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Effects of low dietary cation-anion difference induced by ruminal ammonium chloride infusion on performance, serum, and urine metabolites of lactating dairy cows

Kun Wang[1,a], Xuemei Nan[1,a], Puyi Zhao[1], Wei Liu[1], James K. Drackley[2], Shijie Liu[3], Kaizhan Zhang[4], and Dengpan Bu[1,5,6,*]

* **Corresponding Author:** Dengpan Bu
Tel: +86-010-62813901, **Fax:** +86-010-62815988,
E-mail: budengpan@126.com

[1] State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China
[2] Department of Animal Sciences, University of Illinois, Urbana IL 61801, USA
[3] China Feed Industry Association, Beijing 100125, China
[4] Beijing Sino Farm, Beijing 100125, China
[5] CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing 100193, China
[6] Hunan Co-Innovation Center of Animal Production Safety, CICAPS, Changsha, Hunan 410128, China

[a] These authors contributed equally to this work.

ORCID
Kun Wang
https://orcid.org/0000-0002-6855-8743
Xuemei Nan
https://orcid.org/0000-0002-1483-4036
Puyi Zhao
https://orcid.org/0000-0002-0518-3788
Wei Liu
https://orcid.org/0000-0001-5616-2154
James K. Drackley
https://orcid.org/0000-0002-4560-5594
Shijie Liu
https://orcid.org/0000-0002-3673-5194
Kaizhan Zhang
https://orcid.org/0000-0001-8798-1558
Dengpan Bu
https://orcid.org/0000-0003-1548-5509

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Objective: The objective of the present study was to determine ammonium chloride tolerance of lactating dairy cows, by examining effects of negative dietary cation anion difference (DCAD) induced by ruminal ammonium chloride infusion on performance, serum and urine minerals, serum metabolites and enzymes of lactating dairy cows.
Methods: Four primiparous lactating Chinese Holstein cows fitted with ruminal cannulas were infused with increasing amounts (0, 150, 300, or 450 g/d) of ammonium chloride in a crossover design. The DCAD of the base diet was 279 mEq/kg dry matter (DM) using the DCAD formula (Na + K – Cl – S)/kg of DM. Ammonium chloride infusion added the equivalent of 0, 128, 330, and 536 mEq/kg DM of Cl in treatments. According to the different dry matter intakes (DMI), the resulting actual DCAD of the four treatments was 279, 151, –51, and –257 mEq/kg DM, respectively.
Results: DMI decreased linearly as DCAD decreased. Yields of milk, 4% fat-corrected milk, energy-corrected milk, milk fat, and milk protein decreased linearly as DCAD decreased. Concentrations of milk protein and milk urea nitrogen increased linearly with decreasing DCAD. Concentration of Cl– in serum increased linearly and concentration of PO43- in serum increased quadratically as DCAD decreased. Urine pH decreased linearly and calculated urine volume increased linearly with decreasing DCAD. Linear increases in daily urinary excretion of Cl^{-}, Ca^{2+}, PO_4^{3-}, urea N, and ammonium were observed as DCAD decreased. Activities of alanine aminotransferase, aspartate aminotransferase, and γ-glutamyl transferase in serum and urea N concentration in serum increased linearly as DCAD decreased.
Conclusion: In conclusion, negative DCAD induced by ruminal ammonium chloride infusion resulted in a metabolic acidosis, had a negative influence on performance, and increased serum enzymes indicating potential liver and kidney damage in lactating dairy cows. Daily ammonium chloride intake by lactating dairy cows should not exceed 300 g, and 150 g/d per cow may be better.

Keywords: Lactating Dairy Cow; Dietary Cation Anion Difference; Ammonium Chloride; Serum and Urine Metabolites

INTRODUCTION

In dairy cows, the concept of negative dietary cation anion difference (DCAD, defined as milliequivalents of Na + K – Cl – S per kg of feed dry matter [DM]) has been used in dry cow nutrition to reduce the incidence of parturient paresis [1,2]. Interest has also grown in the potential effects of DCAD on lactating dairy cows. Tucker et al [3] were the first to evaluate DCAD in lactating dairy cows, and their results reported that milk yield was 8.6% higher when a diet with DCAD of 20 vs –10 mEq (Na + K –Cl)/100 g of DM was fed. Similar re-

sults have been reported that increases in milk yield and dry matter intake (DMI) when DCAD increased from −79.4 to +324.4 mEq/kg of DM [4] and from +120.4 to +464.1 mEq/kg of DM [5]. Likewise, addition of Cl salts has negative effects. Escobosa et al [6] reported that milk production reduced when cows were supplemented with anions (2.28% $CaCl_2$). Yen et al [7] found that high dietary $CaCl_2$ limited intake in swine through a Cl-induced metabolic acidosis, an indication that altered acid-base status affects animal performance. Thus, it is well known that low DCAD results in negative effects on DMI and milk production.

