Metabolic and blood acid-base responses to prepartum dietary cation-anion difference and calcium content in transition dairy cows

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ABSTRACT

Dairy cows commonly undergo negative Ca balance accompanied by hypocalcemia after parturition. A negative dietary cation-anion difference (DCAD) strategy has been used prepartum to improve periparturient Ca homeostasis. Our objective was to determine the influence of a negative DCAD diet with different amounts of dietary Ca on the blood acid-base balance, blood gases, and metabolic adaptation to lactation. Multiparous Holstein cows ($n = 81$) were blocked into 1 of 3 dietary treatments from 252 d of gestation until parturition: (1) positive DCAD diet and low Ca (CON; containing +6.0 mEq/100 g DM, 0.4% DM Ca); (2) negative DCAD diet and low Ca (ND; −24.0 mEq/100 g DM, 0.4% DM Ca); or (3) negative DCAD diet plus high Ca supplementation (NDCA; −24.1 mEq/100 g DM, 2.0% DM Ca). There were 28, 27, and 26 cows for CON, ND, and NDCA, respectively. Whole blood was sampled at 0, 24, 48, and 96 h after calving for immediate determination of blood acid-base status and blood gases. Serum samples collected at −21, −14, −7, −4, −2, −1, at calving, 1, 2, 4, 7, 14, 21, and 28 d relative to parturition were analyzed for metabolic components. Results indicated that cows fed ND or NDCA had lower blood pH at calving but greater pH at 24 h after calving compared with CON. Blood bicarbonate, base excess, and total CO₂ (tCO₂) concentrations of cows in ND and NDCA groups were less than those of cows in CON at calving but became greater from 24 to 96 h postpartum. The NDCA cows had lower blood bicarbonate, base excess, and tCO₂ at 48 h and greater partial pressure of oxygen after calving compared with ND. Cows fed ND or NDCA diets had lower serum glucose concentrations than CON cows before

calving but no differences were observed postpartum. Serum concentrations of total protein and albumin were greater prepartum for cows in ND and NDCA groups than for those in CON. Postpartum serum urea N and albumin concentrations tended to be higher for ND and NDCA cows. Cows fed ND or NDCA diets had elevated serum total cholesterol concentration prepartum. During the postpartum period, triglycerides and NEFA of cows fed ND or NDCA diets tended to be lower than those of CON. Cows fed the NDCA diet had greater postpartum total cholesterol in serum and lower NEFA concentration at calving than ND. In conclusion, feeding a prepartum negative DCAD diet altered blood acid-base balance and induced metabolic acidosis at calving, and improved protein and lipid metabolism. Supplementation of high Ca in the negative DCAD diet prepartum was more favorable to metabolic adaptation to lactation in dairy cows than the negative DCAD diet with low Ca.

Key words: transition cow, dietary cation-anion difference, calcium, acid-base balance

INTRODUCTION

The transition period, a critical physiological stage for dairy cows, not only invokes acute alterations of energy and protein metabolism but also mineral metabolism, being linked closely with health, immune status, and productive performance after parturition (Drackley et al., 2005). Owing to dramatically increased demand of Ca for initiation of lactation, postcalving dairy cows often have hypocalcemia. The failure of adaptation to the substantial Ca drop may lead to milk fever, which has severe clinical symptoms and is life threatening (Horst et al., 1997). More common is subclinical hypocalcemia (SCH, total Ca ≤ 2.125 mmol/L; Leno et al., 2017), which still affects around 50% of cows in second lactation or greater (Goff, 2014). Research has demonstrated that cows with SCH have decreased feed intake, leading to worsening of negative energy balance (NEB) and

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an increased occurrence of metabolic disorders (Chamberlin et al., 2013; Martinez et al., 2018; Trevisi and Minuti, 2018). Hence, maintenance of Ca homeostasis around parturition has attracted widespread interest in the dairy industry due to its considerable negative effect on transition cows.

High Ca ingestion prepartum was assumed to be a potential predisposing factor for hypocalcemia (Goings et al., 1974). A low dietary Ca strategy to prevent hypocalcemia has been used but is problematic to apply because of the difficulty in restricting absorbable Ca to <20 g/d. Researchers later proved that metabolic alkalosis is the primary cause of inhibition of parathyroid hormone (PTH) action in restoring Ca homeostasis (Constable et al., 1997; Goff, 2008). Feeding an acidogenic diet before calving can prevent hypocalcemia through enhancement of postpartum blood Ca (Sakha et al., 2014). Reducing dietary DCAD was applied to induce a compensated metabolic acidosis before calving, which increases Ca flux by stimulating Ca resorption from bone and Ca excretion by the kidney, thereby enhancing PTH function on intestinal Ca absorption and bone resorption after calving (Goff, 2008).

Because of the greater bone Ca mobilization and urinary Ca excretion induced by feeding a negative DCAD diet (Vagnoni and Oetzel, 1998), our hypothesis was that feeding high dietary Ca in conjunction with an acidogenic diet might replenish the mobilized and excreted Ca faster, resulting in a lower occurrence rate of SCH following calving. Although an acidogenic dietary strategy is applied, a sufficient Ca flux in the digestive tract can be available for absorption under regulation of stimulated PTH function. The model created by Lean et al. (2006) via meta-analysis recommended that prepartum dietary Ca be above 2.0% DM to reduce risk of milk fever. Studies conducted to investigate the interaction of prepartum dietary Ca along with an acidogenic diet on Ca homeostasis remain scarce. Results of Oba et al. (2011) indicated that high Ca in a low DCAD diet could shorten recovery time from loss of blood Ca induced by EDTA challenge. Additionally, Amundson et al. (2018) demonstrated that feeding a low DCAD diet with high dietary Ca increased the amount of EGTA required to place cows in a state of SCH, suggesting an increased amount of rapidly available Ca in cows fed a low DCAD, high Ca diet. Recently, we (Glosson et al., 2020) reported little effect of high Ca supplementation in a negative DCAD diet on blood Ca concentration or production of Holstein cows, but their reproductive performance including uterine health and fertility was improved by the higher dietary Ca (Ryan et al., 2020). How the blood acid-base balance system and metabolism around parturition respond to a negative DCAD diet with or without supplemental dietary

Ca is still unclear. To achieve better understanding of the metabolic response of transition cows to prepartum acidogenic diets accompanied by different Ca additions, the objectives of our study were to determine the influences of prepartum dietary negative DCAD and Ca concentration on blood acid-base status and metabolic indicators in blood of transition cows.

