Effect of Dietary Cation-Anion Difference and Dietary Crude Protein on Milk Yield, Acid-Base Chemistry, and Rumen Fermentation

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ABSTRACT

Eight primiparous lactating Holstein cows (47 ± 10 d in milk) fitted with ruminal cannulae were used to determine the effect of dietary cation-anion difference (DCAD) and dietary crude protein (CP) concentration on milk yield and composition, acid-base chemistry, and measures of N metabolism in lactating dairy cows. Treatments were arranged as a 2×2 factorial in a randomized complete block design to provide 15 or 17% CP and DCAD of 25 or 50 mEq (Na + K – Cl)/100 g of feed dry matter [15 or 39 mEq (Na + K) – (Cl + S)/100 g of feed dry matter]. High DCAD improved dry matter intake, milk yield, and concentrations of milk fat and protein. An interaction of DCAD and CP was observed for uric acid excretion, an indicator of microbial protein yield. Uric acid excretion was higher for high DCAD than for low DCAD in low CP diets and was similar for low and high DCAD with high CP. Serum bicarbonate concentration, urinary bicarbonate excretion, blood pH, and serum Na were elevated for high DCAD compared with low DCAD. Fractional excretion of Na, K, Cl, and Ca increased for high DCAD. Blood urea N and urinary urea N were greater for high than for low CP diets. No differences due to DCAD were observed for these parameters. Results of this study suggest that, in early lactation cows, blood acid-base chemistry is altered by differences in DCAD that range between the high and low ends of the desired DCAD range. Modifications of acid-base chemistry and the corresponding changes in protein metabolism may allow for more efficient feeding of protein and better nutritional management of the lactating dairy cow.

Key words: dietary cation-anion difference, dietary crude protein, sodium, potassium

INTRODUCTION

The influence of acid-base chemistry on N metabolism has been documented in several species. Van Slyke et

al. (1943) first identified the role of glutamine as a precursor for ammoniogenesis. Ammonia serves as a major systemic buffer during metabolic acidosis. Acid load stimulates renal ammoniogenesis (Madias and Zelman, 1986) and inhibits hepatic ureagenesis (Kashiwagura et al., 1984), whereas alkaline load stimulates urea production (Guder, 1987). Under conditions of normal acid-base chemistry and dietary conditions, adequate glutamine is available in the diet or can be generated from other amino acids to meet the need for ammoniogenesis (Patience, 1990). Oxidation of excess sulfur-containing amino acids also contributes to overall acid load (Patience, 1990).

Dietary K concentration appears to have an effect on the metabolism of Lys and Arg. Free Lys and Arg concentrations increased in K-depleted rat muscle (Arnould and Lachance, 1980). Robbins et al. (1982) observed greater free muscle Lys, Arg, and Glu concentrations when dietary K was elevated from 0.12 to 0.18%. However, concentration of these AA decreased when dietary K exceeded 0.18%. Alleviation of the growth-depressing effects of Lys-Arg antagonism was also noted in poultry with the addition of dietary K (O'Dell and Savage, 1996). Because increasing dietary K increases DCAD, calculated as mEq (Na + K – Cl)/100 g of DM, improved AA utilization may be possible in lactating dairy cows with greater DCAD.

In addition to the relationship of acid-base status with ammoniogenesis (Madias and Zelman, 1986) and ureagenesis (Kashiwagura et al., 1984; Guder, 1987), greater total N excretion (Welbourne et al., 1986) and decreased N retention (May et al., 1987) have been reported during metabolic acidosis. This relationship between acid-base balance and N metabolism coupled with the effect of DCAD on acid-base balance (Patience, 1988; Tucker et al., 1988) suggests that N metabolism can be affected by changing DCAD. Results from earlier work have been inconsistent concerning the relationship between DCAD and BUN in lactating cows. Roche et al. (2005) reported a linear increase in BUN as DCAD increased from 23 to 88 mEq (Na + K – Cl)/100 g of DM. Apper-Bossard et al. (2006) reported a quadratic response in BUN to DCAD, with increasing BUN from

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9 to 24 mEq (Na + K – Cl)/100 g of DM, and decreasing BUN as DCAD was increased further. Escobosa et al. (1984) also reported higher BUN when DCAD was elevated from –19 to 17 mEq (Na + K – Cl)/100 g of DM, but BUN did not change further when DCAD was raised to 32 mEq/100 g of DM.

