The effect of dietary cation-anion difference concentration and cation source on milk production and feed efficiency in lactating dairy cows

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ABSTRACT

Feed costs currently account for 55% or more of the total cost of milk production in US dairy herds, and dairy producers are looking for strategies to improve feed efficiency [FE; 3.5% fat-corrected milk (FCM) per dry matter (DM) intake]. Increasing dietary cation-anion difference [DCAD; $\text{Na} + \text{K} - \text{Cl}$ (mEq/kg of DM)] has been shown to increase milk production, FCM, and FE. However, the optimal DCAD concentration for maximal FE has yet to be determined. The objectives of this research were to test the effects of DCAD concentration and cation source on dairy FE. Sixty Holstein dairy cows (20 cows per experiment) were used in three 4×4 Latin square design experiments with 3-wk experimental periods. In experiments 1 and 2, we tested the effect of DCAD concentration: cows were fed a basal diet containing ~250 mEq/kg of DM DCAD that was supplemented with potassium carbonate at 0, 50, 100, and 150 mEq/kg of DM or 0, 125, 250, and 375 mEq/kg of DM in experiments 1 and 2, respectively. In experiment 3, we tested the effect of cation source: sodium sesquicarbonate replaced 0, 33, 67, and 100% of the supplemental potassium carbonate (150 mEq/kg of DM DCAD). The DCAD concentration had no effect on milk production, milk protein concentration, or milk protein yield in experiments 1 and 2. Dry matter intake was not affected by DCAD concentration in experiment 1 or by cation source in experiment 3. However, DMI increased linearly with increasing DCAD in experiment 2. We detected a linear increase in milk fat concentration and yield with increasing DCAD in experiments 1 and 2 and by substituting sodium sesquicarbonate for potassium carbonate in experiment 3. Increased milk fat concentration with increasing DCAD led to increases in 3.5% FCM in experiments 1 and 2. Maximal dairy FE was achieved at a DCAD concentration of 426 mEq/kg of DM in experiments 1 and 2 and by substituting Na for K in experiment 3. The results of these experiments suggest that both DCAD concentration and the cation

source used to alter DCAD concentration have effects on milk fat content and yield and dairy FE.

Key words: dietary cation-anion difference, feed efficiency, dairy cow

INTRODUCTION

Dairy feed costs are the largest single expense associated with producing milk (Wolf, 2010; Buza et al., 2014) and have accounted for 55% or more of the total cost of milk production in US dairy herds over the last 4 yr (USDA-NASS, 2014). The use of corn for ethanol production coupled with the severe drought during the 2012 growing season nearly doubled purchased feed costs to dairy and livestock producers (USDA-ERS, 2013). Thus, there is renewed interest among dairy producers in feeding strategies that improve feed utilization and increase milk output per unit feed input.

Erdman et al. (2011) and Harrison et al. (2012) demonstrated improved FE in lactating dairy cows by increasing DCAD ($\text{Na} + \text{K} - \text{Cl}$) using potassium carbonate (K_2CO_3) and potassium carbonate sesquihydrate ($\text{K}_2\text{CO}_3 \cdot 1.5\text{H}_2\text{O}$) supplementation, respectively. Dairy feed efficiency (FE) measured as 3.5% FCM/DMI was improved by 7.7% (0.14 units) when the DCAD concentration increased from 251 to 336 mEq/kg of DM using K_2CO_3 supplementation (Erdman et al., 2011). Similarly, Harrison et al. (2012) reported a 6.7% (0.11 unit) increase in FE by increasing DCAD from 463 and 665 mEq/kg of DM using $\text{K}_2\text{CO}_3 \cdot 1.5 \text{H}_2\text{O}$. Erdman et al. (2011) reported that K addition reduced feed costs by approximately \$1/cow per day—a \$365,000 reduction for a 1,000-cow dairy. Other work has shown that increasing DCAD improved acid-base balance, which resulted in increased DMI, milk yield, and milk fat yield (Sanchez and Beede, 1996; Hu and Murphy, 2004; Hu et al., 2007b).

Although Erdman et al. (2011) and Harrison et al. (2012) illustrated the potential for K supplementation to increase dairy FE, the optimal DCAD concentration for maximal FE has not been determined. The NRC (2001) nutrient requirements for a 680-kg dairy cow producing 45 kg/d milk with 3.5% fat suggest minimal dietary concentrations of 1.06, 0.22, and 0.28% for K, Na, and Cl, respectively. This is equivalent to a DCAD

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concentration of 296 mEq/kg of DM using the DCAD equation $\text{DCAD (mEq/kg of DM)} = (\text{Na} + \text{K} - \text{Cl})$. In a meta-analysis, Hu and Murphy (2004) reported that milk yield was greatest when the DCAD ($\text{Na} + \text{K} - \text{Cl}$) concentration was 340 mEq/kg of DM, 4.0% FCM production was highest at 490 mEq/kg of DM DCAD, and DMI was maximized at 400 mEq/kg of DM DCAD. Sanchez and Beede (1996) suggested that both milk yield and DMI were maximized at a DCAD ($\text{Na} + \text{K} - \text{Cl}$) concentration equal to 380 mEq/kg of DM. Although several studies have investigated production responses to various DCAD concentrations, the optimal DCAD concentration for maximal dairy FE is unknown. Therefore, the objective of experiments 1 and 2 was to determine the optimal DCAD concentration required to maximize FE.