MATERIALS AND METHODS

Experimental Design, Cows, and Diets

Part of the research design and methods, as well as production and reproduction responses have been reported by Glosson et al. (2020) and Ryan et al. (2020). The Institutional Animal Care and Use Committee of the University of Illinois approved all procedures (protocol 16115). Multiparous Holstein cows (second or third gestation, $n = 81$) diagnosed with a single calf were the experimental unit. Cows were enrolled at -50 d before expected parturition and moved into sand-bedded freestalls in pens with an electronic gate individual feeder system (American Calan). Parturient cows were moved into a box stall bedded with wheat straw within the same barn. Calves were removed from cows immediately after birth and fed colostrum following standard protocols. After parturition, fresh cows were housed in individual tie stalls bedded with sand over rubber mattresses. Cows were milked 3 times daily.

The experiment was conducted as a randomized complete block design. Enrolled cows were blocked and balanced according to parity, milk yield of previous lactation, initial BCS at dry off, and days to expected calving. Within each block, cows were assigned randomly to 1 of 3 treatments from -28 d before expected calving to parturition. Treatments were (1) control diet of positive DCAD and low dietary Ca (**CON**; +6 mEq/100 g DM DCAD, 0.4% DM Ca); (2) negative DCAD diet and low Ca (**ND**; -24 mEq/100 g DM DCAD, 0.4% DM Ca); and (3) negative DCAD and high Ca diet (**NDCA**; -24 mEq/100 g DM DCAD, 2.0% DM Ca). There were 28, 27, and 26 cows for CON, ND, and NDCA, respectively. The number of cows per treatment was based on power analysis for DMI and milk yield (Glosson et al., 2020). The amount of anionic salt (Animate, Phibro Animal Health Corporation) fed was adjusted via determination of urine pH by portable pH meter to remain in the range of 5.5 to 6.0. After calving, cows from all treatments received the same lactation diet through 30 DIM. Diets prepartum and postpartum (Table 1) fed as TMR were formulated using a software program (AMTS.Cattle.Pro version 4.16.3.2; AMTS LLC). The main ingredients and water to maintain the final DM content of TMR at about

45% were mixed daily as a basal diet using a TMR mixer (Keenan Systems). Different treatment premixes were added into the basal diet and mixed separately (Data Ranger, American Calan). The diets were prepared and offered to cows at 0600 h once daily and were pushed up frequently to achieve ad libitum intake. The daily feed amount offered to each cow was determined by feed refusal of previous day to target <10% refusal. The DM content of feed ingredients was measured once weekly and adjusted accordingly to maintain the DM ratio of ingredients in the TMR. Diet ingredients and TMR were sampled weekly, stored at -20°C , and composited monthly to analyze nutrients at a commercial laboratory (Dairy One, Ithaca, NY).

Sampling and Analyses

Blood samples (10 mL) were collected from the coccygeal vein or artery into heparinized vacuum tubes (BD Vacutainer Systems) at calving, 24, 48, and 96 h (± 1 h) postpartum. After sampling, blood pH, partial pressure of CO_2 (pCO_2), partial pressure of O_2 (pO_2), base excess, bicarbonate (HCO_3^-), total CO_2 (tCO_2), Na, K, hematocrit and hemoglobin concentrations were determined within 10 min using a test cartridge (CG8+, Abbott Point of Care Inc.) in a handheld analyzer (VetScan iSTAT, Abaxis). A minimal chance for aerobic contamination was present when transferring the sample to the test cartridge, but all samples were handled the same. Therefore, the discussion of results will focus on the differences between treatments and not the absolute value of the blood gas measurements.

Another series of blood samples was taken from the coccygeal vein before the morning feeding every Monday, Wednesday, and Friday and daily from -7 d until calving day to ensure samples were available from the actual -30 , -21 , -14 , -7 , -4 , -2 , and -1 d relative to calving. Postpartum samples were collected at 0, 1, 2, 4, 7, 14, 21, and 28 d after parturition. Blood was sampled into vacuum serum separator tubes (BD Vacutainer Systems) and centrifuged at $1,900 \times g$ for 15 min at 4°C to obtain serum. Aliquots of serum were stored at -20°C until analysis. Commercial reagents (system quantitative determination reagents, Beckman Coulter, Inc.) were used in an auto-analyzer (Beckman Coulter AU680 Analyzer, Beckman Coulter, Inc.) at the Clinical Pathology Laboratory in the University of Illinois College of Veterinary Medicine to measure serum concentrations of total protein, urea N, albumin, glucose, total cholesterol, triglyceride, Na, K, Cl and HCO_3^- . Globulin content in serum was calculated as total protein minus albumin. The Na, K, and Cl were used to calculate anion gap according to the following

Table 1. Ingredients composition (% of DM) of the close-up and lactation (LACT) diets fed as TMR