Total N retention was similar for low and high DCAD in both lactating (Delaquis and Block, 1995a) and dry (Delaquis and Block, 1995b) dairy cows. Sheep fed low CP diets had improved N retention and digestibility when NaHCO₃ was included in the diet, increasing the DCAD (Phillip, 1983). Patience et al. (1986) reported no difference in total N digestibility in high DCAD diets fed to swine, whereas Haydon and West (1990) noted increased N retention when DCAD was elevated, suggesting that the effect of DCAD on N metabolism in ruminants may differ compared with other species.

Although an interaction of acid-base chemistry with N metabolism is evident, the mechanisms are unclear and limited research has been conducted in ruminants, especially the lactating dairy cow. The objective of the study was to determine the effects of DCAD on milk yield and composition, rumen function, and AA metabolism of lactating dairy cows in early lactation fed low or high dietary CP.

MATERIALS AND METHODS

Eight primiparous lactating Holstein cows fitted with ruminal cannulas and averaging 47 ± 10 DIM were used in a randomized complete block design with four 3-wk treatment periods (TP) from March 28 to June 19. Cows were blocked by TP and assigned to a unique treatment combination during each TP. The first 2 wk of each TP were used for acclimation to treatments, with data collection occurring during wk 3. Two cows were assigned to each treatment combination, with cows being paired for no more than one TP.

Dietary treatments were arranged as a 2×2 factorial and were formulated to provide a DCAD of 25 (DCAD25) or 50 (DCAD50) mEq (Na + K – Cl)/100 g of DM [15 or 39 mEq (Na + K) – (Cl + S)/100 g of DM, respectively] and CP concentrations of 15 (CP15) or 17% (CP17), on a DM basis. Diets were formulated to meet or exceed NRC (2001) requirements for all nutrients with the exception of CP15 diets, which were below NRC requirements for CP (Table 1). Ingredient composition differed within CP treatments to allow for the addition of sodium bicarbonate and potassium carbonate to adjust DCAD and still maintain the desired CP and DMI. Sodium bicarbonate, potassium carbonate, Prolak (H. J. Baker & Bro., Inc., Westport, CT), urea, and soybean meal were combined in an experimental premix for adjustment of DCAD and CP.

Table 1. Ingredient composition of diets formulated to contain a DCAD of 25 or 50 mEq (Na + K – Cl)/100 g of DM and 15 or 17% CP

Ingredient, % of DM	15% CP		17% CP	
	DCAD25	DCAD50	DCAD25	DCAD50
Corn silage	40.98	37.29	38.79	36.09
Alfalfa hay	10.47	9.81	10.82	9.79
Whole cottonseed	9.30	9.56	8.84	9.28
Steam-flaked corn	30.93	32.60	29.22	29.75
Mineral-vitamin premix ¹	2.98	2.98	2.98	2.98
Soybean meal	3.53	3.82	6.96	7.62
Urea	0.22	0.22	0.40	0.40
Prolak ²	1.59	1.77	1.99	2.14
K ₂ CO ₃	0.00	0.97	0.00	0.97
NaHCO ₃	0.00	0.97	0.00	0.97

¹Mineral premix contained 64.58% limestone, 10.42% salt, 10.42% distillers grain, 8.33% magnesium oxide, and 6.25% vitamin-trace mineral premix.

²Protein supplement manufactured by H. J. Baker & Bro., Inc., Westport, CT.