In most lactating dairy cow diets, K is the predominant cation because of the high concentrations of K in forages (NRC, 2001). However, the strong ion theory upon which the DCAD principle of diet formulation is based suggests that Na or K would be equally effective. Some previous research suggested that milk yield and milk composition were not affected by cation source and that the most important influence on production responses is the overall DCAD concentration (Tucker et al., 1988a; West et al., 1992; Hu and Kung, 2009). However, other studies have reported that there may be significant interactions affecting the milk yield and DMI response to DCAD with different ratios of Na:K in the diet (Sanchez et al., 1994, 1997). Increasing dietary DCAD using K supplements such as K_2CO_3 is typically 3 to 4 times more expensive (per kilogram basis) than Na supplementation using sodium bicarbonate (NaHCO_3). Thus, in addition to knowing the optimal DCAD concentration for maximal performance and FE, the relative effectiveness of Na versus K as strong ion sources is economically important. Therefore, the objective of experiment 3 was to compare the relative effectiveness of Na and K as cation sources when used to increase DCAD.

MATERIALS AND METHODS

Research Facilities and Animals

The experiments conducted in this report were approved by the University of Maryland Animal Care and Use Committee. Three 4×4 Latin square design experiments were conducted using 60 Holstein dairy cows (20 cows; 8 primiparous and 12 multiparous per experiment). At the start of the experiments, cows produced 39.9 ± 1.6 , 39.8 ± 1.9 , and 41.4 ± 1.4 kg/d of milk, and were 89 ± 25 , 95 ± 75 , and 95 ± 25 DIM in experiments 1, 2, and 3, respectively.

Cows were housed and individually fed in tie-stalls fitted with water mattresses (Ryder Supply Company, Chambersburg, PA) that were bedded with wood shavings. Lighting in the research barn was controlled such that the cows received a minimum of 12 h/d of light during each study. Cows had continuous access to water via shared drinking cups in their tie stalls. Cows were milked twice daily at approximately 0630 and 1600 h. Experiments 1 and 2 were conducted from late-January to mid-April (2012 and 2013, respectively), and experiment 3 was conducted from mid-May to late-July 2012.

Experimental Diets and Feeding

Three basal TMR were formulated to meet or exceed the NRC (2001) nutrient requirements for dairy cows producing 40 kg/d milk containing 3.7% fat and 3.1% protein (Table 1). The basal TMR contained 60, 65, and 69% forage (DM basis) and 40, 35, and 32% concentrate in experiments 1, 2, and 3, respectively. In experiments 1 and 3, forage consisted of only corn silage; however, in experiment 2, forage consisted of 62 to 64% corn silage and 6% alfalfa hay. In all experiments, the concentrate portion comprised ground shelled corn and soybean meal (48% CP, as-fed basis) with the remainder of the ingredients (Table 1), including corn gluten meal, macro and trace mineral supplements, rumen-protected fat (Megalac, Arm and Hammer Animal Nutrition, Piscataway, NJ), and feed additives (Table 1), combined and added as a premix in the TMR.

In experiment 1, treatments consisted of a basal diet formulated to contain approximately 250 mEq/kg of DM DCAD (Table 1) or the basal diet plus 50, 100, and 150 mEq/kg of DM added DCAD using supplemental feed-grade K_2CO_3 (DCAD Plus, Church & Dwight Co. Inc., Piscataway, NJ) that resulted in final estimated DCAD of approximately 250, 300, 350, and 400 mEq/kg of DM (Table 1). The DCAD concentrations selected for experiment 1 were designed to be below and above the 296 mEq/kg suggested by the NRC (2001) and to reflect DCAD concentrations found in typical lactating dairy cow rations as well as DCAD concentrations that have been shown in other studies to optimize response variables such as milk yield and DMI (Sanchez and Beede, 1996; Hu and Murphy, 2004).

Similarly, in experiment 2, treatments consisted of a basal diet containing approximately 250 mEq/kg of DM DCAD (Table 2) or the basal diet plus 125, 250, and 375 mEq/kg of DM added DCAD using supplemental K_2CO_3 , which resulted in final estimated DCAD of approximately 250, 375, 500, and 625 mEq/kg of DM (Table 1). The DCAD concentrations chosen for experiment 2 were selected to provide larger incremental increases in DCAD compared with experiment 1.

Table 1. Ingredient composition of experimental diets (DM basis)

Ingredient	Experiment 1				Experiment 2				Experiment 3			
	Added DCAD (mEq/kg of DM) ¹				Added DCAD (mEq/kg of DM) ¹				Supplemental DCAD K:Na ²			
	0	50	100	150	0	125	250	375	100:0	67:33	33:67	0:100
Corn silage	59.71	59.71	59.71	59.71	63.80	63.30	62.70	62.20	65.00	65.00	65.00	65.00
Alfalfa hay	0.00	0.00	0.00	0.00	6.00	5.90	5.90	5.80	0.00	0.00	0.00	0.00
Ground corn	17.70	17.30	16.89	16.49	10.10	10.00	9.90	9.80	11.50	11.44	11.37	11.31
Soybean meal, 48%	18.63	18.63	18.63	18.63	16.40	16.20	16.00	15.80	18.63	18.63	18.63	18.63
Potassium carbonate ³	0.00	0.40	0.81	1.21	0.00	0.82	1.64	2.46	0.91	0.60	0.30	0.00
Sodium sesquicarbonate ⁴	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.74	1.10
Corn gluten meal, 60%	0.74	0.74	0.74	0.74	0.50	0.50	0.50	0.50	0.74	0.74	0.74	0.74
Limestone ⁵	0.79	0.79	0.79	0.79	0.52	0.52	0.52	0.52	0.79	0.79	0.79	0.79
Biophos ⁶	0.10	0.10	0.10	0.10	0.37	0.37	0.37	0.37	0.10	0.10	0.10	0.10
Magnesium oxide	0.00	0.00	0.00	0.00	0.12	0.12	0.12	0.12	0.00	0.00	0.00	0.00
Dynamate ⁷	0.08	0.08	0.08	0.08	0.13	0.13	0.13	0.13	0.08	0.08	0.08	0.08
Salt-white	0.50	0.50	0.50	0.50	0.40	0.40	0.40	0.40	0.50	0.50	0.50	0.50
TM-433 ⁸	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02
4-Plex ⁹	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02
ADE Mix ¹⁰	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Vitamin E ¹¹	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01
Selenium ¹²	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.06	0.05	0.05	0.05	0.05
Megalac ¹³	1.41	1.41	1.41	1.41	1.40	1.40	1.40	1.40	1.41	1.41	1.41	1.41
Omigen-AF ¹⁴	0.17	0.17	0.17	0.17	0.20	0.20	0.20	0.20	0.17	0.17	0.17	0.17
Rumensin-22 g/kg ¹⁵	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.05

¹Supplemental DCAD (mEq/kg of DM) calculated as follows: DCAD = Na + K – Cl.

