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RESEARCH ARTICLE

Effects of Dietary Cation-Anion Difference on Milk Performance, Digestion and Blood Parameters in Lactating Cows Under Heat Stress

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Abstract

The current study determined the effects of dietary cation-anion difference (DCAD) on milk performance, total apparent digestibility, and blood parameters in lactating cows subject to heat stress. Eight Chinese Holstein cows (22.04±2.38 kg of milk/d, 512±76 kg of body weight, 219±20 d in milk) at the late stage of lactation were allocated to group 1 or 2. We used a randomized complete block design with a 2x2 factorial arrangement. The experiment consisted of two periods. Each period lasted 21 days, including the first 14 days for adaptation to the diet and the following seven days for trail. During period 1, group 1 fed with DCAD at 335 mEq/kg dry matter (the basal diet=CON) and group 2 fed 507 mEq/kg dry matter (high DCAD). During period 2, group 1 diet (the basal diet = CON) was swapped group 2 diet (high DCAD). The high DCAD had no significant effects on the respiratory frequency, rectal temperature, blood pH value, the acid-base balance, milk yield, milk composition, and feed intake ($P>0.05$). However, the high DCAD was associated with lower somatic cell count (SCC) in milk ($P=0.04$) and lower immune cell counts in blood, which was conducive to the improvement of milk quality. The apparent digestibility of dry matter, organic matter, energy, neutral detergent fiber, and ethyl extract was greater in the high DCAD group ($P<0.05$). In summary, increasing DCAD in the diet could stabilize milk production and feed intake, improve milk quality and apparent digestibility in lactating cows subject to heat stress.

Keywords: *Dietary cation-anion difference, Milk performance, Digestive performance, Blood physiology*

Isı Stresi Altındaki Laktasyon Dönemi İneklerde Diyet Katyon-Anyon Farkının Süt Performansı, Sindirim ve Kan Parametrelerine Etkisi

Öz

Bu çalışmada, ısı stresine maruz kalan laktasyon dönemi ineklerde diyet katyon-anyon farkının (DKAF), süt performansı, toplam görünür sindirilebilirlik ve kan parametreleri üzerine etkileri belirlendi. Geç laktasyon dönemindeki 8 Çin Holstein ineği (22.04±2.38 kg süt/gün, 512±76 kg vücut ağırlığı, 219±20 gün laktasyon süresi) grup 1 veya grup 2 olarak ayrıldı. 2x2 faktöriyel düzenleme ile rastgele bir tam blok tasarımı kullanıldı. Çalışma, her bir grup için ilk 14 gün diyete uyum ve sonraki yedi gün takip olmak üzere 21 gün süren iki periyottan oluşturuldu. Birinci periyotta grup 1, 335 mEq/kg kuru maddeli DKAF (bazal diyet=Kontrol) ile, grup 2, 507 mEq/kg kuru maddeli DKAF (yüksek DKAF) ile beslendi. İkinci periyot boyunca, grup 1 diyeti (bazal diyet=Kontrol) grup 2 diyeti (yüksek DKAF) ile yer değiştirildi. Yüksek DKAF'ın, solunum frekansı, rektal ısı, kan pH değeri, asit-baz dengesi, süt verimi, süt bileşimi ve yem alımı üzerine anlamlı bir etkisi yoktu ($P>0.05$). Fakat, yüksek DKAF, sütte daha düşük oranda somatik hücre sayısı (SHS) ($P=0.04$) ve kanda daha düşük oranda immün sistem hücre sayısı ile ilişkilendirildi, bu durum süt kalitesinin iyileştirilmesine katkı sağladı. Kuru madde, organik madde, enerji, nötr deterjan lifi ve etil özütünün görünür sindirilebilirliği, yüksek DKAF grubunda daha fazlaydı ($P<0.05$). Özetle, ısı stresine maruz kalan laktasyondaki ineklerde diyette artan DKAF, süt üretimini ve yem alımını stabilize edebilir, süt kalitesini ve görünür sindirilebilirliği iyileştirebilir.