Before the start of the study, cows were trained to eat through electronic feeding gates (American Calan, Inc., Northwood, NH). Cows were fed experimental diets once daily (0800 h) as a TMR, with the amount of feed offered and refused recorded and adjusted daily based on the previous day's consumption to allow for 10%orts. The TMR was pushed up at least 4 times daily. Feed DM content was determined weekly by drying samples at 60°C for 72 h in a forced air oven and was used for adjustment of ration components. Cows were housed in a free-stall barn with fans activated by thermostat when the ambient temperature exceeded 24°C and high-pressure misters activated by a humidistat when fans were activated and relative humidity was below 85%. Cows were milked twice daily at approximately 0400 and 1500 h.

Sampling

Forage, concentrate, treatment mixes, and TMR were sampled 3 times weekly, dried at 60°C for 72 h, and composited by week for later analysis. Samples were ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). Feed N was determined using a Kjeltac System (Foss Tecator AB, Höganäs, Sweden), and CP was calculated as the percentage N $\times$ 6.25 (AOAC, 1990). Acid detergent fiber and NDF were determined according to Van Soest et al. (1991). Samples were extracted using a combination of nitric and acetic acids, and chloride was determined using a chloridometer (Haake Buchler Instruments, Inc., Saddle Brook, NJ) as defined by Cotlove et al. (1958). Phosphorus concentration was determined by colorimetry (Beckman DU Series 500 spectrophotometer; Beckman Instruments Inc., Fullerton, CA) follow-

ing wet ashing (AOAC, 1990). Other minerals (Na, K, Cl, Ca, Mg) were measured by atomic absorption spectrophotometry (model number 3030, Perkin-Elmer, Norwalk, CT) following wet ashing (AOAC, 1990).

Milk yield was measured d 15 through 21 of each TP using the Alpro electronic weight system (DeLaval, Kansas City, MO). Milk samples were collected from 4 consecutive milkings starting with the p.m. milking on d 18 through the a.m. milking on d 20 of each TP and shipped to Southeast Milk Inc. (Bellevue, FL) for analyses of fat and protein using infrared analysis (B2000, Bentley Instruments Inc., Chaska, MN).

Blood was collected into evacuated tubes on d 20 of each TP by jugular venipuncture at 0, 2, 4, 6, and 10 h postfeeding. Whole blood was analyzed for pH within 30 min of collection using an AVL OPTI Critical Care Analyzer (Osmetech Inc., Roswell, GA) at the University of Georgia Veterinary Diagnostic Laboratory. Serum was separated by centrifugation at $2,500 \times g$ for 20 min and was then analyzed for Ca, P, Mg, Na, K, Cl, bicarbonate, BUN, creatinine, glucose, and uric acid using a Boehringer Mannheim/Hitachi 912 automated chemistry analyzer (Roche Laboratory Systems, Indianapolis, IN). Serum collected 6 h postfeeding was analyzed for free AA using a Beckman 6300 Amino Acid Analyzer (Beckman Coulter Inc., Fullerton, CA) according to the method of Slocum and Lee (1983) at the University of Missouri Experiment Station Chemical Laboratories in Columbia. Urine samples collected via manual stimulation at 1300 h on d 19 of each TP were analyzed for pH, Ca, P, Mg, Na, K, Cl, bicarbonate, urea N, creatinine, glucose, and uric acid at the University of Georgia Veterinary Diagnostic Laboratory in Tifton. Fractional excretion of minerals was calculated using individual concentrations as follows: (urinary mineral/serum mineral) $\times$ (serum creatinine/urinary creatinine) $\times$ 100 (Vander, 1991).

Ruminal fluid samples were collected at 0, 2, 4, 6, 8, 10, and 12 h postfeeding on d 20 of each TP. Samples were filtered through 2 layers of cheesecloth and 25% metaphosphoric acid was added in a 1:4 acid:rumen fluid ratio before freezing for later VFA analysis (Erwin et al., 1961). Volatile fatty acid concentrations were determined using liquid gas chromatography (Hewlett-Packard 5890A Gas Chromatograph, Hewlett-Packard Company, Avondale, PA).