Ingredient	Treatment ¹			
	CON	ND	NDCA	LACT
Corn silage	32.10	32.10	32.10	36.61
Wheat straw	36.31	36.31	36.31	2.34
Alfalfa hay	—	—	—	11.26
Corn gluten feed	8.25	8.25	8.25	8.34
Corn grain	—	—	—	18.81
Soybean hulls	6.60	6.60	6.60	4.32
Soybean meal	5.78	5.78	5.78	—
ProVAAl AAAdvantage ²	2.48	2.48	2.48	1.87
Canola meal	—	—	—	11.79
Rumen-protected lysine	—	—	—	0.63
Rumen-protected methionine	—	—	—	0.15
Animate ³	—	4.13	4.13	—
Calcium carbonate	—	—	2.87	—
Wheat middlings	6.20	3.38	0.83	—
Clay	1.24	0.41	—	—
Sodium chloride	0.08	—	—	—
Sodium monophosphate	—	0.08	0.16	—
Magnesium sulfate·7H ₂ O	0.58	—	—	—
Magnesium oxide	0.33	—	—	—
UI prepartum mineral premix ⁴	0.17	0.02	0.02	—
UI lactation mineral mix ⁵	—	—	—	3.70
Potassium carbonate	—	—	—	0.13
Monensin	0.02	0.02	0.02	0.01
Vitamin A ⁶	0.01	0.01	0.01	0.03
Vitamin D ⁶	0.01	0.01	0.01	0.01
Vitamin E ⁶	0.29	0.29	0.29	0.05

¹The control diet (CON) was formulated to offer a positive DCAD that averaged $+6$ mEq/100 g and dietary Ca at 0.40% DM. The negative DCAD (ND) and negative DCAD plus Ca supplementation (NDCA) diets were formulated to offer a negative DCAD that averaged -24 mEq/100 g with dietary Ca at either 0.40% DM or 2.0% DM.

²Perdue Agribusiness Animal Nutrition.

³Composed of 38.0% CP, 3.2% ether extract, 3.1% sugar, 5.8% starch, 15.9% NDF, 1.4% Ca, 0.4% P, 4.8% Mg, 5.4% S, and 13.9% Cl (Phibro Animal Health Corp.).

⁴The University of Illinois mineral and vitamin mix provided 5% Mg, 10% S, 7.5% K, 2.0% Fe, 3.0% Mn, 5,000 mg/kg Cu, 250 mg/kg I, 40 mg/kg Co, 150 mg/kg Se, 2,200 kIU/kg vitamin A, 660 kIU/kg vitamin D₃, and 7,700 IU/kg vitamin E.

⁵The University of Illinois mineral and vitamin mix provided 12.5% Ca, 14.1% Na, 9.6% Cl, 3.2% Mg, 6.5% K, 0.19% S, 26.9 mg/kg Co, 301 mg/kg Cu, 40.2 mg/kg I, 678 mg/kg Fe, 1,519 mg/kg Mn, 8.62 mg/kg Se, 4.47 mg/kg organic Se, 1,621 mg/kg Zn, 43.3 kIU/kg vitamin A, 10.9 kIU/kg vitamin D₃, 466.4 IU/kg vitamin E, 4.23 mg/kg biotin, 46.6 mg/kg thiamine, and 0.35 g/kg monensin.

⁶Vitamins A, D, and E contained 30,000 kIU/kg, 5,009 kIU/kg, and 44,000 IU/kg, respectively.

formula (Pardon et al., 2018): $\text{Anion gap} = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)$. A commercial kit (Wako Chemicals USA Inc.) was used to determine nonesterified fatty acids (NEFA) following the manufacturer's instructions via 96-well plate and microplate spectrophotometer (BioTek Instruments, Inc.). Serum samples from d 0 to 28 DIM were measured for BHB concentration (Cat # RB 1007, Randox Laboratories Ltd.) with the same instrument.

Statistical Analysis

The postpartum period was divided into 3 subperiods: calving, immediate postcalving (defined as 1, 2, and 4 d after calving), and postpartum (defined as 7–28 DIM). Data for different periods were analyzed separately. Statistical analyses were performed using the MIXED procedure of SAS (SAS Institute Inc.). The model included the fixed effects of treatment, time relative to calving as a repeated measurement (with cow as subject), and the interaction of treatment and time, with the random effect of block. Several covariance structures were tested for the repeated measurements, and the one with the lowest Akaike information criterion was selected. Previous milk yield, parity, and initial BCS were used as covariates in the full model but were removed if nonsignificant ($P > 0.15$). For prepartum data, a covariate of pretreatment measurements (–30 d) of serum components was tested in the analysis. All data from 5 cows were excluded after treatment with Ca therapy due to clinical milk fever (2 in CON and 3 in ND). A total of 4 cows died or were euthanized for toxic mastitis, acute ketosis, and a broken hip during the fresh period (2 in CON and 2 in ND). Data were transformed for statistical analysis as necessary to achieve a normal distribution of residuals. Two preplanned orthogonal contrast statements were evaluated for the following treatment effects: (1) CON versus the average of ND and NDCA for the comparison between positive and negative DCAD diets; and (2) ND versus NDCA for the effect of different amounts of Ca in the negative DCAD diet. Results are presented as least squares means. Statistical significance was declared at $P \leq 0.05$, and tendencies were considered when $0.05 < P \leq 0.10$.

RESULTS

The DMI prepartum, published previously (Glosson et al., 2020), was 12.0, 10.0, and 11.0 kg/d for CON, ND, and NDCA, respectively, whereas postpartum DMI was 17.6, 18.4, and 18.8 kg/d for the same treatments. Nutrient composition of the diets is shown in Table 2. The pH of blood in postpartum dairy cows is presented in Table 3 and Figure 1. Blood pH of all treatments was lower at calving, increased after 24 h postpartum and then reached its peak thereafter. Blood pH was lower ($P < 0.05$) for cows fed ND or NDCA at calving than those fed CON. We observed an interaction between treatments and DIM ($P < 0.01$), in which blood pH of cows fed ND or NDCA was higher than CON ($P < 0.05$) at 24 h postpartum but similar thereafter (Figure 1). The blood pH of ND and NDCA treatments did not differ ($P > 0.10$). Cows in ND and NDCA groups