In China, regulatory authorities require demonstrations of product safety to animals before new products can be registered. Such demonstrations may include whether a feed approved for one use, such as for creating a negative DCAD in non-lactating cows, might have detrimental effects if accidentally used in another application within that species, such as feeding ammonium chloride (NH_4Cl) to lactating cows. Therefore, further efforts need to be made to examine effects of NH_4Cl on milk performance, serum, and urine metabolites of lactating dairy cows under Chinese feeding conditions in order to determine NH_4Cl tolerance of lactating dairy cows.

NH_4Cl, as a feed additive, has been widely used in livestock production. Since Leoschke and Elvehjem [8] reported prevention of urinary calculi formation in mink by decreasing urinary pH with addition of NH_4Cl, it has been applied as an acidity regulator in feed for bovines [9,10], goats [11-13], sheep [14], dogs [15], cats [16], and horses [17]. Administration of NH_4Cl also has been widely used to create a model of metabolic acidosis in both humans and animals [18,19]. Common chloride salts used to decrease DCAD for dry cows include $MgCl_2$, $CaCl_2$, and NH_4Cl. Compared to Mg^{+2} and Ca^{+2}, NH_4^+ does not result in overload of essential elements to the cow or environment. However, as a source of nonprotein nitrogen (NPN), NH_4^+ poses the risk of ammonia toxicity. However, Crookshank et al [20] reported that NH_4Cl can be used as a source of NPN without clinical signs of ammonia toxicity when added up to 1% of the total ration. Bartley et al [21] showed that rumen pH was more clearly associated with ammonia toxicity than rumen ammonia. NH_4Cl dissociates into NH_4^+ and Cl- ions in the rumen without increasing rumen pH as occurs when urea is hydrolyzed to ammonia [22]. Kertz et al [23] found that low rumen pH traps ammonia within the rumen, in which case high rumen ammonia should not produce sublethal or lethal ammonia toxicity.

The main objective of this study was to determine NH_4Cl tolerance of lactating dairy cows, by examining effects of negative DCAD induced by ruminal NH_4Cl infusion on performance, serum and urine minerals, serum metabolites and enzymes of lactating dairy cows. The second objective of this study was to determine whether NH_4Cl could pose risk of ammonia toxicity under the conditions of the present study.

MATERIALS AND METHODS

Animals, diets, and experimental design

All animals involved in this study were cared for according to principles of the Chinese Academy of Agricultural Sciences Animal Care and Use Committee (Beijing, China). Four primiparous Chinese Holstein cows (body weight [BW] = 556±39 kg, days in milk = 357±2 d) that had been fitted previously with ruminal cannulas (10-cm center diameter; Bar Diamond, Parma, ID, USA) were housed in a free-stall barn equipped with a computerized monitoring system (RIC system, Insentec B.V., Marknesse, the Netherlands). The system automatically identified individual cows by ear tags and recorded their feeding time and duration, as well as the quantity of feed intake at each meal. The basal diet (Table 1) was formulated to meet or exceed nutrient requirements for energy, protein, minerals, and vitamins according to the Feeding Standards of Dairy Cattle (Ministry of Agriculture of P. R. China recommendations, 2004). The diet was fed as a total mixed ration (TMR) 3 times daily (0730, 1330, and 2000 h) to ensure *ad libitum* intake, allowing for 5% orts, along with free access to water. Cows were milked 3 times daily, at 0800, 1400, and 2030 h.