Anahtar sözcükler: *Diyet katyon-anyon farkı, Süt performansı, Sindirim performansı, Kan fizyolojisi*

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INTRODUCTION

The basic metabolic heat production of dairy cows is very sufficient, such as growth and development, lactation, pregnancy, rumen fermentation, which provides a large amount of metabolic heat production, but the ability to dissipate heat is weak [1,2]. In a hot and humid climate, the deep body temperature of cows rises in a short period of time [3]. Dairy cows need to maintain a balance between heat production and heat dissipation. The main way to reduce metabolic heat production is to reduce feed intake [4]. The reduction of DMI will cause insufficient nutrient supply for the physiological activities related to lactation [5], which will further lead to changes in milk production [1,6] and milk composition [7] of dairy cows. The health and animal welfare issues of some dairy cows are related to the hot climate, increase of water consumption, rectal temperature and respiratory rate [8], these would result in the loss of body weight. Clinical mastitis is positively correlated with deep body temperature [9], the somatic cell count (SCC) is an important indicator for monitoring mastitis in dairy cows. Paape et al.[10] and Bouraoui et al.[11] reported that SCC in milk was increased in summer compared with the non-heat stress period. Lying is the most important dairy cow behavior [12]. In a hot and high humidity environment, the cows have poor lying comfort and increased heat dissipation requirements, resulting in increased standing time [13,14].

A decrease in dry matter intake (DMI) in dairy cows under heat stress could lead to a reduction of mineral element intake, as a result of reduced feed consumption. The increase in respiratory frequency of cows could cause a decrease of salivary bicarbonate, which could even lead to a change of blood acidity and alkalinity and affect the cation-anion status in the body. Therefore, manipulating dietary cation-anion difference (DCAD) is an appropriate dietary strategy to meet the increasing demand for mineral elements under heat stress [15,16]. There is evidence showing that the difference between cations and anions in diet has a linear relationship with the 4% fat corrected milk (FCM) yield of early and middle lactation cows in both cold and hot environments. West et al.[17] reported that DMI and milk yield reached the greatest when the DCAD level in diet was at 380 mEq/kg DM.

Previous studies have focused on early and mid-lactation heat-stressed cows, and the effect of higher ion differences on heat stress in late-lactation cows is not clear. Therefore, we conducted this study to explore whether positive DCAD in a high temperature and high humidity environment can increase the DMI and milk production of dairy cows in late lactation.

MATERIAL AND METHODS

Ethical Statement

The use of the animals and the experimental procedures were approved by the Animal Ethics Committee of the College of Animal Science and Technology, Hunan Agricultural University (NO:20180715), in accordance with the China National Standard - Laboratory Animals - Guideline of Welfare and Ethics.

Animals and Experimental Design

Eight lactating Chinese Holstein dairy cows with same parity (2±0), similar milk yield (22.04±2.38 kg/d), body weight (512±76 kg), and days in milk (219±20 d) were allocated into two treatments in a two period cross-over design during the summer season (from August to September). Two treatments were referred to two levels of DCAD in two diets: the basal diet (control=CON) had 335 mEq/kg DM, and high DCAD diet had 507 mEq/kg DM.

The calculation of the DCAD value for the diets was based on the Na-K-Cl formula described by Mongin [18]:

$$\text{DCAD mEq/kg} = 10\text{x}[(\%\ \text{Na}/0.023) + (\%\ \text{K}/0.039) - (\%\ \text{Cl}/0.035)]$$

The experiment consisted of two periods. Each period lasted 21 days, including the first 14 days for adaptation to the diet and the following seven days for sampling. Extra three days were allowed between the first and the second period. In the second period, two diets were swapped, so each group received both the diets in two period, and there were eight replicates for each diet.

Two diets were formulated to meet the nutrient requirement for lactating cows according to NRC 2001 [19] recommendations.

The ingredients of two diets, as shown in *Table 1*, were almost identical, except for the changes in the amounts of $NaHCO_3$ and KCO_3 to create the differences in DCAD level between two diets. The ratio of concentrate to roughage was 56:44 (dry matter basis). Total mixed ration (TMR) was prepared daily, and fed three times per day (08:00 h, 14:00 h and 20:00 h). Fresh drinking water was accessible all times.

Temperature-Humidity Index (THI) Monitoring

Two wet and dry bulb thermometers (Tianjin Tianma Instrument Factory. Effective ranges: dry bulb (Td) temperature -10-50°C and, wet bulb (Tw) temperature -10-50°C) were hanged on the feeding bars at 1.5 m above the floor to ensure necessary ventilation, be away from sunlight and rain, and avoid cow's touching. Temperature and humidity were recorded daily at 08:00, 14:00 and 20:00 before the feeding throughout the experimental periods. The THI was then calculated using a formula by National Research Council 2001 [19]:

$$\text{THI} = (\text{Td} + \text{Tw}) \times 0.72 + 40.6$$

where Td and Tw are the temperature readings on the dry bulb and wet bulb thermometers. The daily averages of THI, Td, and Tw were calculated.