²Each treatment diet was supplemented with 150 mEq/kg of DM with varying ratios of supplemental K:Na (mEq/kg of DM) using potassium carbonate sesquihydrate and sodium sesquicarbonate as cation sources.

Each treatment contained an estimated added DCAD (Na + K – Cl) of 150 mEq/kg of DM.

³Contained 56% K and 88% DM (Church & Dwight Co. Inc., Piscataway, NJ).

⁴Contained 27% Na (Church & Dwight Co. Inc.).

⁵Contained 36% Ca and 0.02%P.

⁶Contained 17% Ca and 21% P.

⁷Contained 11.5% Mg, 18% K, and 22.5% S (Mosaic Co., Plymouth, MN).

⁸Contained 0.16% Co, 4.0% Cu, 3.0% Fe, 0.35% I, 15% Mn, and 16% Zn (Southern States Cooperative, Inc., Richmond, VA).

⁹Contained 0.20% Co, 0.99% Cu, 0.031% Fe, 1.57% Mn, and 2.83% Zn (Southern States Cooperative Inc.).

¹⁰Contained 5,454,545 IU/kg of vitamin A, 1,818,182 IU/kg of vitamin D, 9,091 IU/kg of vitamin E.

¹¹Contained 56,818 IU/kg of vitamin E.

¹²Contained 0.3 IU/g of selenium, 28% Ca.

¹³Contained 9% Ca; 84.5% fat (Church & Dwight Co. Inc.).

¹⁴Contained (per kilogram) 0.41 mg of biotin, 15 mg of choline, 31 mg of D-pantothenic acid, 1.4 mg of folic acid, 3.2 mg of menadione, 102 mg of niacin, 30 mg of riboflavin, 4.5 × 10¹⁰ cfu of *Saccharomyces cerevisiae*, 15.5 mg of thiamine, 8.2 mg of vitamin B₆, and 41 µg of vitamin B₁₂ (Prince Agri Products Inc., Quincy IL).

¹⁵Contained 20% monensin Na, 1% mineral oil, and carriers such as rice hulls, limestone, and fermentation nutrients (Elanco, Greenfield, IN).

In experiment 3, treatments consisted of a basal diet and the addition of 150 mEq/kg of DM DCAD using 4 different ratios of added K and Na (100:0, 66.7:33.3, 33.3:66.7, and 0:100; mEq/kg of DM) using K_2CO_3 and sodium sesquicarbonate (SQ-810, Church & Dwight Inc.) as the K and Na cation sources, respectively. Each treatment combination resulted in a final estimated DCAD of approximately 400 mEq/kg of DM (Table 1). In all experiments, treatments were applied in a 4×4 Latin square design balanced for carryover effects with 3-wk experimental periods. Before feeding, the basal TMR for the cows was mixed in portable mixer wagon. The treatment supplements were then added to the basal TMR and mixed in a Calan Data Ranger (American Calan, Northwood, NH) for the cows within each treatment group before delivery to individual feed tubs. Amounts of feed offered and feed refusals were recorded once daily at 0930 h.

Measurements

Measurements included weekly individual cow BW and daily feed intake and feed refusals. Silage and concentrate samples were taken weekly for DM analysis to adjust the as-fed TMR to maintain a constant forage-to-concentrate ratio and to measure feed DM such that daily DMI could be calculated for each cow. Milk production was recorded electronically at each milking at 0630 and 1600 h. Milk samples were collected during the last 4 consecutive milkings of each experimental period (d 20 and 21) and analyzed for fat, protein, other solids (OS; lactose plus minerals), and SCC in all experiments (Lancaster Dairy Herd Information Association, Manheim, PA). Milk MUN was measured only in experiments 2 and 3 (Lancaster Dairy Herd Information Association).

Individual samples of the corn silage, ground corn, soybean meal, vitamin premix, and the treatment mixes were collected weekly and composited by experimental period for analysis of diet DM, CP, ADF, NDF, lignin, ether extract, Ca, P, Mg, Na, K, Cl, and S at a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD). Actual DCAD was calculated based on the K, Na, and Cl concentrations of the individual feeds or mixtures weighted proportionally to their contribution to the diet DM in the TMR.

Statistical Model

Mean data for DMI, milk production, fat and protein percentages, SCC, MUN, fat and protein yields, 3.5% FCM, and FE for each cow from the last week of each experimental period were used in the statistical analyses. Data from each experiment were analyzed using

PROC MIXED in SAS (version 9.3; SAS Institute Inc., Cary, NC) with the statistical model

$$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk},$$

where Y_{ijk} = the response from the i th cow, the j th period, and the k th treatment; μ = the grand mean; C_i = the effect of the i th cow; P_j = the effect of the j th period; T_k = the effect of the k th treatment level; and e_{ijk} = random error.

Treatment was analyzed as a fixed effect, whereas cow and period were analyzed as random effects. In experiments 1 and 2, the treatments were designed to provide 50 mEq/kg of DM and 125 mEq/kg of DM increments in DCAD, respectively, such that the dose response to DCAD concentration was tested. In experiment 3, the treatments were designed to provide equidistant substitution of Na for K at a constant rate of supplemental DCAD such that the production responses to K versus Na could be tested. Therefore, both linear and quadratic orthogonal contrasts were tested in all 3 experiments. In all studies, a probability of $P \leq 0.05$ was considered statistically significant, whereas probabilities of $P \leq 0.10$ were considered trends.