The experimental design (Table 2) was as used previously [24,25]. Cows were administered the 2 treatments in a crossover design: an NH4Cl solution at varying concentrations versus water as control. Each experimental period of the crossover design lasted 4 wk (Table 2). A cow in each period was thus considered a main plot. To prepare the cows for the first period, there was an initial 1-wk adaptation during which all cows were infused with water. During the 4-wk period, graded amounts of NH_4Cl (0, 150, 300, and 450 g/d) were administered to the treated cows with each amount coinciding with 1 of the 4 wk (i.e., the sub-plots of the split plot). Control cows continued to be infused with the same amount of water. For the treated cows, we determined based on preliminary experiments that it was not desirable physiologically to randomize the administration of these 4 amounts. Therefore, as in the study by Drackley et al [25], we allowed the cows to adapt to each amount of NH_4Cl before receiving a higher amount. After period 1, all cows were returned to water infusion for a 2-wk washout period. Then period 2 was initiated for another 4 wk with the same 4 cows in opposite treatment groups. With this design, the effects of week of administration and amount of NH_4Cl were confounded. Because control cows received water infusion each week, against which the treated cows were compared, the confounding is not relevant (see description of statistical analysis).

During the experiment periods, cows were fed with a TMR 3 times daily (0730, 1330, and 2000 h) to ensure *ad libitum* intake and infused 3 times daily after feeding 30 min. The infused NH_4Cl solution was freshly prepared before each infusion by dissolving one-third of the daily dose of NH_4Cl for each

Table 1. Ingredient and chemical composition of basal diets (% of DM)

Item	Content
Ingredient	
Corn silage	21.59
Alfalfa hay	11.39
Extruded soybeans	1.68
Soybean meal	10.15
Flaked corn	15.61
Ground corn	8.23
Corn DDGS	8.57
Whole cottonseed	6.51
Rapeseed meal	1.24
Apple pomace	1.72
Beet pulp	4.79
Salt	0.38
Calcium carbonate 38%	1.05
Sodium bicarbonate	1.39
Magnesium oxide	0.24
Potassium bicarbonate	0.91
Dicalcium phosphate	0.05
Monensin	0.01
Zeolite	0.05
Fat[1)]	1.77
Yeast culture[2)]	0.62
Syrup	1.48
Premix[3)]	0.57
CP	16.27
ADF	23.33
NDF	34.39
NE_L[4)] (Mcal/kg)	1.80
Ether extract	5.24
Ash	8.24
Ca	0.75
P	0.32
Na	0.54
K	1.33
Cl	0.61
Mg	0.44
S	0.20
DCAD[5)] (mEq/kg DM)	279

DM, dry matter; DDGS, distillers dried grains with solubles; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; NEL, net energy for lactation; DCAD, dietary cation anion difference.
[1)] Bergafat, a saturated free fatty acid supplement (Berg+Schmidt, Germany).
[2)] Diamond V XP yeast culture supplement (Diamond V, Cedar Rapids, Iowa, USA).
[3)] Premix composition per kilogram: 1,230 mg of Cu (minimum [min]), 4,950 mg of Zn (min), 1,760 mg of Mn (min), 50 mg of I (min), 61 mg of Se (min), 37 mg of Co (min), 504,800 IU of vitamin A (min), 88,800 IU of vitamin D_3 (min), and 2,100 IU of vitamin E (min), 700 mg of vitamin B_3 (min).
[4)] Estimated according to NRC [52].
[5)] Calculated by the formula: DCAD, mEq/kg DM = (%Na/0.0023 + %K/0.0039) – (%Cl/0.00355 + %S/0.0016).

treatment amount in 600 mL of distilled water. Solutions were infused into the rumen manually by opening the ruminal cannulas and administering the solutions into the rumen.

Sampling and measurements

The TMR and orts from individual cows were sampled on the last 3 d of each week and analyzed for DM content by drying samples at 50°C for 48 h in a forced-air oven [26]. The samples were ground to pass through a 6-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA), and composited by cow. Subsamples of TMR and orts were ground to pass through a 1-mm screen to analyze crude protein (CP), ether extract, acid detergent fiber, and ash according to AOAC International [27]. The contents of neutral detergent fiber were obtained according to Van Soest et al [28], with α-amylase and without sodium sulfite. The dietary contents of Ca, P, Mg, Na, K, Cl, and S were determined at an official laboratory (National Food Safety Supervision and Inspection Center, Beijing, China) by Inductively Coupled Plasma-Optical Emission Spectrometer (Optima 8000DV, Perkin-Elmer, Shanghai, China).