had lower blood bicarbonate and base excess values at calving than cows fed CON ($P < 0.05$). From 24 to 96 h after calving, bicarbonate and base excess were significantly greater for ND and NDCA ($P < 0.05$) compared with CON, with a significant difference between ND and NDCA groups at 48 h ($P < 0.05$, Figure 2). For blood gases, $t\text{CO}_2$ concentration showed a similar pattern as bicarbonate. Blood $t\text{CO}_2$ was significantly lower in cows fed ND or NDCA diet ($P < 0.05$) than CON at calving and the difference reversed at 24 h. The $p\text{CO}_2$ at 48 h after calving differed among treatments ($P < 0.05$) with cows fed CON showing the lowest $p\text{CO}_2$ and cows in ND group were greater than NDCA. Blood $p\text{O}_2$ of cows that received the CON diet did not differ from those fed ND or NDCA diets ($P = 0.62$) at 96 h after calving, and $p\text{O}_2$ was greater for cows fed NDCA when compared with ND ($P = 0.02$). There was no significant difference for Na and K concentrations among treatments ($P > 0.10$). When all time points were included, we found a significant DIM effect and treatment $\times$ DIM interaction ($P < 0.01$) for hematocrit and hemoglobin concentrations, although no significant difference was observed for the 2 preplanned contrasts over all times.

Serum components for cows fed close-up dietary treatments are shown in Table 4. Serum anion gap throughout the study did not differ among treatments ($P > 0.10$). During the prepartum period, serum bicarbonate concentrations were lower for cows that consumed ND or NDCA ($P < 0.01$) than those fed CON, with a significant difference between ND and NDCA groups ($P < 0.01$). Cows fed ND and NDCA diets had reduced bicarbonate at calving compared with those fed CON ($P < 0.01$). Conversely, in immediate postcalving period (24, 48, and 96 h postpartum), bicarbonate concentration became significantly higher in ND and NDCA groups ($P < 0.01$).

Prepartum total protein and albumin concentrations of cows fed ND or NDCA diets were significantly greater than those fed CON ($P = 0.047$ and $P = 0.01$). Postpartum urea N concentration tended to be greater with the negative DCAD treatments than CON ($P = 0.10$), with a tendency toward higher albumin in the immediate postcalving period (24–96 h postpartum) period in comparison with CON ($P = 0.10$). For serum glucose, feeding ND or NDCA diets resulted in lower glucose concentration than CON prepartum, although no effect of treatments on glucose was observed at calving and postpartum.

During the prepartum period, cows given ND or NDCA diets had significantly greater total cholesterol concentration than those given CON (Table 5). Immediate postcalving total cholesterol concentrations were greater in NDCA than in ND ($P = 0.03$), and tended to

Table 2. Nutrient content of the experimental diets¹ ($\pm$ SD; DM basis)

Nutrient ²	Treatment			
	CON	ND	NDCA	LACT
DM, % as fed	45.9 $\pm$ 2.5	46.8 $\pm$ 2.6	46.3 $\pm$ 2.7	48.3 $\pm$ 1.7
CP, %	15.1 $\pm$ 1.3	16.6 $\pm$ 1.5	16.4 $\pm$ 1.0	17.8 $\pm$ 0.6
ADF, %	33.6 $\pm$ 2.2	33.9 $\pm$ 3.6	33.3 $\pm$ 1.9	18.9 $\pm$ 1.9
aNDFom, %	49.3 $\pm$ 2.6	47.7 $\pm$ 4.1	46.9 $\pm$ 4.8	30.4 $\pm$ 1.4
Starch, %	14.1 $\pm$ 2.2	12.7 $\pm$ 2.2	12.8 $\pm$ 1.2	30.8 $\pm$ 1.5
Ash, %	9.39 $\pm$ 1.0	8.59 $\pm$ 0.3	11.8 $\pm$ 1.6	8.72 $\pm$ 0.8
TDN, %	60.4 $\pm$ 1.8	61.3 $\pm$ 2.7	59.1 $\pm$ 1.8	68.7 $\pm$ 0.95
NE _L , Mcal/kg	1.36 $\pm$ 0.04	1.41 $\pm$ 0.06	1.36 $\pm$ 0.04	1.58 $\pm$ 0.02
Ca, %	0.4 $\pm$ 0.06	0.44 $\pm$ 0.05	1.97 $\pm$ 0.6	1.15 $\pm$ 0.32
P, %	0.42 $\pm$ 0.03	0.43 $\pm$ 0.04	0.44 $\pm$ 0.05	0.49 $\pm$ 0.04
Mg, %	0.43 $\pm$ 0.08	0.44 $\pm$ 0.03	0.43 $\pm$ 0.05	0.33 $\pm$ 0.02
K, %	1.19 $\pm$ 0.06	1.16 $\pm$ 0.07	1.15 $\pm$ 0.05	1.48 $\pm$ 0.08
Na, %	0.10 $\pm$ 0.03	0.08 $\pm$ 0.02	0.11 $\pm$ 0.03	0.55 $\pm$ 0.03
S, %	0.33 $\pm$ 0.15	0.50 $\pm$ 0.06	0.52 $\pm$ 0.04	0.31 $\pm$ 0.02
Cl, %	0.29 $\pm$ 0.06	0.91 $\pm$ 0.10	0.91 $\pm$ 0.09	0.61 $\pm$ 0.05
Fe, mg/kg	419 $\pm$ 38	457 $\pm$ 79	529 $\pm$ 182	370 $\pm$ 62
Cu, mg/kg	18.1 $\pm$ 4.5	17.8 $\pm$ 1.1	19.2 $\pm$ 1.7	16.4 $\pm$ 0.8
Mn, mg/kg	103 $\pm$ 24	98 $\pm$ 9.7	99 $\pm$ 16	87 $\pm$ 7
Zn, mg/kg	94 $\pm$ 17	92 $\pm$ 6.6	96 $\pm$ 12	88 $\pm$ 8
DCAD, mEq/100 g of DM	6.0 $\pm$ 9.5	-24.0 $\pm$ 6.0	-24.1 $\pm$ 5.3	25.4 $\pm$ 2.3

¹The control diet (CON) was formulated to offer a positive DCAD that averaged +6 mEq/100 g and dietary Ca at 0.40% DM. The negative DCAD (ND) and negative DCAD plus Ca supplementation (NDCA) diets were formulated to offer a negative DCAD that averaged -24 mEq/100 g with dietary Ca at either 0.40% or 2.0% DM. LACT = lactation diet.