Table 1. *Ingredients and nutrient levels of the diets*

Ingredients (g/kg DM)	**Basal Diet CON=Control**	**High DCAD**
Corn silage	420	415
Alfalfa hay	124	123
Oatmeal hay	154	151
Whole cottonseed	185	181
Corn grain, finely ground	180	178
Steam-flaked corn	56.2	53.9
DDGS	68.6	67.3
Soybean meal	81.2	80.0
Extruded full-fat soybean	6.1	6.0
Premix†	31.5	29.6
$NaHCO_3$	2.0	5.8
K_2CO_3	0	8.2
Chemical Composition (g/kg DM)		
OM	908	918
CP	168	161
NDF	454	423
ADF	241	230
EE	72.7	69.6
Ash	91.5	81.7
Ca	4.76	5.23
P	3.94	4.22
Na	3.42	4.48
K	13.0	17.5
Cl	5.51	4.78
DCAD (mEq/kg DM) ‡	335	507

†: One kg premix contains: 400 mg Zn, 100 mg Cu, 200 mg Fe, 3600 mg Mg, 350 mg Mn, 96 mg Cr, 4.0 mg Co, 50 mg Se, 500 mg lysine, 500 mg methionine, 250.0000 IU Vit. A, 100.000 IU Vit. D_3, 4.000 IU Vit. E

‡: DCAD: mEq/kg DM = 10 × [(% Na/0.023) + (% K/0.039)] - [% Cl/0.0355]

Measurement of Rectal Temperature and Respiratory Rate of Cows

The rectum (about 6-8 cm from the annas) temperature (RT) of eight cows was recorded using an electronic thermometer at 08:00, 14:00, and 20:00 every day during the sampling period. The respiratory rate (RR) of the cows was counted by the research officers using a stopwatch and a counter at 08:30, 14:30, and 20:30, each for 60 sec during the sampling period. The RR was calculated by observing thoracoabdominal movements for 1 min and expressed in breaths/minute [20].

Feed and Fecal Sample Collections and Analysis

The mounts of feed offered and refusal were recorded throughout each of the experimental periods to calculate daily feed intake and the average feed intake for the experimental period. The feed and refusal samples were collected on days of 1, 3, 5, and 7 of the sampling period, pooled, and stored at -20°C for analyses later on.

During the sampling period, a 200 g of fecal sample was taken daily from the rectum of each cow. Then 20 mL of 10% sulfuric acid was added into the sample to prevent nitrogen loss. The samples for each cow were pooled, subsampled, and stored at -20°C for analyses of later on.

The feed and fecal samples were determined for dry matter (DM) content first, then ground to pass through a 40 mesh sieve. The dried samples were analyzed using the AOAC [21] procedures for DM, crude ash content, crude protein (2300 Automatic Kjeldahl Nitrogen Analyzer Denmark FOSS Co., Ltd.), crude fat, and gross energy (SDACM3100 calorimeter, Hunan Sande Science and Technology Co., Ltd., China). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the methods described by Van Soest et al.[22] Amylase (Termamyl 120L, TypeS, Denmark) and anhydrous sodium sulfite were added to the analytic processes. Concentrations of Na and K elements were determined using an inductive coupled plasma optical emission spectrometer (ICP-OES) [23]. Water-soluble chlorides were determined by $AgNO_3$ titration [24]. Phosphorus was determined using a vanadium molybdenum yellow colorimetric assay [25], and Ca was determined by complexometric titration with disodium ethylenediamine tetraacetate [26]. The ash undissolved in 2M hydrochloric acid in feed and fecal samples was used as an internal marker for the calculation of the total apparent digestibility [27].

Milk Yield and Milk Composition

Milk yields at 0800 h, 1400 h, and 2000 h were recorded daily throughout the whole experimental period. After milking, a 100 mL of milk sample was collected respectively at 0800 h, 1400 h, and 2000 h on day 7 of the sampling period and pooled at a ratio of 4:3:3. The pooled milk sample was mixed with a drop of potassium dichromate as preservative and then refrigerated at 4°C. Stored 4°C sample was used for determinations of milk fat, lactose, somatic cell count, solid dry matter, and solid content of milk fat by infrared spectrophotometry (Mino-78110 Automatic Milk Composition Analyzer, FOSS Co., Denmark) on the next day.