Milk production was recorded daily and milk samples were collected on the last 3 d of each experimental week. Milk samples were collected at each milking of every sampling day, and the 3 samples from each day were pooled in a proportion of 4:3:3 by volume (this ratio reflecting the milk yield of morning, afternoon, and night) into 50-mL subsamples to which was added 1 milk preservative tablet (Bromopol, D and F Control Systems, San Ramon, CA, USA). This subsample was then stored at 4°C for future analysis of milk composition by infrared analysis [29] with a Foss-Milkoscan TM Minor (MilkoScan FT120, Foss Electric A/S, Hillerod, Denmark). Milk urea N was measured by an assay kit (urease method) purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Blood was sampled from the coccygeal vein on d 7 of each experimental week 3 h after the a.m. feeding. Blood samples collected in serum separator tubes (Serum Clot Activator, Greiner Bioone GmbH, A-4550 Kremsmunster, Austria) were allowed to clot for 30 min at room temperature and stored in the refrigerator overnight, and serum was harvested by centrifugation at 3,000×*g* for 15 min at 4°C [30]. Serum was stored at –20°C for future analysis of alanine aminotransferase (ALT; Reitman-Frankel colorimetric method), aspartate aminotransferase (AST; Reitman-Frankel colorimetric method), γ-glutamyl transferase (GGT; Szasz method), urea N (urease method), creatinine (picric acid colorimetric method), and uric acid (phosphotungstic acid colorimetric method), using an automated chemistry analyzer (Hitachi 7080, Beijing CIC Clinical Laboratory, Beijing, China). Assay kits for ALT, AST, GGT, urea N, creatinine, uric acid, and serum ammonium (protein precipitation method) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Urine samples were collected from each cow on the last 3 d of each experimental week [9]. Grab samples of midstream urine were collected between 1100 and 1145 h from each cow after eliciting micturition by manual stimulation of the vulva.

Table 2. Schematic of experimental design and application of treatments

Cow	Preliminary	Week									
		Period 1				Washout		Period 2			
	-1	1	2	3	4	5	6	7	8	9	10
	Amount of NH_4Cl (g/d)										
10,658	W	0	150	300	450	W	W	0	0	0	0
10,662	W	0	150	300	450	W	W	0	0	0	0
10,725	W	0	0	0	0	W	W	0	150	300	450
10,714	W	0	0	0	0	W	W	0	150	300	450

W, cows were infused with water only.
For 0 g/d of NH_4Cl, cows were infused with water, too.

Urine pH was determined immediately by a portable pH meter (Seven Go portable pH meter, Mettler Toledo, Switzerland). An aliquot (50 mL) was stored at 4°C; other 10-mL aliquots were diluted immediately with 40 mL of 0.036 *N* H_2SO_4 and stored at –20°C for analysis of urine urea nitrogen (UUN), creatinine, urine ammonium, allantoin and uric acid. Urine samples (50 mL aliquots) from each cow were pooled together. A 10-mL urine subsample and serum samples stored at –20°C were analyzed for Na^+ (ion-selective electrode), K^+ (ion-selective electrode), Cl^- (ion-selective electrode), Ca^{2+} (*o*-cresolphthalein complexone), PO_4^{3-} (molybdate reaction), and Mg^{2+} (xylidyl blue reaction) concentrations with an electrolyte analyzer (AC9000, Audicom medical technology co., LTD, Shanghai, China). Allantoin and uric acid were measured using the procedure described in Chen and Gomes [31]. Assay kits for UUN (urease method), creatinine (picric acid colorimetric method) and urine ammonium (protein precipitation method) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Urine volumes used to compute daily excretion of UUN, urine ammonium, allantion, and uric acid were estimated as (BW×29)/urine creatinine concentration (mg/L) as described by Valadares et al [32]. The creatinine clearance rate (L/min) was calculated using the formula described by Spek et al [33].