²Experimental diet samples were collected weekly and composited monthly for the nutrient analysis throughout the trial. aNDFom = amylase-treated NDF, OM basis.

be greater in NDCA compared with ND in the postpartum subperiod ($P = 0.09$). A tendency was observed for decreased postpartum serum triglycerides concentration for cows fed ND or NDCA compared with CON for both immediate postcalving and postpartum periods ($P = 0.07$ and $P = 0.09$). For the postpartum period,

triglycerides of NDCA tended to be greater than that of ND ($P = 0.06$).

We observed a significant increase of NEFA concentrations prepartum for cows fed ND or NDCA diet ($P = 0.01$) compared with CON, whereas NDCA diet resulted in greater prepartum NEFA than ND ($P =$

Table 3. Least squares means for blood acid-base balance and gas indices from 0 to 4 d after calving for cows fed close-up dietary treatments CON, ND, or NDCA starting at -28 d relative to expected calving

Variable in blood ¹	Treatment ²			SEM	DIM	Trt ³ $\times$ DIM	Contrast ⁴	
	CON	ND	NDCA				C1	C2
pH	7.417	7.411	7.420	0.005	<0.01	<0.01	0.77	0.21
pCO ₂ , mm Hg	45.76	47.34	45.93	0.82	0.61	0.02	0.23	0.099
pO ₂ , mm Hg	46.6	43.1	49.2	4.6	0.27	0.17	0.62	0.02
Base excess, mmol/L	4.94	5.81	5.25	0.45	<0.01	<0.01	0.27	0.38
HCO ₃ ⁻ , mmol/L	29.44	30.38	29.77	0.42	<0.01	<0.01	0.19	0.30
tCO ₂ , mmol/L	30.68	31.73	31.04	0.44	<0.01	<0.01	0.17	0.25
Na, mmol/L	142.79	142.55	142.88	0.22	<0.01	0.14	0.77	0.31
K, mmol/L	4.19	4.25	4.32	0.05	<0.01	<0.01	0.11	0.26
Hematocrit, % PCV	27.69	27.57	27.00	0.48	<0.01	<0.01	0.45	0.36
Hemoglobin, g/dL	9.46	9.36	9.23	0.17	<0.01	<0.01	0.39	0.55

¹pCO₂ and pO₂ = partial pressure of CO₂ or O₂; tCO₂ = total CO₂; PCV = packed cell volume.

²The control diet (CON) was formulated to offer a positive DCAD that averaged +6 mEq/100 g and dietary Ca at 0.40% DM. The negative DCAD (ND) and negative DCAD plus Ca supplementation (NDCA) diets were formulated to offer a negative DCAD that averaged -24 mEq/100 g with dietary Ca at either 0.40% or 2.0% DM.

³Trt = treatment.

⁴The preplanned contrast statements were (C1) CON versus the average of NDCA and ND and (C2) NDCA versus ND.

0.03). At calving, the difference reversed, such that cows given NDCA diet had lower NEFA than those fed ND ($P = 0.04$). Postpartum NEFA concentrations tended to be lower for cows fed ND or NDCA treatments compared with those fed CON ($P = 0.06$). Mean serum BHBA concentrations did not differ significantly among treatments.

DISCUSSION

Blood Acid-Base Balance and Gases

Blood acid-base balance is of great significance for metabolic activity, providing a stable and appropriate pH environment for enzymes, membrane permeability, and organ functions. We found that feeding ND or NDCA diets to dry cows decreased the blood pH at calving compared with the positive DCAD CON diet, consistent with previous research (Gelfert et al., 2007) and confirming a condition of compensated metabolic acidosis. Urine pH in this study followed the same pattern (Glosson et al., 2020), in which it was maintained in the range of 5.5 to 6.0 with administration of the negative DCAD diet before calving. In contrast, Seifi et al. (2010) found no change of blood pH for cows fed a negative DCAD diet. The reason seems to be their much higher DCAD (-8.2 mEq/100 g) compared with the DCAD in our trial (-24.0 mEq/100 g). Likewise, Grünberg et al. (2011) also found no difference in blood pH but their DCAD was only -9.0 mEq/100 g.

Figure 1. Least squares means for blood pH from 0 to 4 d after calving for cows fed different prepartum dietary treatments. The control diet (CON) was formulated to offer a positive DCAD that averaged $+6$ mEq/100 g and dietary Ca at 0.40% DM. The negative DCAD (ND) and negative DCAD plus Ca supplementation (NDCA) diets were formulated to offer a negative DCAD that averaged -24 mEq/100 g with dietary Ca at either 0.40% or 2.0% DM. * represents the significant difference ($P < 0.05$) of contrast CON versus (ND and NDCA). Error bars represent SE.

The HCO_3^- buffer system is significantly correlated with blood pH and is critical to blood acid-base balance (Yıldızdaş et al., 2004). From the iSTAT and serum biochemical analysis, our preplanned comparisons revealed decreased blood HCO_3^- prepartum and at calving for cows fed either of the 2 negative DCAD diets. Our results agree with the positive correlation between DCAD and blood HCO_3^- reported in Charbonneau et al. (2006). Greater blood anions (Cl^- , SO_4^{2-}) concentration resulting from absorption of anions from the lower DCAD diet have a large influence on humoral acid-base balance. In that case, bicarbonate has to be decreased while H^+ is increased to maintain blood electric neutrality in the normal range (Stewart, 1983; Gelfert et al., 2010). To stabilize membrane potential of the glomerulus and body fluid electric neutrality, the kidney must excrete excess anions absorbed from the negative DCAD diet (Wu et al., 2008). The significantly lower blood and urine pH induced by consumption of the negative DCAD diets before calving is similar to previous findings (Liesegang et al., 2007; Martins et al., 2016). Furthermore, in contradiction with an earlier report (Horst et al., 1997), when the NDCA diet was given, the blood pH did not differ significantly from ND at calving in the present study. Although nonsignificant, the slight difference in blood pH at calving in cows fed the 2 negative DCAD diets could be attributable to the mild alkalinizing influence of Ca^{2+} on blood acid-base status, because the majority of supplemental calcium in the NDCA diet originated from calcium carbonate. Feeding either ND or NDCA successfully led to compensated metabolic acidosis. The anion gap was calculated as $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)$ as defined by Pardon et al., (2018), but no significant effect on the prepartum anion gap occurred with the administration of ND and NDCA diets. The reduction of prepartum bicarbonate likely counteracted the inclusion of Cl from the negative DCAD diets, and the anion SO_4^{2-} was not included in the calculation formula for anion gap.

