/113)	24.1	13.8	25.0	22.2	0.51	0.37	0.57

¹Cows were fed the following diets according to treatment: -70_short = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with -70 mEq/kg of DM from 255 d of gestation to calving; -180_short = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with -180 mEq/kg of DM from 255 d of gestation to calving; -70_long = diet containing -70 mEq/kg of DM from 232 d of gestation to calving; -180_long = diet containing -180 mEq/kg of DM from 232 d of gestation to calving.

²DUR = effect of feeding duration (42 vs. 21 d); DCAD = effect of level of DCAD (-70 vs. -180); DUR × DCAD = effect of interaction between DUR and DCAD (-70_{short} plus -180_{long} vs. -180_{short} plus -70_{long}).

³Data collected until 42 d postpartum.

⁴Based on at least 1 blood sample with value below the indicated threshold with samples collected on d 0, 1, 2, 3, and 4 postpartum. i = ionized; t = total.

Figure 5. Daily risk of subclinical hypocalcemia based on concentration of ionized Ca (iCa) ≤ 1.0 mM in whole blood (A), plasma total Ca (tCa) ≤ 2.0 mM in plasma (B), or tCa < 2.15 mM in plasma (C) in cows fed prepartum acidogenic diets of short (21 d) or long (42 d) durations and with -70 or -180 mEq/kg of DM. (A) Mean daily risks averaged 12.8 , 9.1 , 10.0 , and $13.3 \pm 6.4\%$ for -70_{short} , -180_{short} , -70_{long} , and -180_{long} ; effects of duration ($P = 0.91$), DCAD ($P = 0.96$), day ($P < 0.001$), and interactions between duration and DCAD ($P = 0.59$), duration and day ($P = 0.61$), DCAD and day ($P = 0.11$), and duration and DCAD and day ($P = 0.97$). (B) Mean daily risks averaged 31.6 , 21.7 , 21.7 , and $17.1 \pm 5.5\%$ for -70_{short} , -180_{short} , -70_{long} , and -180_{long} ; effects of duration ($P = 0.21$), DCAD ($P = 0.21$), day ($P < 0.001$), and interactions between duration and DCAD ($P = 0.73$), duration and day ($P = 0.15$), DCAD and day ($P = 0.71$), and duration and DCAD and day ($P = 0.66$). (C) Mean daily risks averaged 50.2 , 49.1 , 54.2 , and $44.3 \pm 6.3\%$ for -70_{short} , -180_{short} , -70_{long} , and -180_{long} ; effects of duration ($P = 0.95$), DCAD ($P = 0.39$), day ($P < 0.001$), and interactions between duration and DCAD ($P = 0.50$), duration and day ($P = 0.95$), DCAD and day ($P = 0.88$), and duration and DCAD and day ($P = 0.32$). Error bars represent SEM.

gested to be the cause of less appetite, although in some cases experiments used high-Cl and -S products and not necessarily a particular salt (Vagnoni and Oetzel, 1998; Martinez et al., 2018). Vagnoni and Oetzel (1998) supplemented cows with different acidogenic products to reduce the DCAD of diets fed to dry cows. Those authors showed that acidogenic diets reduced DMI regardless of the source of strong anions fed; they suggested that the decrease in DMI may be explained by discomfort generated by the metabolic acidosis rather

than the palatability of the acidogenic products (Vagnoni and Oetzel, 1998). We recently showed that the reduction in appetite and changes in feeding behavior are induced by the changes in acid-base status and not by feeding acidogenic product per se (Zimpel et al., 2018). Cows fed an acidogenic product to reduce the DCAD had depressed DMI, but supplementing alkalogenic salts to avoid the decrease in DCAD induced by the acidogenic diet prevented the decline in DMI (Zimpel et al., 2018). In consequence to the decrease

in DMI, cows fed the -180 mEq/kg of DM DCAD had less caloric intake and, consequently, a smaller NE balance that led to a greater BCS loss prepartum.