Statistical Analysis

Means for DMI, milk yield and composition, serum AA concentration, and fractional excretion were analyzed using the PROC GLM procedure of SAS (SAS Institute, 1999). Sums of squares were partitioned to DCAD, CP, TP, cow within treatment, and DCAD $\times$

CP. Serum mineral and metabolite data and rumen parameters included repeated measures and were analyzed using the PROC MIXED procedure of SAS (SAS Institute, 1999). Sums of squares in these models were partitioned to DCAD, CP, TP, cow within treatment, time postfeeding, DCAD $\times$ CP, DCAD $\times$ time, CP $\times$ time, and DCAD $\times$ CP $\times$ time. Cow within treatment was the random variable with time postfeeding as the repeated measure. Main effects were tested using cow within treatment as the error term. Interactions were tested using the residual error from the model.

RESULTS AND DISCUSSION

Chemical Composition of Diets

The DCAD was higher than originally formulated for all diets (Table 2) because of higher Na and lower Cl concentrations than expected. All diets met or exceeded NRC (2001) guidelines for Na, K, and Cl (0.19, 1.02, and 0.25%, respectively) and the calculated DCAD using these values [$27 \text{ mEq (Na + K - Cl)/100 g of DM}$]. The DCAD was formulated to represent the high (50 mEq (Na + K - Cl)/100 g of DM) and low (25 mEq/100 g of DM) end of the optimal range suggested by Sanchez and Beede (1996). The CP17 diets were formulated to represent NRC (2001) recommendations for CP, and CP15 diets were formulated below NRC (2001) recommendations.

DMI, Milk Yield, and Milk Composition

A positive response to DCAD ($P < 0.01$) was observed for DMI and DMI/100 kg of BW (Table 3). This is consistent with earlier work in which West et al. (1992) reported a linear improvement in DMI as DCAD increased from 12 to 46 mEq/100 g of DM. Apper-Bossard et al. (2006) also reported a linear increase in DMI with greater DCAD in high concentrate diets similar to those in the present study.

Yield of milk, ECM, milk fat, and milk protein, as well as milk fat and protein percentage were higher for DCAD50 vs. DCAD25 (Table 3). Milk yield increased from 23.9 to 26.2 kg/d for low vs. high DCAD. In earlier work, milk yield responded positively when DCAD was increased in high concentrate diets (Apper-Bossard et al., 2006), especially in early and mid lactation cows. Although milk fat percentage was low across treatments, it increased from 2.44 to 2.92% with high DCAD. Greater milk yield and milk fat percentage also resulted in a 0.15 kg/d improvement in fat yield. Roche et al. (2005) observed a linear increase in milk fat percentage and milk fat yield with increasing DCAD in pasture-fed cows. In high concentrate diets, Apper-Bossard et al. (2006) also reported a linear increase in milk fat

Table 2. Chemical composition¹ of diets formulated to contain a DCAD of 25 or 50 mEq (Na + K – Cl)/100 g of DM and 15 or 17% CP

Item	15% CP		17% CP	
	DCAD25	DCAD50	DCAD25	DCAD50
DM, %	60.1 ± 2.7	61.8 ± 2.6	61.7 ± 2.6	62.5 ± 2.5
	% of DM			
CP	14.43 ± 0.56	14.93 ± 0.41	17.07 ± 0.70	17.53 ± 0.37
NDF	29.65 ± 2.04	30.39 ± 1.82	29.77 ± 1.36	28.29 ± 1.83
ADF	16.62 ± 1.84	16.52 ± 1.37	16.44 ± 0.61	16.40 ± 1.80
RUP, ² % of CP	36.6	37.2	35.4	36.2
Soluble protein, ² % of CP	31.8	30.9	32.2	30.7
NE _L , ² Mcal/kg	1.69	1.69	1.69	1.69
Ca	0.99 ± 0.12	0.99 ± 0.09	1.01 ± 0.03	0.99 ± 0.07
P	0.43 ± 0.01	0.45 ± 0.02	0.44 ± 0.03	0.45 ± 0.01
Mg	0.22 ± 0.00	0.23 ± 0.02	0.23 ± 0.01	0.24 ± 0.01
K	1.04 ± 0.11	1.54 ± 0.09	1.06 ± 0.08	1.58 ± 0.13
Na	0.24 ± 0.03	0.51 ± 0.03	0.27 ± 0.02	0.51 ± 0.02
Cl	0.28 ± 0.02	0.28 ± 0.02	0.28 ± 0.01	0.27 ± 0.01
S ³	0.24	0.24	0.25	0.26
DCAD	29.1	53.7	31.0	55.0
DCAD:S ⁴	14.2	38.7	15.4	38.8