Blood Samples and Assays

Blood samples were taken from the tail vein into three tubes two hours after the morning feeding on day 7 of the sampling period. One sample with heparin as anticoagulant was sent to Xiangya Hospital of Central South University to analyze gas indicators in blood. The second sample with EDTA as anticoagulant was sent to the Institute of Subtropical Ecology, Chinese Academy of Sciences for hematological analysis. The third sample was centrifuged at 3000 rpm for 15 min at 4°C, plasma was collected, transported in liquid nitrogen, stored in -20°C. The plasma sample was carried out blood biochemical analysis (Mindray BS-200) at Hunan Co-Innovation Center of Animal Pro Application.

Statistical Analysis

The number of animals was calculated with G*Power Software by using Student *t*-test with P<0.05 and power (1-β) = 0.80.

Statistical analysis of data was performed using SAS 9.4 software (SAS Research Institute Ltd.) in a mixed linear model. In the model, the treatment and period were the fixed factors, and animal as a random factor [5]. The differences in the means with P values <0.05 are declared significant, and difference in the means with P values <0.10 but >0.05 are declared a tendency. GraphPad Prism 16.0 was used to draw graphics.

RESULTS

THI, Respiratory Frequency and Rectal Temperature of Cows

The temperature, humidity, and calculated THI in the cowshed during the experimental period are shown in *Fig. 1*. The dry bulb thermometer readings ranged from 24.93°C to 34.25°C and the average for the period was at 28.94±2.5°C (SD). The wet bulb thermometer readings ranged from 19.87 to 28.88 and the average was 25.61±2.24°C. The calculated THI ranged 73.94 to 85.00, and the average was 80.12±3.35. The THI decreased slightly from day 34 to day 45 due to the changes of weather. During the whole experiment period, the THI was always greater than 72.

The respiratory rate and rectal temperature of cows are shown in *Table 2*. There were no significant differences in the respiratory rate and rectal temperature between CON and high DCAD groups.

Apparent Digestibility, Milk production Performance, and Efficiency

Dietary intake and apparent nutrient digestibility of the cows are listed in *Table 3*. The difference in DCAD had no significant influence on the intakes DM, OM, CP, energy, ethyl extract, NDF, and ADF (P>0.05). However, the apparent digestibility of those nutrients was all significantly greater in the high DCAD group compared with the basal diet group.

As shown in *Table 4*, the DCAD difference had no significant effect on milk yield and milk compositions, such as protein, fat, lactose, and non-fat milk solids, and milk conversion efficiency (P>0.05). However, SCC was lower in the high DCAD group compared with those in the basal diet group (P<0.05).