Statistical analysis

Data were analyzed statistically by using PROC MIXED of SAS (version 9.3, SAS Institute Inc., Cary, NC, USA) as described by Drackley et al [25]. The model for an observation on cow performance, y_{ijkl} was as follows:

$$y_{ijkl} = \mu + t_i + p_j + c_k + \varepsilon_{ijk} + a_l + at_{il} + e_{ijkl}$$

Where t_i is the effect of treatment (control or NH_4Cl, considered fixed), p_j is the effect of period (j = 1,2, considered random), c_k is the effect of the k'th cow (k = 1,2,…4, considered random), and ε_{ijk} is the main plot error modeled as an interaction of cow with period and treatment. The fixed effect of amount, a_l, is confounded with the passage of time over the 4 wk in which this factor was applied (Table 2). As noted by Drackley et al [25], it is expected that cows receiving the water-only infusion over the 4 wk would have relatively constant performance and metabolism. Therefore, the interaction at_{il} is the element of interest in this model, where the differences between treated cows and control cows over the 4 wk in which the amount of NH_4Cl was stepped up can be determined.

To model effects of infusion amount and its interaction with treatment, the REPEATED statement within PROC MIXED of SAS was used with cow (period×treatment) as the subject effect. For all variables, the covariance structure leading to the best fit of the model was the compound symmetry option. Polynomial contrasts were constructed to partition the treatment by amount interaction into single degree of freedom interactions of the linear and quadratic effects of amount with treatment, and the p-values associated with these contrasts were tabulated. Degrees of freedom were determined by using the Kenward-Roger method [34]. Model residuals were examined and all variables were normally distributed. Least squares means were calculated and are presented with their standard errors throughout. Significance was declared at $p< 0.05$ and trends at $0.05<p<0.10$.

RESULTS

DCAD, DMI, and health

The DCAD of the base diet was 279 mEq/kg DM using the DCAD formula (Na + K – Cl – S)/kg of DM. NH_4Cl infusion added the equivalent of 0, 128, 330, and 536 mEq/kg DM of Cl from the 0, 150, 300, and 450 g/d treatments. Accordingly, based on actual DMI, the DCAD of the 4 treatments were 279, 151, –51, and –257 mEq/kg DM, respectively. Data for measured variables are reported and discussed in terms of these DCAD values.

Mean DMI decreased linearly as DCAD decreased (Table 3). During both periods 1 and 2, when infusion amount came to 300 g/d, the treatment cows began to have severely depressed DMI. By the last day of period 2, the 2 treatment cows were deemed too sick to complete the trial. Blood samples were

Table 3. Effects of low DCAD on dry matter intake and performance of lactating dairy cows

Item	DCAD (mEq/kg DM)				SEM	Treatment by amount, p value	
	279	151	–51	–257		Linear	Quadratic
DMI (kg/d)	21.6	21.9	17.0	15.7	1.58	0.0001	0.376
Milk (kg/d)	30.2	30.1	27.6	17.8	2.94	0.001	0.060
4% FCM[1] (kg/d)	29.4	30.1	26.2	17.4	2.92	0.002	0.080
ECM[2] (kg/d)	32.2	32.6	28.9	19.4	3.07	0.002	0.084
Fat (%)	3.80	4.00	3.66	3.77	0.071	0.374	0.512
Fat (kg/d)	1.16	1.20	1.01	0.68	0.118	0.003	0.120
Protein (%)	3.40	3.26	3.43	3.94	0.149	0.002	0.022
Protein (kg/d)	1.02	0.98	0.95	0.66	0.082	0.008	0.141
Lactose (%)	4.86	4.90	4.85	4.41	0.116	<0.0001	0.004
Lactose (kg/d)	1.48	1.48a	1.34	0.80	0.162	0.0004	0.039
MUN (mg/dL)	21.04	22.77	22.69	27.03	1.280	0.002	0.703
Total solids (%)	11.98	12.10	11.88	12.18	2.797	0.611	0.683
Solids-not-fat (%)	8.54	8.45	8.55	8.84	0.085	0.258	0.336

DCAD, dietary cation anion difference; DM, dry matter; SEM, standard error of the mean; DMI, dry matter intake; FCM, fat-corrected milk; ECM, energy-corrected milk; MUN, milk urea nitrogen.
[1] 4% FCM (kg/d) = 0.4 × milk (kg/d)+15 × fat (kg/d), (NRC [52]).
[2] ECM (kg/d) = 0.327 × milk (kg/d)+12.95 × fat (kg/d)+7.65 × protein (kg/d).

obtained and cows received medical attention. After receiving therapy for 1 wk, the 2 sick cows had recovered.