Reducing the DCAD of prepartum diets from 183 to -74 mEq/kg of DM improved yields of milk and ECM in the first 3 wk of lactation in dairy cows (Leno et al., 2017). Nevertheless, increasing the duration of feeding acidogenic diets prepartum in the present experiment from 21 to 42 d reduced milk yield in the first 42 d postpartum by 2.5 kg/d. Although yields of 3.5% FCM and ECM did not differ with treatments. In observational studies, production of fat and protein-corrected milk was optimized when cows were fed transition diets for approximately 25 d (Degaris et al., 2008). In controlled experiments, feeding acidogenic diets up to 42 d prepartum did not influence early lactation milk yield compared with feeding for 21 d (Weich et al., 2013; Wu et al., 2014). Although cows in Weich et al. (2013) and Wu et al. (2014) received diets with DCAD of -150 and -200 mEq/kg of DM, respectively, the responses in acid-base balance were more moderate than those observed in the current experiment in cows fed the -180 mEq/kg of DM diet. Also, cows fed long had gestation length decreased by 2 d compared with those fed short, and it is possible that a reduction in gestation length might have affected mammogenesis and subsequent milk yield (Capuco et al., 1997; Vieira-Neto et al., 2017a). The last weeks of gestation determine most mammary development, and number of milk secreting cells depends on length of dry period. Capuco et al.

(1997) characterized the cytological changes of bovine mammary gland during the weeks after milk cessation and documented that nearly all alveolar cells display characteristics of cells competent for milk synthesis and milk production during the last week prepartum. The mechanism that underlies the decrease in milk yield from a longer exposure to metabolic acidosis remains unclear, but it is possible that the 2 fewer days of gestation in cows fed long might have affected differentiation of the mammary epithelial cells into secretory cells and, in consequence, milk yield.

Another possibility is that longer exposure to metabolic acidosis might have influenced either prolactin signaling (Yang et al., 2013) or the growth hormone/IGF-1 endocrine axis (Challa et al., 1993b) that is important to mammary development (Neville and Waters, 1983; Akers et al., 1990). In mouse cell culture systems transfected with human prolactin and growth hormone receptors, acidosis at pH 6.8 disrupted prolactin receptor signaling (Yang et al., 2013). Those authors suggested that proton-induced disruption of prolactin signaling occurred by impeding ligand-receptor binding in tissues (Yang et al., 2013). Metabolic acidosis has been found to inhibit growth hormone secretion in rats (Challa et al., 1993b), and impair growth hormone/IGF-1 endocrine axis function in humans (Challa et al., 1993a; Brüngger et al., 1997). Therefore, it is plausible to speculate that the prolonged metabolic acidosis for 42 d might have influenced mammogenesis either by influencing prolactin signaling in the mammary tissue

Table 7. Effects of level of DCAD and duration (DUR) of prepartum feeding on reproduction in Holstein cows¹

Item ²	Short		Long		P-value ³		
	-70	-180	-70	-180	DUR	DCAD	DUR × DCAD
Inseminated, % (no.)	89.7 (29)	93.1 (29)	89.3 (28)	85.2 (27)	0.55	0.89	0.51
Days to AI, LSM ± SEM	81.6 ± 3.4	80.0 ± 3.5	80.2 ± 3.4	84.2 ± 3.8	0.64	0.68	0.33
Pregnant, ⁴ % (no.)							
First AI	41.7 (24)	33.3 (27)	28.0 (25)	34.8 (23)	0.53	0.96	0.44
All AI	35.6 (59)	34.3 (67)	21.3 (75)	23.9 (71)	0.03	0.86	0.71
Pregnant d 305, ⁵ % (no.)	72.4 (29)	79.3 (29)	57.1 (28)	63.0 (27)	0.08	0.46	0.87
Days to pregnancy							
LSM ± SEM	150 ± 14	146 ± 14	174 ± 16	151 ± 13	—	—	—
Median (95% CI)	156 (80 – 176)	139 (80 – 148)	153 (116 – 263)	153 (80 – 201)	—	—	—

¹Cows were fed the following diets according to treatment: -70_short = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with -70 mEq/kg of DM from 255 d of gestation to calving; -180_short = diet containing +110 mEq/kg of DM from 232 to 254 d of gestation, followed by a diet with -180 mEq/kg of DM from 255 d of gestation to calving; -70_long = diet containing -70 mEq/kg of DM from 232 d of gestation to calving; -180_long = diet containing -180 mEq/kg of DM from 232 d of gestation to calving.

²Data collected for 305 d postpartum.

³DUR = effect of feeding duration (42 vs. 21 d); DCAD = effect of level of DCAD (-70 vs. -180); DUR × DCAD = effect of interaction between DUR and DCAD (-70 _short plus -180 _long vs. -180 _short plus -70 _long).