Regarding the postpartum period, the blood pH, bicarbonate, and base excess of cows fed the 2 negative DCAD diets demonstrated a greater increase and became significantly higher than CON in immediate post-calving period (24, 48, 96 h postpartum). At this same time urine pH was increasing but was still markedly lower than CON in the same cows in the same study (Glosson et al., 2020). Noticeably increased blood pH, bicarbonate, and base excess after calving indicated a quick recovery in acid-base status. We speculate that the rapid increase might be ascribed to the alkalinizing effect of greater blood ionized Ca concentration observed (0.98, 1.11, and 1.05 mM at 1 DIM for CON, ND, and NDCA groups, respectively; Glosson et al.,

2020). Another reason for the rapid increase could be the faster recovery of DMI of the positive DCAD lactation diet (25.4 mEq/100 g DM) after parturition for cows fed the negative DCAD diets prepartum (17.6, 18.4, and 18.8 kg/d for cows fed CON, ND, and NDCA, respectively; Glosson et al., 2020). With the assistance of higher postpartum blood ionized Ca concentration, chewing, rumination, and smooth muscle contraction of the digestive tract could be more functional and effective (Hansen et al., 2003).

As for blood gases, the measured values using iSTAT analyzer in this study were in good agreement with the venous reference range reported by Higgins (2011) and Lee et al. (2015). Blood $t\text{CO}_2$ concentration reflects the sum of CO_2 , carbonic acid, and bicarbonate in blood circulation but is dominated by the effect of bicarbonate. Although the difference of $p\text{CO}_2$ among treatments was not significant at calving, our results showed that $t\text{CO}_2$ and $p\text{CO}_2$ shared similar fluctuation with bicarbonate levels, in that negative DCAD diets with or without

Figure 2. Least squares means for blood acid-base balance and gas indices during 0 to 4 d after calving for cows fed different prepartum dietary treatments. The control diet (CON) was formulated to offer a positive DCAD that averaged +6 mEq/100 g and dietary Ca at 0.40% DM. The negative DCAD (ND) and negative DCAD plus Ca supplementation (NDCA) diets were formulated to offer a negative DCAD that averaged -24 mEq/100 g with dietary Ca at either 0.40% or 2.0% DM. * represents the significant difference ($P < 0.05$) of contrast CON versus (ND and NDCA); # represents the significant difference ($P < 0.05$) of contrast ND versus NDCA. $p\text{CO}_2$ = partial pressure of CO_2 ; $t\text{CO}_2$ = total CO_2 . Error bars represent SE.

additional Ca brought about significant reduction of $t\text{CO}_2$. This finding is in line with the investigation of Martins et al. (2016), who reported that blood $t\text{CO}_2$ was reduced along with the decrease of dietary DCAD.

Salles et al. (2012) also reported that blood $p\text{CO}_2$ was linearly related to dietary DCAD when DCAD was reduced from 40.0 to -10.0 mEq/100 g DM. Blood CO_2 resulting from respiration can dissolve into carbonic acid, and be further transformed to bicarbonate and H^+ , which acidifies the humoral environment. Hence, under the condition of metabolic acidosis, the $t\text{CO}_2$ and $p\text{CO}_2$ reduction might represent feedback regulation from low blood pH and result from increased expiration of CO_2 (Block, 1994). Postpartum splanchnic consump-

tion of O_2 , particularly in the liver, could be more than double the average prepartum, with the majority of the increase occurring in the first 11 DIM (Reynolds et al., 2003). This alteration may be ascribed to the nutrient delivery from increased consumption and digestion of greater feed intake, lactation metabolic activity, and hepatic oxidative metabolism of transition dairy cows. In the current study, feeding NDCA brought about greater blood $p\text{O}_2$ in the first 4 DIM, which might reflect more sufficient O_2 supply in circulation for metabolic requirements. Although no effect of treatments was observed on hemoglobin, which binds O_2 for blood transport, the increase of O_2 concentration provided some evidence that a prepartum acidogenic diet with high Ca could

Table 4. Least squares means for serum metabolic profile for cows fed close-up dietary treatments CON, ND, or NDCA starting at -28 d relative to expected calving