Rumen pH (6.06, 6.16, 5.97, 5.88) tended to decrease linearly (p = 0.084) and concentration of ammonia (14.97, 20.81, 25.54, 39.30 mg/L) in the rumen contents increased linearly (p<0.0001) as infusion increased [35].

Milk yield and composition

Effects of decreasing DCAD on milk yield and composition are shown in Table 3. Yields of milk, 4% fat-corrected milk (FCM), and energy-corrected milk (ECM) decreased linearly. The quadratic effects approached significance, indicating that the magnitude of decrease tended to be greater when DCAD was from –51 to –257 mEq/kg DM. Similarly, milk fat yield and milk protein yield decreased linearly. Concentrations of milk protein and milk urea nitrogen (MUN) increased linearly with decreasing DCAD. Milk lactose concentration decreased as DCAD decreased, with decrease being greater as DCAD was from –51 mEq/kg DM or greater (quadratic effect, p = 0.004). Yield of milk lactose decreased in a quadratic fashion similar to milk lactose concentration, with the largest decrease when DCAD was –257 mEq/kg DM. No effects were observed on concentrations of milk fat, total solids, and solids-not fat.

Serum and urine minerals

Table 4 presents mean values of serum ions, urine irons and urine metabolites of dairy cows as DCAD decreased. Urine pH decreased linearly and the quadratic effects approached significance, indicating that the magnitude of decreases tended to increase when DCAD was –51 or –257 mEq/kg DM. Concentration of Cl^- in serum increased linearly; the quadratic effect approached significance because the magnitude of increase tended to be greater when DCAD was –257 mEq/kg DM. Concentration of Ca^{2+} in serum tended to decrease linearly, but Mg^{2+} in serum tended to increase linearly with decreasing DCAD. Concentration of PO_4^{3-} in serum increased quadratically as DCAD decreased, with the largest increases when DCAD was –257 mEq/kg DM. Calculated urine volume increased linearly as DCAD decreased. Daily excretion of Cl^-, Ca^{2+}, PO_4^{3-}, UUN, and urine ammonium increased linearly as DCAD decreased, whereas daily excretion of K^+ and urine acid in urine decreased linearly.

Serum metabolites and enzymes

Blood biochemical indices of dairy cows as DCAD decreased are shown in Table 5. Linear increases were observed in serum activities of ALT, AST, and GGT, as well as the concentration of blood urea nitrogen, as DCAD decreased. However, the concentration of uric acid in serum decreased linearly with decreasing DCAD. Concentration of creatinine in serum tended to increase linearly while concentration of serum ammonium was unaffected with decreasing DCAD.

DISCUSSION

Urea and NH_4Cl, as sources of NPN, undergo different dissociation processes in the rumen. When urea is hydrolyzed by microbial ureases it forms NH_3, but NH_4Cl dissociates into NH_4^+ and Cl^-. The NH_3 is converted to NH_4^+ with the addition of a hydrogen ion, and rumen pH is unchanged or elevated [22] and vice versa. Bartley et al [21] reported that rumen pH was more related to ammonia toxicity than was rumen ammonia. Visek [36] showed that elevated pH facilitates greater absorption of ammonia across the rumen wall. In our study,

Table 4. Effects of low DCAD on serum ions, urine irons and urine metabolites of lacating dairy cows

Item	DCAD (mEq/kg DM)				SEM	Treatment by amount, p value	
	279	151	–51	–257		Linear	Quadratic
Serum ions (mmol/L)							
K^+	4.23	4.34	4.32	4.08	0.059	0.472	0.090
Na^+	137.5	137.8	138.4	142.4	1.14	0.138	0.137
Cl^-	95.6	98.0	99.1	109.6	3.10	0.003	0.072
Ca^{2+}	2.30	2.30	2.28	2.19	0.026	0.080	0.852
PO_4^{3-}	1.68	1.67	1.78	2.25	0.137	0.020	0.029
Mg^{2+}	0.99	0.98	0.98	1.01	0.007	0.056	0.669
Urine pH	7.92	7.54	5.83	5.70	0.573	<0.0001	0.086
Urine ions (mmol/d)							
K^+	5,710	4,932	5,723	4,047	397.5	0.028	0.349
Na^+	3,001	2,325	3,168	3,704	284.4	0.441	0.270
Cl^-	3,356	5,232	7,854	7,716	1,078.32	0.0003	0.444
Ca^{2+}	14	100	214	731	160.8	0.0009	0.100
PO_4^{3-}	14.2	11.0	27.8	190.0	43.2	0.016	0.070
Mg^{2+}	114.5	123.1	151.2	154.4	10.0	0.790	0.517
Urine volume (kg/d)	42.7	44.8	92.5	127.9	20.5	0.025	0.709
Creatinine clearance (L/min)	1.86	1.72	1.76	1.52	0.071	0.104	0.700
UUN (mmol/d)	4,465	4,541	5,274	6,451	562.8	0.001	0.363
Urine ammonium (mmol/d)	30	80	826	2541	586.1	0.004	0.147
Allantoin (mmol/d)	167	246	465	367	65.8	0.287	0.510
Uric acid (mmol/d)	46.3	48.9	49.4	29.8	4.64	0.015	0.129