⁴Pregnancy based on the diagnosis on d 67 after each AI according to the number of cows receiving at least 1 AI with pregnancy diagnosis performed.

⁵Pregnancy based on the diagnosis on d 67 according to the number of cows enrolled in the experiment.

Figure 6. Survival curves for days from calving to pregnancy according to duration of prepartum feeding (short vs. long; A) and level of DCAD (-70 vs. -180 mEq/kg of DM; B). (A) Median (95% CI) and mean $\pm$ SEM days to pregnancy were, respectively, 144 (113 to 156) and 149 ± 10 d, resulting in 75.9% pregnant in cows fed short and 153 (114 to 201) and 168 ± 11 d resulting in 60.0% pregnant in cows fed long. The adjusted hazard ratio (AHR) was 1.55 (95% CI = 0.98 to 2.45; $P = 0.06$). (B) Median (95% CI) and mean $\pm$ SEM days to pregnancy were, respectively, 156 (116 to 165) and 162 ± 11 d resulting in 64.9% pregnant in cows fed the diets with -70 mEq/kg of DM and 144 (113 to 179) and 156 ± 11 d resulting in 71.4% pregnant in cows fed the diets with -180 mEq/kg of DM. The AHR was 0.89 (95% CI = 0.57 to 1.38; $P = 0.59$). Interaction between duration of feeding and level of DCAD did not affect time to pregnancy ($P = 0.94$).

or by reducing growth hormone/IGF-1 either directly or by the decreased DMI and the less-favorable nutrient balance.

Few experiments have evaluated the effect of level of DCAD on yield and composition of colostrum. Recently, Martinez et al. (2018) fed prepartum cows diets

with approximately +130 or -130 mEq/kg of DM and demonstrated that reducing the DCAD of prepartum diets did not influence colostrum yield or composition. Weich et al. (2013) showed that colostrum yield was unaffected by feeding acidogenic diets containing -150 mEq/kg of DM either for the last 21 or 42 d of gestation. In the present experiment, reducing the DCAD from -70 to -180 mEq/kg of DM reduced first milking colostrum yield by 1.4 kg/d, suggesting perhaps that a more severe metabolic acidosis with subsequent reduction in prepartum DMI might have effects on colostrum synthesis. Cows fed the -180 mEq/kg of DM diet had measures of acid-base balance suggesting more severe metabolic acidosis than those of Weich et al. (2013) or those of Martinez et al. (2018), as reported by Rodney et al. (2018). Prepartum blood pH, base excess, and bicarbonate were all less in cows fed -180 mEq/kg of DM in the present experiment than those fed acidogenic diets reported by Rodney et al. (2018), and urinary pH in cows fed -180 mEq/kg of DM was less than those reported by Rodney et al. (2018) or Weich et al. (2013). Therefore, it is possible that the more acidogenic diet fed in the current experiment induced a more pronounced metabolic acidosis that reduced nutrient intake which might have been detrimental to colostrum synthesis.

Although yield of first milking colostrum was depressed in cows fed -180 compared with -70 mEq/kg of DM, the more acidogenic diet did not affect yields of milk or milk components in the first 42 d of lactation. Our results suggest that the negative effects of a more pronounced metabolic acidosis and subsequent reduced nutrient intake during late gestation are only present in the immediate postpartum, which reduces colostrum yield, but does not compromise subsequent lactation performance.

Acidogenic diets induce a typical compensated metabolic acidosis with a moderate decline in blood pH, reduced blood bicarbonate and base excess, increased net acid urinary excretion, and reduced blood $p\text{CO}_2$ (Vagnoni and Oetzel, 1998; Charbonneau et al., 2006). The decrease in blood pH from feeding diets with negative DCAD occurs because the increased absorption of strong anions, such as Cl^- , leads to a loss of bicarbonate and an increase in free H^+ , which consequently reduces base excess and blood pH (Riond, 2001; Constable, 2014). This decrease in blood pH triggers a series of compensatory mechanisms, one of which is controlled by the respiration with increases in exhaled CO_2 , thereby reducing $t\text{CO}_2$ concentrations in blood. Renal compensation also occurs with excess of acid excreted in the urine resulting in reduced urinary pH, all of which were observed when cows were fed the diets with negative DCAD. Because cows fed the -180 mEq/kg of

DM treatment received a more acidogenic diet, it was anticipated that their responses in acid-base balance would be more pronounced than those receiving the -70 mEq/kg of DM diet.