To determine the dose response to DCAD, treatment means for FCM, milk fat yield, milk yield, and FE from experiments 1 and 2 were pooled and fitted by quadratic regression. To account for differences in production and FE between experiments 1 and 2, individual study effects were quantified as random effects using PROC MIXED (version 9.3; SAS Institute Inc.), and study-adjusted means were calculated at each DCAD concentration. Using the study-adjusted values, quadratic regression analysis was conducted using PROC REG (version 9.3; SAS Institute Inc.) to determine the dose response to DCAD concentrations for each variable.

RESULTS

Experiments 1 and 2: Optimal DCAD Concentration

The ingredient and chemical composition (DM basis) of the dietary treatments from experiment 1 and 2 are presented in Tables 1 and 2, respectively. As expected, diets within each study were similar in chemical composition except for K and DCAD. Dietary K increased linearly from 1.30 to 1.79% and from 1.29 to 2.64% in experiments 1 and 2, respectively. Measured DCAD concentrations in the treatment diets were 277, 319, 368, and 406 mEq/kg of DM in experiment 1 and 257, 360, 488, and 603 mEq/kg of DM in experiment 2. The range in measured DCAD concentrations among treatments was 129 mEq/kg of DM in experiment 1 and 346 mEq/kg of DM in experiment 2, being somewhat lower

Table 2. Chemical composition of experimental diets (DM basis)

Item	Experiment 1					Experiment 2					Experiment 3				
	Added DCAD (mEq/kg of DM) ¹					Added DCAD (mEq/kg of DM) ¹					Supplemental DCAD K:Na ²				
	0	50	100	150	SEM	0	125	250	375	SEM	100:0	67:33	33:67	0:100	SEM
DM (%)	47.9	47.9	47.9	47.9	0.004	53.7	54	54.2	54.5	0.182	52.9	52.8	52.7	52.7	0.050
NE _L (Mcal/kg)	1.74	1.73	1.72	1.72	0.003	0.73	0.73	0.73	0.73	0.083	0.77	0.77	0.77	0.77	— ³
CP (%)	16.3	16.3	16.2	16.2	0.019	15.7	15.6	15.4	15.3	0.113	15.9	15.9	15.9	15.9	0.003
NDF (%)	26.7	26.7	26.6	26.6	0.025	33.6	33.4	33.1	32.8	0.178	27.1	27.1	27.1	27.1	0.003
ADF (%)	15.3	15.3	15.3	15.3	0.006	21.3	21.1	20.9	20.8	0.018	17	17	17	17	0.003
Lignin (%)	2.29	2.28	2.27	2.26	0.006	3.45	3.42	3.39	3.36	0.015	2.37	2.37	2.37	2.37	—
Ash (%)	5.53	5.82	6.32	6.47	0.218	6.01	5.96	5.91	5.86	0.032	7.13	7.12	7.21	7.23	0.028
Fat ⁴ (%)	2.3	2.27	2.25	2.23	0.015	2.84	2.81	2.79	2.77	0.005	0.02	0.02	0.02	0.02	—
Na (%)	0.24	0.24	0.25	0.25	0.003	0.17	0.17	0.17	0.17	0.002	0.26	0.39	0.57	0.71	0.099
K (%)	1.30	1.46	1.64	1.79	0.107	1.29	1.69	2.19	2.64	0.002	1.79	1.57	1.39	1.19	0.128
Cl (%)	0.57	0.57	0.57	0.57	—	0.53	0.52	0.52	0.51	0.293	0.55	0.55	0.55	0.55	—
S (%)	0.18	0.18	0.18	0.18	—	0.2	0.19	0.19	0.19	0.001	0.18	0.18	0.18	0.18	—
Ca (%)	0.75	0.75	0.75	0.75	—	0.86	0.86	0.85	0.84	0.001	0.7	0.7	0.7	0.7	—
P (%)	0.38	0.38	0.38	0.38	—	0.43	0.43	0.43	0.42	0.003	0.38	0.38	0.38	0.38	—
Mg (%)	0.2	0.2	0.19	0.19	0.003	0.29	0.29	0.29	0.29	—	0.18	0.18	0.18	0.18	—
DCAD (mEq/kg of DM) ⁵	277	319	368	406	13.0	257	360	488	603	74.5	416	416	448	458	5.17
DCAD-S (mEq/kg of DM) ⁶	164	205	255	293	13.0	196	312	428	544	74.8	360	360	392	402	5.14

¹Supplemental DCAD from potassium carbonate calculated as DCAD = Na + K – Cl (mEq/kg of DM).

²Each treatment diet was supplemented with 150 mEq/kg of DM with varying ratios of supplemental K:Na (mEq/kg of DM) using potassium carbonate sesquihydrate and sodium sesquicarbonate as cation sources.

³Dash indicates SEM <0.001.

⁴Measured as crude fat, which would not include the 1.19% fatty acids from Megalac (Arm and Hammer Animal Nutrition, Piscataway, NJ).

⁵Dietary Na + K – Cl.

⁶Dietary Na + K – Cl – S.

than the expected ranges of 150 and 375 mEq/kg of DM, respectively.

The DCAD concentration had no effect ($P > 0.05$) on BW, milk production, or milk protein yield (Table 3). However, we observed a trend ($P = 0.067$) for reduced milk protein percentage with increased DCAD in experiment 1. Dry matter intake was not affected by DCAD concentration in experiment 1. However, in experiment 2, DMI increased linearly in response to increasing DCAD ($P < 0.05$). In addition, increasing DCAD in experiment 2 resulted in a significant decrease in MUN ($P = 0.001$). The DCAD had no effect on OS percentage, OS yield, or SCC ($P > 0.05$).