¹Mean value ± standard deviation.²Calculated values using CPM-Dairy (The Department of Animal Science, Cornell University, Ithaca, NY; The Center for Animal Health and Productivity, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA; and The William H. Miner Agricultural Research Institute, Chazy, NY).³Estimated values according to NRC (2001).⁴In mEq (Na + K) – (Cl + S)/100 g of DM.

percentage with higher DCAD. Roche et al. (2005) proposed that higher DCAD would raise rumen pH, shifting fermentation in favor of acetate and butyrate production—substrates utilized for de novo fatty acid synthesis.

An interaction of DCAD and CP ($P < 0.01$) was observed for milk protein percentage (Table 3). Higher milk protein percentage was observed for DCAD50 in CP15 diets, consistent with earlier work (West et al., 1991; Delaquis and Block, 1995a) in which DCAD elicited a positive linear response for milk protein percent-

age. Milk protein percentage was similar for DCAD25 and DCAD50 within CP17 diets. Higher DCAD fed in conjunction with lower CP may increase the availability of CP for milk protein synthesis via greater microbial growth that results from improved ruminal conditions in higher DCAD diets (West et al., 1987; Tucker et al., 1988). In higher CP diets, the quantity of MP reaching the small intestine was likely less limiting, thereby reducing the effect of DCAD on milk protein synthesis. Delaquis and Block (1995a) reported similar results for cows fed diets with CP concentrations similar to the

Table 3. Dry matter intake, milk yield, milk composition, and milk component yield for cows fed diets formulated to contain a DCAD of 25 or 50 mEq (Na + K – Cl)/100 g of DM and 15 or 17% CP

Item	15% CP		17% CP		SEM	<i>P</i> -value		
	DCAD25	DCAD50	DCAD25	DCAD50		DCAD	CP	DCAD × CP
DMI								
DM, kg/d	17.8	19.2	17.7	19.0	0.35	**	NS	NS
DM, kg/100 kg of BW	3.8	4.1	3.8	4.1	0.08	**	NS	NS
Milk yield, kg/d								
Milk	24.0	25.7	23.8	26.6	0.4	**	NS	NS
ECM ¹	20.5	23.4	20.6	24.1	0.4	**	NS	NS
Fat	0.60	0.76	0.61	0.77	0.01	**	NS	NS
Protein	0.70	0.76	0.71	0.79	0.01	**	NS	NS
Milk composition, %								
Fat	2.44	2.95	2.44	2.89	0.03	**	NS	NS
Protein	2.89	2.94	2.99	2.98	0.01	*	**	**

¹ECM = 0.3246 × (kg milk) + 12.86 × (kg fat) + 7.04 × (kg protein); Tyrrell and Reid (1965).* $P < 0.05$; ** $P < 0.01$; NS: $P > 0.10$.