DCAD, dietary cation anion difference; DM, dry matter; SEM, standard error of the mean; UUN, urine urea nitrogen.

although ruminal ammonia concentration increased markedly, ruminal pH decreased as infusion increased. Lower rumen pH can trap ammonia within the rumen, and high rumen ammonia does not produce sublethal or lethal ammonia toxicity [23]. Rumen ammonia dissociated from NH_4Cl would be absorbed gradually through ruminal epithelium cell to blood and then excreted into urine. Therefore, the concentration of ammonia in serum was not affected by the infusion of NH_4Cl, and signs consistent with ammonia toxicity did not appear. Therefore, we attribute most of the negative effects of increasing NH_4Cl infusion to the negative DCAD and resulting uncompensated metabolic acidosis.

Decreasing dietary DCAD had negative effects on the late-lactating cows in this study. The linear decreases of milk yield, 4% FCM, ECM, milk fat yield, milk protein yield, and milk lactose yield likely were attributable in large part to the decrease of DMI. Because NH_4Cl was infused into the rumen of lactating dairy cows, the decrease of DMI could not have been caused by palatability issues. Instead, decreased DMI likely was attributable to the metabolic acidosis that was induced by low DCAD. Cows infused with the highest amount of NH_4Cl showed obvious signs of distress due to the negative DCAD.

Dietary cation-anion balance has well-known effects on acid-base status and production of cows and other animals. The optimum DCAD in ruminants depends on their production status. Block [1] reported that the incidence of milk fever was reduced from 47.4% in cows fed a cationic diet to 0% when cows received an anionic diet during the dry period. Conversely, Tucker et al [3] showed that a cationic diet (200

Table 5. Effects of low DCAD on biochemical indices of blood of lacating dairy cows

Item	DCAD (mEq/kg DM)				SEM	Treatment by amount, p value	
	279	151	–51	–257		Linear	Quadratic
Alanine aminotransferase (U/L)	26	28	28	46	4.7	0.011	0.080
Aspartate aminotransferase (U/L)	79	85	95	114	7.7	0.0004	0.261
γ-Glutamyl transferase (mmol/L)	31.0	30.9	31.9	36.2	1.25	0.003	0.169
Urea N (mmol/L)	4.88	5.54	5.40	6.36	0.307	0.005	0.704
Uric acid (umol/L)	37.8	32.7	25.6	20.6	3.80	0.046	0.077
Creatinine (umol/L)	53	57	56	67	3.0	0.084	0.832
Ammonia (umol/L)	123.6	122.1	111.1	141	6.2	0.096	0.564

DCAD, dietary cation anion difference; DM, dry matter; SEM, standard error of the mean.

mEq/kg DM) resulted in greater milk yield than when diets containing –100, 0, or 100 mEq/kg were fed. In a meta-analysis, Hu and Murphy [37] reported that milk yield was greatest when the DCAD (Na + K – Cl) 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. In our study, DMI, milk yield, ECM yield, and 4% FCM yield did not decrease when DCAD decreased from 279 to 151 mEq/kg DM, but decreased markedly when DCAD was –51 mEq/kg DM. The optimum DCAD may relate to stage of lactation and milk production, so lack of production change when DCAD was 151 mEq/kg DM may be attributable to the late stage of lactation (days in milk = 357±2 d) of our cows.