In experiments 1 and 2, milk fat percentage increased linearly with increasing DCAD ($P < 0.05$) such that fat percentage was highest (2.86 and 3.62%, respectively) in cows fed the highest DCAD concentrations in each experiment (406 and 603 mEq/kg of DM, respectively). Milk fat yield increased linearly in response to increased DCAD ($P < 0.05$) in experiment 1, in which fat yield was highest (1,108 g/d) at a measured DCAD concentration of 406 mEq/kg of DM. In experiment 2, fat yield was both linearly ($P < 0.001$) and quadratically ($P = 0.048$) altered by DCAD such that the maximal fat yield (1,379 g/d) occurred at a measured DCAD of 603 mEq/kg of DM.

Yields of 3.5% FCM linearly increased ($P < 0.05$) with increasing DCAD in both experiments. However, a quadratic effect of DCAD on 3.5% FCM was observed in experiment 2 ($P < 0.05$), in which 3.5% FCM reached a plateau at a DCAD concentration of 360 mEq/kg of DM. In experiment 1, the increase in 3.5% FCM at a constant DMI resulted in a linear increase in FE ($P = 0.042$), with the greatest FE shown in the 406 mEq/kg of DM treatment. However, the DCAD concentration required for maximal FE could not be determined from experiment 1 because the highest treatment DCAD concentration yielded the maximum FE. In experiment 2, DMI increased linearly with DCAD but 3.5% FCM plateaued at 360 mEq/kg of DM, which resulted in a FE that was greatest at DCAD concentrations of 360 and 488 mEq/kg of DM, but the differences in FE only trended toward significance (quadratic, $P = 0.085$).

Experiment 3: Cation Source

The ingredient and chemical composition of the dietary treatments in experiment 3 are presented in Tables 1 and 2, respectively. As expected, diets were similar in chemical composition (Table 2) except for Na and K. Calculated treatment DCAD concentrations (using the Na + K - Cl equation) were 417, 418, 447, and 457 mEq/kg of DM for the 100:0, 67:33, 33:67, and 0:100 K:Na treatments. Although the diets were ex-

Table 3. Effects of DCAD concentration on feed intake, milk production, milk composition, and dairy feed efficiency (3.5% FCM/DMI)

Item	Experiment 1					Experiment 2							Probability	
	Added DCAD (mEq/kg of DM)					Added DCAD (mEq/kg of DM)								
	0	50	100	150	SEM	Lin ¹	Quad ²	0	125	250	375	SEM	Lin ¹	Quad ²
Observations (no.)	19	20	20	20				20	20	20	20			
BW (kg)	615	610	614	607	18.5	0.208	0.876	636	635	638	630	9.2	0.194	0.205
DMI (kg/d)	22	22.3	22.6	22.3	0.46	0.202	0.209	22.3	22.9	23.1	23.6	0.52	0.007	0.902
Milk (kg/d)	39.4	39	39.6	39.3	1.28	0.937	0.979	38.9	39.5	39.5	38.8	1.52	0.937	0.228
3.5% FCM (kg/d)	33.2	33.9	34.5	34.9	1.16	0.048	0.836	37.1	39.2	39.2	39.1	0.98	0.008	0.037
Fat (%)	2.59	2.77	2.72	2.86	0.18	0.025	0.758	3.28	3.50	3.50	3.62	0.15	0.001	0.418
Fat yield (g/d)	996	1,050	1,070	1,108	63.1	0.015	0.802	1,252	1,362	1,366	1,379	39.0	0.001	0.048
Protein (%)	3.05	3.03	3.02	2.99	0.06	0.067	0.729	2.95	2.99	2.95	2.92	0.06	0.167	0.152
Protein yield (g/d)	1,192	1,177	1,191	1,167	33.3	0.449	0.813	1,143	1,174	1,158	1,124	35.5	0.395	0.101
OS ³ (%)	5.64	5.65	5.67	5.65	0.06	0.685	0.650	5.71	5.72	5.77	5.71	0.05	0.666	0.299
OS yield (g/d)	2,220	2,202	2,244	2,217	77.9	0.829	0.907	2,216	2,256	2,271	2,211	83.1	0.993	0.181
SCC (linear score)	4.06	3.62	3.73	3.79	0.94	0.753	0.627	2.8	2.8	2.19	2.69	0.78	0.702	0.647
MUN ⁴ (mg/dL)	—	—	—	—	—	—	—	15.5	14.0	13.6	12.0	0.36	0.001	0.959
3.5% FCM/DMI	1.52	1.53	1.53	1.58	0.04	0.042	0.334	1.67	1.71	1.71	1.66	0.04	0.759	0.085

¹Linear orthogonal contrasts.

²Quadratic orthogonal contrasts.

³Other solids (lactose plus minerals).

⁴Milk was not analyzed for MUN during experiment 1.