Table 4. Blood serum mineral and metabolite concentrations for cows fed diets formulated to contain a DCAD of 25 or 50 mEq (Na + K – Cl)/100 g of DM and 15 or 17% CP

Item	15% CP		17% CP		SEM	P-value		
	DCAD25	DCAD50	DCAD25	DCAD50		DCAD	CP	DCAD × CP
Na, mEq/L	142.92	143.53	142.08	144.50	0.58	*	NS	NS
K, mEq/L	4.08	4.22	4.14	4.24	0.09	NS	NS	NS
Cl, mEq/L	101.10	99.53	101.05	100.18	0.94	NS	NS	NS
DCAD, (Na + K – Cl) mEq/L	45.90	48.22	45.16	48.57	0.93	**	NS	NS
Ca, mg/dL	10.06	9.93	10.28	9.99	0.17	NS	NS	NS
Mg, mg/dL	2.53	2.27	2.55	2.16	0.08	**	NS	NS
Glucose, mg/dL	68.43	67.80	68.10	66.45	2.03	NS	NS	NS
Bicarbonate, mmol/L	22.54	24.46	22.06	24.57	0.73	**	NS	NS
BUN, mg/dL	8.68	8.35	12.48	12.73	0.73	NS	**	NS
pH	7.468	7.497	7.456	7.495	0.009	**	NS	NS

* $P < 0.05$; ** $P < 0.01$; NS: $P > 0.10$.

high CP diets fed in the current study. In addition to improvements in milk protein percentage, greater milk protein yield was noted for high DCAD diets in the current experiment. Additional buffering provided by DCAD may reduce the role of protein in blood buffering, resulting in greater proportions of protein available for milk protein synthesis. Improved milk protein may also result from a protein-sparing effect with increased DCAD. Honeyfield and Froseth (1985) reported decreased plasma basic AA concentrations and improved pig growth and efficiency with greater dietary Na. In the current study, significantly lower blood AA concentrations were not observed, suggesting that enhanced microbial growth that resulted from greater ruminal buffering improved the availability of MP for milk protein synthesis, especially in the low CP diet.

Blood and Urinary Minerals and Metabolites

Serum Na concentration (Table 4) and fractional excretion of Na (Table 5) were elevated in cows receiving

high DCAD, probably because the high DCAD diets contained higher Na concentrations (Table 2). Greene et al. (1983) reported that elevated dietary K concentration could also result in increased absorption and excretion of Na, offering an alternative explanation for the increased absorption of Na. A decrease in both serum Mg concentration and Mg fractional excretion was observed with high DCAD. Jittakhot et al. (2004) reported depressed Mg absorption when dietary K was elevated. Decreased absorption would reduce serum Mg concentration with less Mg available for excretion. Blood bicarbonate concentration and excretion were both elevated by high DCAD. Similar relationships between DCAD and blood bicarbonate concentration and urinary excretion have been reported in earlier work (West et al., 1991; Roche et al., 2005; Apper-Bossard et al., 2006). Serum DCAD and blood pH were also greater for high DCAD diets (Table 4). Additional blood buffering is associated with improved DMI (Delaquis and Block, 1995a), especially in early and mid lactation cows.

Table 5. Fractional and urinary excretion of mineral and metabolites for cows fed diets formulated to contain a DCAD of 25 or 50 mEq (Na + K – Cl)/100 g of DM and 15 or 17% CP

Item	15% CP		17% CP		SEM	P-value		
	DCAD25	DCAD50	DCAD25	DCAD50		DCAD	CP	DCAD × CP
Fractional excretion, ¹ %								
Na	0.41	2.04	0.53	1.48	0.20	**	NS	NS
K	37.17	78.00	39.63	77.84	4.74	**	NS	NS
Cl	0.28	1.08	0.33	0.82	0.10	**	NS	NS
Ca	1.08	0.22	0.71	0.50	0.21	*	NS	NS
Mg	13.44	9.93	12.33	9.54	1.62	†	NS	NS
Urine metabolite/urine creatinine ²								
Urea N	4.13	4.56	7.87	8.09	0.47	NS	**	NS
Bicarbonate	0.38	2.67	0.73	2.44	0.26	**	NS	NS
Uric acid	0.68	0.84	0.73	0.74	0.04	*	NS	*

¹Fractional excretion = (urinary mineral/plasma mineral) × (plasma creatinine/urinary creatinine) × 100 (Vander, 1991).²Expressed as (mg of urine metabolite/100 mL of urine)/(mg of urine creatinine/100 mL of urine).† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; NS: $P > 0.10$.