The concentration of milk protein measured by infrared analysis in our study represents CP or total nitrogen rather than true protein. Although the concentration of milk total protein increased with the higher infusion amounts, the concentration of MUN also increased with decreasing DCAD. So, the concentration of milk true protein likely did not increase with increasing amount of NH_4Cl infused. It seemed that NH_4^+ as a source of NPN had little influence on the synthesis of milk protein under conditions of our study.

In general, responses of mineral ions in serum and ion excretion in urine followed well described patterns. Increased serum Cl^- concentration at the highest infusion rate may have resulted from the inability of the kidneys to excrete additional Cl^-, as the amounts excreted were similar for DCAD of –151 and –257. The kidneys can efficiently eliminate excess anions from the blood, thus infusion of NH_4Cl induced a sharp reduction in urinary pH. The effect of increased dietary anions (Cl and S) to decrease urine pH is well documented [38-40]. Monitoring the pH of urine is considered a sensitive method for assessing the acid-base balance of animals [41].

Urinary excretion of Ca^{2+} increased sharply with decreasing DCAD, with serum Ca^{2+} concentration decreasing at the lowest DCAD. Previous studies revealed that decreased DCAD increased urinary Ca^{2+} excretion in lactating [42], nonlactating [43], and close-up prepartum [44,45] dairy cows. The metabolic acidosis induced by the negative DCAD diets in this study likely decreased Ca^{2+} reabsorption via the kidney tubules, so more Ca^{2+} was excreted into the urine. Although negative DCAD diets prepartum are believed to increase serum Ca^{2+} in newly calved cows by increasing Ca mobilization from bone to maintain blood Ca^{2+} [46], the extreme acidosis induced in this study likely created more urinary excretion of Ca^{2+} than could be maintained in the blood.

Because bone hydroxyapatite is the source of increased Ca^{2+} concentrations excreted in urine, increased concentration of PO_4^{3-} in serum and increased urinary PO_4^{3-} excretion was expected. Block [1] observed an increase in concentration of serum PO_4^{3-} during the peripartum period for cows receiving anionic diets. Metabolic acidosis induced by low DCAD diets may result in an increase in parathyroid hormone release, which in turn would increase phosphorus mobilized from bone, increase serum PO_4^{3-}, and result in excess PO_4^{3-} being lost in urine. In contrast, other studies [40,47,48] reported no significant effect of prepartum DCAD on serum PO_4^{3-}. The different results may be attributable to the different lactation stage of test cows and differences in the degree of acidosis induced.

The liver as a main organ in ruminant metabolism is sensitive to nutritional modifications. Serum ALT, AST, and GGT are frequently used as markers of liver damage resulting from metabolic disease or stressors [49,50]. Activities of ALT, AST, and GGT in serum are increased when liver is damaged, resulting in liberation of these cellular enzymes into the serum. In our trial, ALT, AST, and GGT activities increased linearly with decreasing DCAD, suggesting that the cows incurred some degree of liver damage. Renal function indices such as serum urea and creatinine are used to evaluate the functional integrity of the kidney, with elevated values being an indication of defective functional state [51]. Linearly increased serum urea and the tendency to linearly increase creatinine concentrations observed in this study also may imply some degree of renal toxicity imposed by negative DCAD. However, increasing ruminal infusion of ammonium ions and decreased DMI may have resulted in excess ammonia and more urea formation, which confounds the interpretation.

CONCLUSION

Negative DCAD caused by acute changes in the amount of NH_4Cl infused into the rumen had negative influence on performance of lactating dairy cows. In particular, a DCAD of –51 mEq/kg DM as induced by infusion of 300 g/d of NH_4Cl caused severe metabolic acidosis, decreased DMI, and decreased milk yield. The most negative DCAD also caused some indications of liver and kidney damage. Infusion of NH_4Cl did not affect concentration of milk true protein and did not seem to cause ammonia toxicity under the conditions of the present study. While a small amount of NH_4Cl that does not create a negative DCAD can be tolerated, short-term ingestion of amounts that create a negative DCAD could be detrimental in lactating dairy cows. As a result, we recommended that daily NH_4Cl intake by lactating dairy cows should not exceed 300 g, and a more appropriate daily intake may be 150 g.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript. Liu S is an employee of China Feed Industry Association, and Zhang K is an employee of Beijing Sino Farm.

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