Concentrations of iCa in blood prepartum and on the day of calving were greater in cows fed -180 than -70 mEq/kg of DM, but these differences did not influence the risk of hypocalcemia. Two of 58 cows (3.5%) fed short developed milk fever, and this incidence was less than values reported in the literature in herds with variable prepartum nutritional management (Lean et al., 2006; USDA, 2007; Reinhardt et al., 2011). It is well established that, as the prepartum DCAD decreases, blood iCa increases and risk of milk fever decreases (Charbonneau et al., 2006; Lean et al., 2006). Because all cows in the experiment were fed acidogenic diets prepartum, it is not surprising that incidence of milk fever was low, and risk of hypocalcemia did not differ among treatments. In fact, treatment did not affect the incidence of any of the individual diseases evaluated, morbidity, or multiple diseases. Hypocalcemia is known as a gateway disease and predisposes cows to other peripartum problems (Curtis et al., 1985; Martinez et al., 2012; Ribeiro et al., 2013). Lack of differences in hypocalcemia likely explains the absence of effects on morbidity.

Pregnancy per AI after all AI was greater for cows fed short than those fed long. The reduced P/AI resulted in a tendency for reduced pregnancy rate and extended days open. One possible explanation for the decreased reproductive performance of the cows fed long compared with those fed short is the longer exposure to the metabolic acidosis that reduced DMI during the dry period. The reduced nutrient intake caused a more negative NE balance prepartum. These cows also lost numerically more BW postpartum concurrent with less milk yield, perhaps suggesting that DMI was lower in early lactation in cows fed long than short. Because postpartum DMI was not measured, we can only speculate what might have happened with nutrient intake on that period. It is well characterized that reduced nutrient intake and more negative NE balance disrupts reproduction by delaying first postpartum ovulation and compromising P/AI (Staples et al., 1990). Wathes et al. (2007) proposed that the decreased reproductive performance in cows in more negative nutrient balance is in part linked to reduced systemic concentrations of IGF-1, which affects ovarian activity and early embryo development. Perhaps, the reduced nutrient intake observed during the dry period, when cows in long were fed acidogenic diets, might have also reflected in less intake postpartum, which could have affected subsequent reproductive performance. Another possibility is that cows fed acidogenic diets for 42 d had shorter gestation

length, which has been associated with impaired reproductive performance (Norman et al., 2011; Vieira-Neto et al., 2017a). Even though the negative association between shorter duration of gestation and reduced reproductive performance has been shown, the underlying mechanism remains unclear. Finally, it is possible that prolonged acidosis for 42 d prepartum might have influenced tissue metabolism that has ramifications to reproduction in the subsequent lactation.

CONCLUSIONS

Feeding diets with negative DCAD induced a compensated metabolic acidosis in dairy cows regardless of the duration of feeding, but the acidosis was more severe in those fed the -180 than -70 mEq/kg of DM. Cows fed the more acidogenic diet had increased iCa concentrations prepartum and at calving, but treatments did not influence morbidity or the incidence or daily prevalence of subclinical hypocalcemia. Reducing the DCAD decreased DMI, resulting in a more negative energy balance in the last weeks of gestation. Cows fed the more acidogenic diet yielded less colostrum, perhaps as consequence of less nutrient intake during the last weeks of gestation. Nevertheless, feeding the diet with -180 mEq/kg of DM did not influence yields of milk, milk components, health, or reproduction in dairy cows compared with cows fed a diet with -70 mEq/kg of DM, thereby suggesting that this range of DCAD is likely adequate for diets fed prepartum. Extending the duration of feeding diets with negative DCAD had minor effects on blood iCa and measures of acid-base status pre- and postpartum, but reduced milk yield in the first 42 d postpartum and P/AI in all AI in the first 305 d of lactation. It is possible that the reduction in milk yield and reproductive performance caused by feeding acidogenic diets for 42 d prepartum might have a common underlying cause, although the current experiment does not allow us to identify it. Collectively, the present data suggest that feeding acidogenic diets beyond 21 d might be detrimental to dairy cows and there is no need to reduce the DCAD from -70 to -180 mEq/kg of DM. Further experiments are needed to investigate the underlying mechanisms by which feeding acidogenic diets for 42 d influences production and reproduction in dairy cows.

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