Table 6. Serum, essential, and total AA concentrations for cows fed diets formulated to contain a DCAD of 25 or 50 mEq (Na + K – Cl)/100 g of DM and 15 or 17% CP

Item	15% CP		17% CP		SEM	P-value		
	DCAD25	DCAD50	DCAD25	DCAD50		DCAD	CP	DCAD × CP
Essential AA, µg/mL								
Lys	25.84	27.52	23.57	24.13	1.47	NS	†	NS
Arg	29.17	24.92	27.12	22.80	2.52	NS	NS	NS
Met	33.72	35.19	32.65	33.94	1.83	NS	NS	NS
Phe	0.45	0.18	0.46	0.21	0.17	NS	NS	NS
Val	1.12	1.05	0.59	0.87	0.15	NS	*	NS
Trp	7.08	7.38	6.18	6.51	0.41	NS	†	NS
Thr	22.80	23.72	21.88	21.00	1.03	NS	NS	NS
Ile	0.01	0.01	0.00	0.03	0.00	†	NS	*
His	13.94	13.56	13.02	12.95	0.56	NS	NS	NS
Leu	13.63	12.05	11.63	12.81	0.74	NS	NS	†
Total essential AA, µg/mL	147.76	145.59	137.10	135.24	4.16	NS	*	NS
Total AA, µg/mL	403.20	412.61	392.28	391.71	15.24	NS	NS	NS
Essential AA:total AA	0.37	0.35	0.35	0.35	0.01	NS	NS	NS

† $P < 0.10$; * $P < 0.05$; NS: $P > 0.10$.

High DCAD resulted in greater fractional excretion of Cl and K, and reduced urinary Ca excretion (Table 5). Greater fractional excretion of Ca for low DCAD diets is consistent with earlier work (Wang and Beede, 1992; Roche et al., 2005). High DCAD, which included greater dietary K content, increased urinary K excretion (Table 5). West et al. (1992) reported greater urinary K excretion as DCAD increased, especially in treatments in which DCAD was raised using dietary K. Urinary excretion of Cl is related to the requirement for an anion to be excreted in conjunction with a cation, and would therefore be caused by the increased urinary K excretion. There was no effect of DCAD on BUN or urinary urea N excretion. Increased BUN in lactating cows was reported when DCAD was increased from –19.9 to 16.8 mEq (Na + K – Cl)/100 g of DM (Escobosa et al., 1984). However, no difference was observed when DCAD was further elevated to 32 mEq/100 g of DM. Differences in BUN observed by these authors might reflect a threshold level of DCAD above which hepatic ureagenesis is not improved by further altering blood pH.

A DCAD × CP interaction occurred for urinary uric acid:urinary creatinine ratio (Table 5). A much larger effect for DCAD was observed in CP15 diets compared with CP17 diets. Urinary uric acid excretion is an indicator of microbial protein flow to the duodenum (Johnson et al., 1998). Greater DMI for DCAD50 resulted in greater intake of CP, which could contribute to increased microbial CP synthesis. The absence of an increase in uric acid:urinary creatinine ratio for DCAD50 within CP17 diets, however, suggests that DCAD has a greater effect on the efficiency of ruminal protein metabolism. This increase in the efficiency of protein metabolism may be due to additional ruminal

buffering provided by the high DCAD fed in conjunction with high concentrate diets. No change was observed for microbial CP synthesis for DCAD50 within CP17 diets even though ruminal ammonia was more plentiful, as evidenced by greater BUN (Table 4) and urinary urea N (Table 5). Greater microbial protein yield was also likely to contribute to the improvement in milk protein percentage and yield observed in CP15-DCAD50 treatment combination (Table 3). No difference in uric acid:urinary creatinine ratio was observed between CP treatments. The large increase in uric acid excretion for DCAD50 within CP15 diets resulted in similar uric acid excretion between CP treatments.