Variable ¹	Treatment ²			SEM	DIM	Trt ³ × DIM	Contrast ⁴	
	CON	ND	NDCA				C1	C2
Anion gap, mmol/L								
Prepartum	17.06	17.37	16.86	0.27	0.31	0.94	0.84	0.11
At calving	19.10	19.11	18.91	0.58			0.88	0.77
Immediate postcalving	18.01	18.29	18.51	0.50	0.06	0.46	0.41	0.67
Postpartum	18.06	17.70	17.93	0.43	<0.01	0.67	0.57	0.65
Bicarbonate, mmol/L								
Prepartum	24.75	21.00	21.96	0.28	<0.01	0.80	<0.01	<0.01
At calving	24.99	21.62	22.08	0.51			<0.01	0.50
Immediate postcalving	26.41	28.81	28.01	0.52	<0.01	0.03	<0.01	0.18
Postpartum	25.34	25.75	25.93	0.39	0.02	0.84	0.11	0.63
Urea N, mg/dL								
Prepartum	14.63	14.95	15.37	0.32	<0.01	0.88	0.14	0.29
At calving	14.34	14.29	15.52	0.60			0.40	0.11
Immediate postcalving	12.40	12.09	12.95	0.50	0.02	0.27	0.82	0.16
Postpartum	11.39	12.16	12.38	0.48	0.23	0.28	0.10	0.73
Total protein, g/dL								
Prepartum	6.21	6.33	6.35	0.05	<0.01	0.74	0.047	0.88
At calving	5.92	6.01	6.03	0.07			0.25	0.81
Immediate postcalving	5.97	5.85	6.04	0.10	<0.01	0.08	0.83	0.15
Postpartum	6.76	6.66	6.71	0.11	<0.01	0.29	0.41	0.63
Albumin, g/dL								
Prepartum	3.31	3.39	3.37	0.02	<0.01	0.86	0.01	0.64
At calving	3.39	3.45	3.43	0.03			0.14	0.70
Immediate postcalving	3.22	3.30	3.33	0.05	0.56	0.12	0.10	0.64
Postpartum	3.28	3.31	3.36	0.07	0.01	0.27	0.50	0.59
Globulin, g/dL								
Prepartum	2.89	2.96	3.00	0.05	<0.01	0.99	0.19	0.62
At calving	2.54	2.58	2.65	0.06			0.29	0.42
Immediate postcalving	2.73	2.63	2.70	0.10	<0.01	0.06	0.59	0.63
Postpartum	3.48	3.35	3.30	0.12	<0.01	0.33	0.24	0.76
Glucose, mg/dL								
Prepartum	64.7	61.7	63.1	0.7	<0.01	0.61	<0.01	0.12
At calving	109.6	99.4	111.3	6.4			0.54	0.15
Immediate postcalving	57.2	55.1	57.8	1.2	<0.01	0.85	0.58	0.11
Postpartum	57.3	57.6	57.5	0.8	0.09	0.52	0.78	0.97

¹Blood samples were taken at d -30 (used as a covariate in the model), -21 , -14 , -7 , -4 , -2 , -1 , calving, d 1, 2, 4, 7, 14, 21, and 28 relative to parturition. Samples of d 1, 2 and 4 were analyzed separately as immediate postcalving.

²The control diet (CON) was formulated to offer a positive DCAD that averaged $+6$ mEq/100 g and dietary Ca at 0.40% DM. The negative DCAD (ND) and negative DCAD plus Ca supplementation (NDCA) diets were formulated to offer a negative DCAD that averaged -24 mEq/100 g with dietary Ca at either 0.40% or 2.0% DM.

³Trt = treatment.

⁴The preplanned contrast statements were (C1) CON versus the average of NDCA and ND and (C2) NDCA versus ND.

contribute to improvement of the metabolic adaptation to parturition in early lactating dairy cows.

Blood Metabolic Profile

Alterations of total protein, albumin, globulin, and urea N in transition cows reflect protein availability and N utilization status. Our study confirmed enhancement of blood total protein and albumin concentrations before calving by feeding negative DCAD diets. A recent trial of Guo et al. (2019) found that serum total protein and albumin concentrations of sows were increased at 1 and 18 DIM by an acidogenic lactation diet. Our findings contradict results reported by Grünberg et al. (2011) who found that feeding a DCAD diet of -9 mEq/100 g led to a reduction of plasma protein. This might be explained by the differences in DCAD of the diets between the 2 studies. Cows in our study were fed a prepartum diet with a negative DCAD value of -24 mEq/100 g. The changes in albumin and total protein were associated with the higher prepartum blood Ca concentration that resulted from the acidogenic ration (Glosson et al., 2020). Approximately 40% of Ca in circulation is bound with albumin (80%) and globulin (20%; Bazydlo et al., 2014). With respect to postcalving data, cows previously fed negative DCAD diets also

had a tendency toward higher serum albumin and urea N concentrations. A study conducted by Li et al. (2008) revealed that a prepartum diet with low DCAD (-26.5 mEq/100 g) resulted in a decline of digested N deposition efficiency compared with a positive DCAD diet, thereby contributing more available N for milk protein synthesis (Martins et al., 2015). Furthermore, albumin was found to be inversely related to postpartum disease and has been recommended as a disease risk indicator for transition cows by Van Saun (2006). Cows with greater serum albumin concentrations during close-up and fresh periods had lower incidence of diseases. The puerperal response of improved protein metabolism in response to the feeding of a negative DCAD diet could be one of the reasons for the reduction in adverse health score reported in Glosson et al. (2020). Blood albumin was also suggested by Bikle et al. (1986) to be a binding protein for transport of vitamin D, which might benefit bone Ca mobilization and intestinal Ca absorption (Weiss et al., 2015).

Wu et al. (2014) demonstrated a significantly negative effect of a low DCAD diet prepartum on blood glucose with an extended feeding time of more than 3 wk. We also observed that the consumption of negative DCAD diets decreased prepartum blood glucose concentration, perhaps associated with decreased DMI (Yu et al.,

Table 5. Least squares means for serum lipid metabolites for cows fed close-up dietary treatments CON, ND, or NDCA starting at -28 d relative to expected calving