Table 4. Relative effectiveness of cation source on feed intake, milk production, milk composition, and dairy feed efficiency (3.5% FCM/DMI) in experiment 3¹

Item	K:Na ratio of supplement				SEM	Probability	
	100:0	67:33	33:67	0:100		Lin ²	Quad ³
Observations (no.)	20	20	20	20			
BW (kg)	644	636	639	642	12.0	0.848	0.163
DMI (kg/d)	22.3	22.3	22.1	22.0	0.46	0.598	0.851
Milk (kg/d)	37.5	37.3	36.3	37.9	1.28	0.903	0.219
3.5% FCM (kg/d)	34.6	35.2	34.3	36.7	1.06	0.132	0.262
Fat (%)	3.06	3.20	3.20	3.36	0.17	0.005	0.885
Fat yield (g/d)	1,132	1,173	1,144	1,250	49.1	0.041	0.354
Protein (%)	2.99	2.99	3.01	3.07	0.07	0.181	0.476
Protein yield (g/d)	1,114	1,106	1,086	1,156	33.9	0.332	0.114
OS ⁴ (%)	5.74	5.73	5.71	5.67	0.04	0.092	0.505
OS yield (g/d)	2,147	2,141	2,077	2,151	74.2	0.786	0.349
SCC (linear score)	4.63	5.57	4.95	4.79	0.98	0.960	0.430
MUN (mg/dL)	15.3	14.9	15.6	14.8	0.38	0.518	0.495
3.5% FCM/DMI	1.56	1.58	1.55	1.67	0.04	0.036	0.125

¹Each treatment diet was supplemented with 150 mEq/kg of DM with varying ratios of supplemental K:Na (mEq/kg of DM) using potassium carbonate and sodium sesquicarbonate as cation sources.

²Linear orthogonal contrasts.

³Quadratic orthogonal contrasts.

⁴Other solids (lactose plus minerals).

pected to have equal DCAD concentrations, the 33:67 and 0:100 K:Na treatments had 32 and 46 mEq/kg of DM greater DCAD concentrations than the 100:0 and 67:33 K:Na treatments, respectively. This was due to a greater than expected measured Na content in these treatments, in which the expected increment in diet Na was 0.115 and 0.23% (+50 and 100 mEq/kg of DM Na) compared with the measured increments of 0.18 and 0.32%, which increased the DCAD from Na by 78 and 139 mEq/kg of DM, respectively. As shown in experiment 1, increasing DCAD by 50 mEq/kg of DM resulted in an average FE increase of only 0.02 units; thus, the 46 mEq/kg of DM DCAD difference between the highest and lowest DCAD treatments would not be large enough to cause a 0.11-unit change in FE, which was observed in this study.

Treatment had no effect ($P > 0.05$) on DMI, BW, or milk production (Table 4). As for milk composition, treatment had no effect on milk protein yield, protein percentage, OS yield, OS percentage, MUN, or SCC ($P > 0.05$). However, milk fat percentage and fat yield increased linearly with increased Na supplementation ($P < 0.05$), where fat percentage and fat yield were greatest (3.36% and 1,156 g/d) in cows fed the 0:100 K:Na treatment (in which Na was the sole supplemental cation). Despite a significant effect on milk fat percentage and yield, cation source did not affect 3.5% FCM ($P = 0.132$ and 0.262 for linear and quadratic effects, respectively). Although we detected no change in DMI or 3.5% FCM individually, FE (FCM/DMI) increased linearly ($P = 0.036$) with increasing Na.

DISCUSSION

Previous studies have reported that DMI increases linearly in response to increasing DCAD concentrations using cation sources such as disodium phosphate, sodium bicarbonate, sodium carbonate, and K_2CO_3 (Delaquis and Block, 1995; Wildman et al., 2007b; Apper-Bossard et al., 2010). The results from experiment 2 confirm those results as DMI increased linearly from 22.3 to 23.6 kg as DCAD increased from 257 to 603 mEq/kg of DM. However, DMI was not affected by increased DCAD concentrations during experiment 1. It is possible that the DCAD effect on DMI observed in experiment 2 occurred as a result of the larger increments in treatment DCAD concentrations compared with experiment 1 (125 and 50 mEq/kg of DM, respectively). In experiment 3, cation source did not significantly affect DMI. This result supports several other studies that showed that DMI is not affected by K:Na ratios (O'Connor et al., 1988; West et al., 1992; Wildman et al., 2007a). However, with the exception of the work of Wildman et al. (2007a) and Hu and Kung (2009), dietary DCAD was not held constant in previous experiments such that DMI effects of K, Na, and DCAD were confounded.

Previous work has shown that increasing DCAD concentration significantly increased milk production in lactating dairy cows (Sanchez and Beede, 1996; Tucker et al., 1988a; Hu and Murphy, 2004). However, DCAD had no effect on milk yield in experiments 1 or 2. It is quite possible that the DCAD effect on milk production

seen in other studies occurred because of larger increments in treatment DCAD concentrations (Delaquis and Block, 1995; Roche et al., 2005). For example, Wildman et al. (2007a) reported that increased DCAD (Na + K - Cl) improved milk yield; however, the increment in DCAD between the 2 dietary treatments was 250 mEq/kg of DM. In a meta-analysis conducted by Hu and Murphy (2004), the authors reported a significant effect of DCAD on milk production, but the experimental DCAD concentrations ranged from -191 to 636 mEq/kg of DM, with the majority of the milk production responses being the result of treatment means from cows fed very low DCAD (≤ 100 mEq/kg of DM) diets, which would be atypical of diets fed on commercial dairies. In the current studies, treatment increments were only 50 and 125 mEq/kg of DM DCAD with the range in measured DCAD of 129 and 346 mEq/kg of DM in experiments 1 and 2, respectively. It is difficult to compare the results from different DCAD experiments because of the lack of similarity between DCAD concentration ranges, cation sources, basal diets, and experimental animals (e.g., parity, stage of lactation, breed).

Although DCAD concentration did not affect milk yield in experiment 1 or 2, DCAD has been shown to alter milk yield in previous studies, so a regression analysis was conducted on the study-adjusted milk yields from experiments 1 and 2 to determine which DCAD concentration maximized milk yield (Sanchez and Beede, 1996; Hu and Murphy, 2004). The results of the regression analysis suggest that milk yield was greatest (39.51 kg/d) at a DCAD of 415 mEq/kg of DM ($P = 0.0444$; $R^2 = 0.6173$; Figure 1). The results observed in this regression analysis are similar to those reported by Sanchez and Beede (1996), which suggested that a DCAD concentration of 380 mEq/kg of DM maximized milk yield.