Blood AA

With the exception of Ile, there were no effects of DCAD on serum concentrations of any individual essential AA, total essential AA, or total AA (Table 6). A DCAD and CP interaction was noted for Ile ($P < 0.05$) and Leu ($P < 0.10$). For CP15 treatments, DCAD50 resulted in decreased Leu concentration. Greater milk protein percentage for DCAD50 within CP15 diets may have decreased serum Leu concentration; however, there would be an expectation for a decrease in additional AA to Leu. Crude protein treatment effects were observed for Lys ($P < 0.10$), Val ($P < 0.05$), and Trp ($P < 0.10$), as well as for total essential AA ($P < 0.05$). For each of these AA, serum concentrations were lower for CP17 compared with CP15. It is unclear why these differences occurred, but a greater contribution by microbial growth to the total MP in CP15 diets compared with CP17 diets, particularly when fed with higher DCAD, may create differences in AA profile.

Table 7. Rumen VFA concentration and acetate:propionate ratio over 12 h postfeeding for cows fed diets formulated to contain a DCAD of 25 or 50 mEq (Na + K - Cl)/100 g of DM and 15 or 17% CP

Item	15% CP		17% CP		SEM	P-value		
	DCAD25	DCAD50	DCAD25	DCAD50		DCAD	CP	DCAD × CP
VFA, mol/100 mol								
Acetate	55.24	56.24	54.00	54.89	1.34	NS ¹	NS	NS
Propionate	30.98	29.16	31.07	29.95	1.42	NS	NS	NS
Butyrate	9.56	10.79	10.06	10.77	0.38	*	NS	NS
Isobutyrate	1.27	1.35	1.15	1.03	0.13	NS	NS	NS
Valerate	1.39	1.26	2.01	1.45	0.35	NS	NS	NS
Isovalerate	1.56	1.20	1.71	1.91	0.17	NS	NS	NS
Total VFA, mM	58.37	63.74	60.45	58.76	3.22	NS	NS	NS
Acetate:propionate ratio	1.82	2.03	1.81	1.87	0.14	NS	NS	NS

**P* < 0.05; NS: *P* > 0.10.

Rumen VFA

No DCAD or CP effects were observed for ruminal acetate, propionate, or branched-chain VFA molar proportion (Table 7). A numeric trend for greater acetate:propionate ratio and a significant increase in molar proportion of butyrate for DCAD suggests changes in fermentation patterns contributing to increased milk fat percentage and yield (Table 3), as suggested by Roche et al. (2005) in grazing dairy cows. These results are consistent with the work of Miettinen and Huhtanen (1996) in which higher milk fat yield was reported with higher butyrate and lower propionate concentrations, and is consistent with the greater milk fat yield noted for DCAD50 diets (Table 3). No interactions with main effects or time postfeeding were observed. Tucker et al. (1988) reported no difference in acetate, propionate, or butyrate concentration or for acetate:propionate ratio for DCAD. Both the low and high DCAD in the current study were greater than those used by Tucker et al. (1988).

CONCLUSIONS

Greater DMI and milk yield, as well as blood bicarbonate and pH for high DCAD, further solidifies the role of DCAD in altering acid-base chemistry and production in early-lactation dairy cows. Increased milk protein percentage suggests that increasing DCAD improves protein utilization, possibly through improved rumen conditions for microbial growth. Improved microbial CP synthesis as measured by urinary uric acid excretion for low dietary CP concentrations may offer the opportunity for more efficient N feeding. A better understanding of the interaction between DCAD and CP would allow us to reduce dietary protein recommendations without negatively affecting milk yield, thereby reducing the cost of milk production and the negative impact that dairying can have on the environment through N losses.

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