Variable ¹	Treatment ²			SEM	DIM	Trt ³ × DIM	Contrast ⁴	
	CON	ND	NDCA				C1	C2
Total cholesterol, mg/dL								
Prepartum	82.4	86.4	87.8	1.9	<0.01	0.15	0.03	0.57
At calving	62.3	63.2	65.5	2.0			0.32	0.35
Immediate postcalving	59.4	58.7	65.5	2.2	<0.01	0.16	0.29	0.03
Postpartum	101.4	99.3	109.8	4.8	<0.01	0.82	0.72	0.09
Triglycerides, mg/dL								
Prepartum	22.5	22.7	23.3	0.8	<0.01	0.02	0.56	0.62
At calving	9.8	10.4	10.3	0.6			0.49	0.91
Immediate postcalving	8.9	7.9	8.3	0.3	0.04	0.76	0.07	0.41
Postpartum	10.3	9.1	9.9	0.4	0.82	0.59	0.09	0.06
NEFA, mEq/L								
Prepartum	0.28	0.30	0.35	0.02	<0.01	0.26	0.01	0.03
At calving	0.83	1.00	0.79	0.08			0.49	0.04
Immediate postcalving	0.66	0.69	0.66	0.07	0.03	0.10	0.81	0.61
Postpartum	0.66	0.57	0.60	0.05	<0.01	0.28	0.06	0.83
BHB, mmol/L								
At calving	0.54	0.59	0.51	0.05			0.92	0.13
Immediate postcalving	0.75	0.78	0.69	0.04	0.40	0.46	0.81	0.19
Postpartum	0.60	0.58	0.54	0.05	<0.01	0.62	0.30	0.46

¹Blood samples were collected at d -30 (used as a covariate in the model), -21 , -14 , -7 , -4 , -2 , -1 , calving, d 1, 2, 4, 7, 14, 21, and 28 relative to parturition. Samples of d 1, 2 and 4 were analyzed separately as immediate postcalving.

²The control diet (CON) was formulated to offer a positive DCAD that averaged $+6$ mEq/100 g and dietary Ca at 0.40% DM. The negative DCAD (ND) and negative DCAD plus Ca supplementation (NDCA) diets were formulated to offer a negative DCAD that averaged -24 mEq/100 g with dietary Ca at either 0.40% or 2.0% DM.

³Trt = treatment.

⁴The preplanned contrast statements were (C1) CON versus the average of NDCA and ND and (C2) NDCA versus ND.

2016). Meanwhile, negative DCAD plus high Ca had the opposite effect on prepartum glucose although the difference did not reach significance ($P = 0.12$). Diets containing anionic salts are widely believed to decrease feed palatability, according to feeding behavior (Vagnoni and Oetzel, 1998) as well as reduced DMI (Grünberg et al., 2011). As a result of lower feed ingestion, cows given low DCAD diets could have decreased glucose in circulation. On the contrary, in our study cows fed the NDCA diet had elevated close-up DMI compared with ND (10.0 vs. 11.0 kg/d DMI for cows fed ND and NDCA diets, respectively; Glosson et al., 2020), which may be the reason for the numerically higher blood glucose. However, no significant differences occurred for postcalving cows. This finding is supported by the research of Grünberg et al. (2011) who found that feeding a low DCAD diet did not affect insulin response to circulating glucose in parturient dairy cows.

Most cows incur NEB after parturition owing to initiation of lactation and suppression of DMI before and after parturition, which causes an increase in catabolism of body adipose stores (Holcomb et al., 2001, Janovick and Drackley, 2010). Previous anionic diet strategies were applied with the purpose of improving lipid metabolism and reducing metabolic disorders with varying results (Seifi et al., 2010; Weich et al., 2013). Our data demonstrated that cows given negative DCAD diets had higher blood total cholesterol compared with CON before parturition. Furthermore, feeding NDCA resulted in a greater postpartum total cholesterol concentration when compared with ND. This result could indicate a more active lipoprotein synthesis and improvement of nutrient metabolism because cholesterol concentration decreases significantly around parturition (Bionaz et al., 2007). Alternatively, because cholesterol synthesis is active in the intestine to aid dietary lipid absorption, higher serum cholesterol in NDCA postpartum could reflect the greater DMI by those cows. In the current study, postcalving serum triglycerides concentration tended to be lower for cows fed prepartum acidogenic diets. Weich et al. (2013) reported a similar tendency toward reduced triglycerides in response to the feeding of a negative DCAD diet.

The prepartum increase of NEFA for ND and NDCA might be a consequence of suppressed DMI in response to consumption of the acidogenic diet. Following parturition, mobilized NEFA due to NEB contributes to fatty infiltration and accumulation in the liver, leading to accumulation of ketones in circulation (BHB, acetoacetic acid, and acetone), which can cause ketosis (Phillippo et al., 1994). Investigation carried out by Zhang et al. (2020) noted that transition dairy cows with low blood Ca concentration (<2.0 mmol/L) at 24 h after calving

had higher NEFA and BHB concentrations during the fresh period. In our study, results suggested that NEFA for cows fed negative DCAD diets were lower postpartum in comparison with CON, indicating an improvement of postpartum lipid metabolism. One possible rationalization for this is that higher blood Ca concentrations observed in cows fed a prepartum acidogenic diet might accelerate the recovery of digestive activities (chewing, rumination, and digestive tract peristalsis), which are typically suppressed throughout the immediate periparturient period (Hansen et al., 2003; Weich et al., 2013). Improved activity might subsequently lead to increased DMI and improved energy balance of dairy cows in the fresh period. Our results coincide with those of Apper-Bossard et al. (2006), who observed higher postpartum energy balance in cows that consumed a low DCAD diet. Besides this, NDCA further decreased serum NEFA concentrations although the lower BHB was not significant. Our results demonstrated that supplementation of high Ca could strengthen the benefits of a strongly negative DCAD diet on lipid metabolism in periparturient cows.

CONCLUSIONS

The findings of this study indicate that the blood acid-base balance could be altered and compensated metabolic acidosis could be induced by feeding acidogenic diets (-24 mEq/100 g DM) to transition dairy cows. The compensated metabolic acidosis recovered to normal immediately within 24 h after calving once the consumption of acidogenic diet was stopped. The negative DCAD diet with a greater amount of Ca addition had a similar effect, and seemed to speed the normalization of acid-base balance after calving. On the basis of elevated serum total protein, albumin, and cholesterol, along with reduced triglycerides and NEFA, protein and lipid metabolism of periparturient cows benefited from the prepartum acidogenic diets. Our results overall provide additional evidence that high Ca supplementation in conjunction with a strongly negative DCAD diet was more favorable to the metabolic adaptation to lactation in dairy cows than the negative DCAD with low Ca.

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