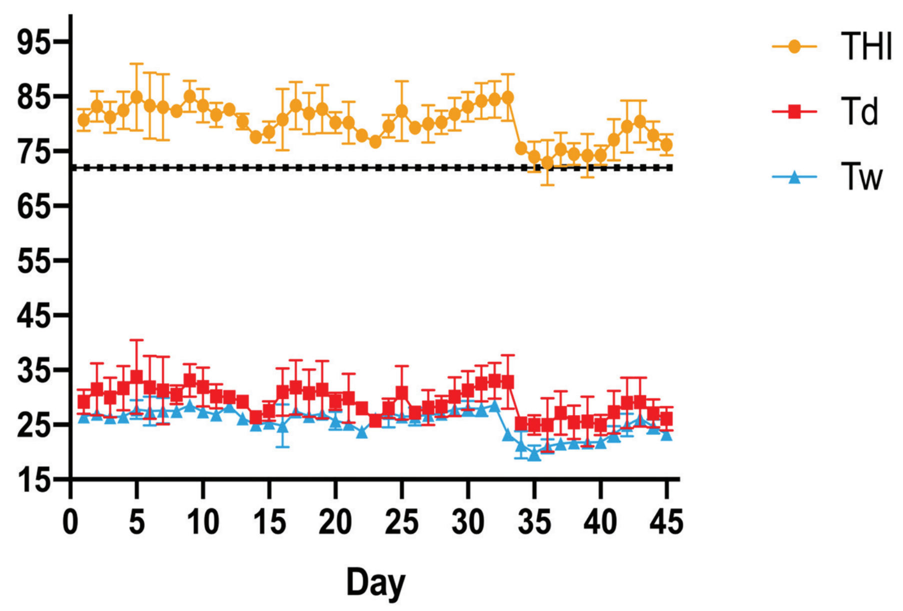

Fig 1. The daily average temperature-humidity index (THI), dry bulb temperatures (Td) and wet bulb temperatures (Tw) during the experimental period (THI:80.15±4.30, Td:29.24±3.86 and Tw:25.64±2.57). The horizontal line at 72 indicates the threshold for heat stress

***Table 2.** Effects of dietary cation-anion difference (DCAD) on the respiratory rate and rectal temperatures in lactating cows*

Item	DCAD (mEq/kg DM)		SEM	P Value
	Basal Diet CON=Control	High		
Respiratory rate (the number of breath/min)	64.0	62.0	1.02	0.130
Rectal temperatures (°C)	38.8	38.9	0.09	0.151

The dietary cation-anion difference (DCAD) values were 335 and 507 mEq/kg DM respectively for CON=contorl and High DCAD groups

Table 3. *Effects of dietary cation-anion difference (DCAD) on feed intake and nutrient apparent digestibility in lactating cows*

Item	DCAD (mEq/kg DM)		SEM	P Value
	Basal Diet CON=Control	High		
Intake, kg/d				
DM	21.3	22.0	0.95	0.625
OM	19.5	19.9	0.87	0.763
CP	3.08	3.28	0.140	0.328
Energy (Mcal/kg)	75.3	80.0	3.42	0.340
EE	1.54	1.53	0.091	0.901
NDF	9.26	9.68	0.432	0.500
ADF	4.26	4.13	0.203	0.500
Total apparent digestibility, %				
DM	66.7	76.5	1.03	<.001
OM	69.4	78.0	0.93	<.001
CP	66.8	76.6	1.56	<.001
Energy	64.2	75.6	1.28	<.001
EE	78.5	83.7	1.18	0.008
NDF	58.9	73.2	2.31	0.001
ADF	66.7	76.5	1.03	<.001

The dietary cation-anion difference (DCAD) values were 335 and 507 mEq/kg DM respectively for CON=contorl and High DCAD groups

Table 4. *Effects of dietary cation-anion difference (DCAD) on milk yield, milk compositions, and milk production efficiency in lactating cows*

Item	DCAD (mEq/kg DM)		SEM	P Value
	Basal Diet CON=Control	High		
Milk yield (kg/d)	20.4	20.1	0.75	0.162
FCM (kg/d)	21.1	21.9	0.71	0.260
CP (%)	3.20	3.28	0.039	0.194
Fat (%)	4.52	4.55	0.104	0.840
Lactose (%)	5.07	5.08	0.017	0.685
SNF (%)	9.04	9.10	0.045	0.299
TS (%)	13.7	13.4	0.15	0.191
SCC (cells × 10^3)	158	107	14.5	0.021
Milk production efficiency (kg/kg)	1.37	1.38	0.021	0.748

SCC: *Somatic cell count;* ***SNF:*** *Non-fat milk solids;* ***TS:*** *Total solids*
The dietary cation-anion difference (DCAD) values were 335 and 507 mEq/kg DM respectively for CON=contorl and High DCAD groups

Table 5. *Effects of dietary cation-anion difference (DCAB) on the gas indexes in blood and hematology in lactating cows*

Item	DCAD (mEq/kg DM)		SEM	P Value
	Basal Diet CON=Control	High		
pH	7.39	7.40	0.012	0.646
Hctc (%)	30.9	30.7	0.69	0.798
ctHB (g/dL)	10.0	9.90	0.220	0.744
$cHCO_3^-t$ (mmol/L)	24.4	24.4	0.82	0.982
pCO_2 (mmHg)	39.5	37.5	1.04	0.195
pO_2 (mmHg)	58.0	65.1	6.87	0.472
sO_2 (%)	64.1	78.0	6.83	0.050
cCa^{2+} (mmol/L)	1.20	1.19	0.012	0.579
cCl^- (mmol/L)	105	105	0.6	0.972
cK^+ (mmol/L)	3.91	3.85	0.104	0.706
cNa^+ (mmol/L)	140	140	0.4	0.823