In experiment 3, cation source had no effect on milk yield, which was similar to results that have been reported in other experiments (West et al., 1992; Sanchez et al., 1997; Hu and Kung, 2009). Wildman et al. (2007a) reported a quadratic effect of K:Na ratio on milk production. At an average DCAD of 410 mEq/kg, milk production was highest when the K:Na ratio was 4:1 (Wildman et al., 2007a). Unlike the study by Wildman et al. (2007a), which included high K:Na ratios (millequivalent basis) of 2:1, 3:1, and 4:1, the present study included supplemental K:Na ratios of only 1:0, 2:1, 1:2, and 0:1.0. Perhaps a cation source effect on milk production is visible only when the extreme K:Na or Na:K ratios are tested. It has been suggested the overall DCAD concentration is more important than individual ion concentrations in altering milk production responses (Tucker et al., 1988a; West et al., 1992).

Milk fat percentage and yield (g/d) showed the greatest responses to DCAD and increased linearly with increasing DCAD concentration. In experiment 1, the average milk fat percentage and yield increased by 0.27 percentage units and 112 g/d, respectively, as DCAD increased from 277 to 406 mEq/kg of DM. In experiment 1, the mean milk fat content across treatments was only 2.74%. This may have been due to the low NDF content of the corn silage being fed that resulted in total diet NDF of only 26.7%. Although dietary NDF percentage was low during experiment 1, the effects of DCAD on milk fat percentage and milk fat yield were similar between experiments 1 and 2. For example, milk fat percentage increased 0.22 percentage units with the first increment in supplemental DCAD (103 mEq/kg). Similarly, milk fat yield increased by 112 g/d in experiment 1 (129 mEq/kg of DM DCAD increase) and 110 g/d in experiment 2 with the first increment in supplemental DCAD (103 mEq/kg of DM). Therefore, the results of experiment 1 and experiment 2 are consistent with each other despite the low dietary NDF percentage of the diets fed in experiment 1.

In experiment 2, milk fat percentage and yield increased by 0.34 percentage units and 127 g/d, respectively, as DCAD increased from 257 to 603 mEq/kg of DM. However, the majority of the response came with the first increment in DCAD using dietary K as the supplemental cation source at 368 mEq/kg of DM. Regression analysis using study-adjusted milk fat yields from experiments 1 and 2 suggests that predicted milk fat yield was greatest (1,391 g/d) at a DCAD of 509 mEq/kg of DM ($P = 0.0035$; $R^2 = 0.9262$; Figure 2).

Figure 1. Quadratic regression equations for study-adjusted mean milk yield and feed efficiency (3.5% FCM/DMI) in experiments 1 and 2. For milk yield (Δ ; kg/d), $Y = -0.00001933 \times \text{DCAD}^2 + 0.0160 \times \text{DCAD} + 36.18$ ($P = 0.0444$; $R^2 = 0.6173$; $n = 8$); maximum milk yield occurred at a DCAD of 415 mEq/kg of DM. For feed efficiency ($\square$), $Y = -0.00000183 \times \text{DCAD}^2 + 0.00156 \times \text{DCAD} + 1.387$ ($P = 0.0102$; $R^2 = 0.7670$; $n = 8$); maximum feed efficiency occurred at a DCAD of 426 mEq/kg of DM.

Figure 2. Quadratic regression equations for study-adjusted mean FCM and milk fat yields in experiments 1 and 2. For FCM yield (Δ ; kg/d), $Y = -0.00004359 \times \text{DCAD}^2 + 0.0425 \times \text{DCAD} + 29.17$ ($P = 0.0011$; $R^2 = 0.9409$; $n = 8$); maximum FCM occurred at a DCAD of 488 mEq/kg of DM. For milk fat yield ($\square$; g/d), $Y = -0.00214478 \times \text{DCAD}^2 + 2.1817 \times \text{DCAD} + 835.8$ ($P = 0.0035$; $R^2 = 0.9262$; $n = 8$); maximum fat yield occurred at a DCAD of 509 mEq/kg of DM.

This result is consistent with the meta-analysis results published by Hu and Murphy (2004), which suggested that a DCAD concentration of 550 mEq/kg of DM would maximize milk fat yield.

Because of the increase in milk fat percentage, 3.5% FCM also increased linearly in response to increasing DCAD concentration in both experiments 1 and 2. Regression analysis using the study-adjusted FCM values from experiments 1 and 2 suggest that 3.5% FCM was greatest (39.6 kg/d) at a DCAD of 488 mEq/kg of DM ($P = 0.0011$; $R^2 = 0.9409$; Figure 2). In their meta-analysis, Hu and Murphy (2004) suggested that the DCAD concentration required to maximize 4.0% FCM was 490 mEq/kg of DM DCAD. Thus, the results from the current regression analysis support the previous suggestion that a DCAD of approximately 488 mEq/kg of DM will maximize either 3.5% or 4.0% FCM (Hu and Murphy, 2004).

In experiment 3, both milk fat percentage and fat yield (g/d) increased linearly when Na was substituted for K as the supplemental cation source. Fat percentage and yield increased by 0.30 percentage units and 118 g/d, respectively, by increasing dietary Na from 0.26 to 0.71% and reducing dietary K from 1.79 to 1.19%. However, measured DCAD ranged from 416 to 458 mEq/kg of DM as dietary Na increased and dietary K decreased, suggesting that part of the cation source response may have been due to a change in DCAD. Milk fat concentration increased with increasing DCAD in experiments 1 and 2 and with substitution of Na for K in experiment 3. The magnitude of the change in milk fat concentration across DCAD and cation treatments were 0.27, 0.34, and 0.30 percentage units for experi-

ments 1, 2, and 3, respectively. However, the range in DCAD concentration across treatments was 129, 346, and 42 mEq/kg of DM, respectively. The relatively small change in DCAD in experiment 3 compared with the comparable magnitude in fat concentration responses across experiments suggests that Na substitution for K, and not DCAD, was the principle reason for the increased milk fat concentration in experiment 3.

Several studies have reported that milk fat concentration and fat yield are not affected by cation source (West et al., 1992; Sanchez et al., 1997; Hu and Kung, 2009). The NRC (2001) suggested that milk yield and DMI are not solely affected by individual dietary Na or K concentrations. Instead, changes in these responses may be the result of the interactive effect of K, Na, and Cl because most physiological processes require a tightly regulated ratio of these cations (NRC, 2001). Therefore, if milk yield and DMI can be improved by manipulating Na:K ratios, it is quite possible that milk fat percentage and fat yield could also be increased by this method.

The dietary treatment that resulted in the highest milk fat production consisted of 1.19% K and 0.71% Na, resulting in nearly equal contributions of K and Na on a milliequivalent per kilogram of DM basis (304 mEq/kg of DM from K, 309 mEq/kg of DM from Na). West et al. (1992) reported that cation source did not affect milk fat production but their treatment with the highest Na percentage (0.87%) also contained 0.89% K, resulting in a Na:K ratio of 1.66 on a milliequivalent basis. A milk fat response to Na in the West et al. (1992) study may not have been detected due to a low overall K:Na ratio and the relatively low dietary K concentration. Therefore, the Na:K ratio may play a key role in altering the rumen environment and increasing milk fat production. The cause of the increased milk fat production with increased Na is unknown. However, it could be speculated that this is a rumen fermentation response, especially because of the known effects of absorbed rumen biohydrogenation intermediates on mammary lipogenesis (NRC, 2001; Bauman and Griinari, 2003). One could speculate that a "sodium effect" exists in the rumen that may have been responsible for increased milk fat production when the dietary K:Na ratio is altered. However, because rumen data were not collected in experiment 3, the potential mechanism of a sodium response cannot be addressed.

Although cation source did not significantly affect MUN concentrations, increasing DCAD concentration resulted in a significant decrease in MUN in experiment 2. Similarly, Spek et al. (2012) found that increasing dietary NaCl resulted in a significant decrease in MUN. Spek et al. (2012) found significant positive linear relationships between dietary sodium chloride and water

intake, which resulted in an increased glomerular filtration rate and urine volume that corresponded with a decrease in MUN. It is possible that increasing dietary K in our study also increased water intake, which would result in increased urine volume and reduced MUN.

The main goal of experiments 1 and 2 was to determine the optimal DCAD concentration to maximize FE expressed as 3.5% FCM per unit of DMI. In experiment 1, DMI was not affected by DCAD concentration. Therefore, the denominator of the dairy FE equation (DMI) was similar between treatments. However, as DCAD concentration had a significant, linear effect on 3.5% FCM, FE increased 0.06 units with increasing DCAD and was maximal at the highest DCAD concentration of 406 mEq/kg of DM. In experiment 2, increasing DCAD increased both DMI and 3.5% FCM, resulting in a curvilinear response to DCAD between diets ranging from 257 to 603 mEq/kg of DM DCAD. However, maximal FE occurred in the treatments with 360 and 488 mEq/kg of DM DCAD. Regression analysis using study-adjusted FE values from experiments 1 and 2 suggested that FE was maximized (1.72) at a DCAD concentration of 426 mEq/kg of DM ($P = 0.0102$; $R^2 = 0.7670$; Figure 1). This result is similar to the maximal FCM responses to DCAD reported by Hu and Murphy (2004) and Sanchez et al., (1994), which were 490 and 380 mEq/kg of DM DCAD, respectively.

The magnitude of the maximal FE response observed in experiments 1, 2, and 3 were 0.06, 0.04, and 0.11, respectively, which were somewhat lower than reported in recent experiments using K supplementation. For example, Harrison et al. (2012) reported that FE improved by 0.11 units when DCAD was increased from 490 to 700 mEq/kg of DM with added dietary K as the cation source. Erdman et al. (2011) reported a 0.14-unit increase in FE by increasing DCAD in a corn silage-based diet from 255 to 336 mEq/kg of DM with added K_2CO_3 . In addition, calculated FE values from published treatment means show that increasing DCAD from 291 to 537 mEq/kg of DM resulted in a 0.09-unit change in FE and increasing DCAD from 310 to 550 mEq/kg of DM resulted in a 0.12-unit change in FE in diets containing 15 and 17% CP, respectively (Wildman et al., 2007a). The differences in FE responses might be attributed to the length of experimental treatment periods. In the present studies, dietary treatments were applied in 3-wk experimental periods, which are probably sufficient to allow for changes in milk fat percentage to be expressed (Rico and Harvatine, 2013) but insufficient to measure the full magnitude of milk production and DMI effects. In the studies of Erdman et al. (2011) and Harrison et al. (2012), treatments were applied at the beginning of lactation and lasted for 140 and 70 d, respectively. Thus, under commercial conditions, the

expected FE response to DCAD would be expected to be greater when dietary changes in DCAD are applied at the herd level and maintained continuously. Because of the potential magnitude of the change, we conclude that adequate DCAD can have a major effect on dairy FE.

CONCLUSIONS

In summary, an increase in milk fat concentration was the most consistent short-term response to increasing DCAD with dietary K and the substitution of Na for K as the cation source. At DCAD concentrations of 250 to 400 mEq/kg of DM, dietary K appeared to have no effect on feed intake, whereas at higher concentrations, feed intake was increased. The combination of the changes in milk fat concentration resulting in increased FCM suggested that maximal FE was achieved at a DCAD of 426 mEq/kg of DM when DCAD was increased using dietary K as the cation source. This value is consistent with other reports in the literature. Finally, dietary Na was shown to be more effective than dietary K in increasing milk fat in diets with DCAD of 400 mEq/kg of DM.

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