BE: *bases excess;* ***HCO^{3-}:*** *bicarbonate;* ***pCO_2:*** *carbon dioxide partial;* ***pO_2:*** *oxygen partial pressure;* ***Hb:*** *hemoglobin;* ***Hct:*** *hematocrit;* ***sO_2:*** *oxygen saturation*
The dietary cation-anion difference (DCAD) values were 335 and 507 mEq/kg DM respectively for CON=contorl and High DCAD groups

Table 6. *Effects of dietary cation-anion difference (DCAD) on the immune cell counts in blood of lactating cows*

Item	DCAD (mEq/kg DM)		SEM	P Value	Normal Range
	Basal Diet CON=Control	High			
Leukocytes (10^9/L)	8.38	6.60	0.382	0.004	5-11.7
Neutrophils (10^9/L)	3.35	2.79	0.196	0.056	1.5-5.2
Lymphocytes (10^9/L)	4.35	3.28	0.241	0.005	1.9-6.4
Monocytes (10^9/L)	0.36	0.28	0.033	0.117	0-0.6
Eosinophils (10^9/L)	0.28	0.22	0.020	0.071	0-3.6
Basophils (10^9/L)	0.04	0.028	0.0048	0.045	0-0.2

The dietary cation-anion difference (DCAD) values were 335 and 507 mEq/kg DM respectively for CON=contorl and High DCAD groups

The Gas Indexes in Blood and Hematology

The gas indexes in blood and hematological indexes in lactating cows are shown in *Table 5*. There were no significant differences in blood pH, cCa^{2+}, cK^+, cNa^+, cCl, Hctc, ctHB, $cHCO_3^-$, pCO_2 and pO_2 between two DCAD groups ($P=0.05$). The SO_2 value in blood was significantly greater in the high DCAD group ($P=0.05$).

The Immune Cell Counts in Blood

Table 6 shows the immune cell counts in blood in lactating dairy cows. Cows on the high DCAD diet had relatively lower counts of leukocytes, lymphocytes, and basophils ($P\leq0.05$), and a tendency of low counts of neutrophils and eosinophils ($P<0.10$) compared with the cows on the basal diet DCAD diet. No significant difference in the monocyte count between two diets was observed ($P=0.117$). Nevertheless, all those immune cell counts fell into the normal ranges.

Metabolites and Lipids in Plasma

The concentrations of total triglycerides (TG), total cholesterols (TCHO), high-density lipoprotein cholesterols (HDL-C), low-density lipoprotein cholesterols (LDL-C), glucose, and lactic acid, and α-amylase and cholinesterase activities are shown in *Table 7*. There were no significant differences in

Table 7. *Effects of dietary cation-anion difference DCAD on lipids and metabolites in plasma of lactating cows*

Item	DCAD (mEq/kg DM)		SEM	P Value
	Basal Diet CON=Control	High		
TG (mmol/L)	0.232	0.245	0.0090	0.37
TCHO (mmol/L)	6.81	6.70	0.476	0.83
HDL-C (mmol/L)	5.64	5.62	0.345	0.94
LDL-C (mmol/L)	2.76	2.66	0.296	0.74
GLU (mmol/L)	3.62	3.86	0.106	0.03
LAC (mmol/L)	1.28	1.34	0.226	0.85
CHE (g/L)	207.9	210.7	6.55	0.68
AMS (U/L)	28.6	29.4	2.69	0.76

__AMS:__ α-amylase; __CHE:__ cholinesterase; __HDL-C:__ high-density lipoprotein cholesterols; __GLU:__ glucose; __LAC:__ lactic acid; __LDL-C:__ low-density lipoprotein cholesterols; __TCHO:__ total cholesterols; __TG:__ total triglycerides
The dietary cation-anion difference (DCAD) values were 335 and 507 mEq/kg DM respectively for CON=contorl and High DCAD groups

those parameters between two DCAD groups (P>0.05) except that glucose concentration in plasma was greater in the high DCAD group compared with that in the CON DCAD group (P<0.05).

DISCUSSION

THI is an objective indicator to thermo and humid environment that can cause a heat stress in animals. When THI exceeds 72, cows begin to suffer heat stress; when THI is between 78 and 89, cows could be under moderate heat stress; once THI exceeds 90, cows are under severe heat stress [28]. The THI in the current study ranged from 73 to 80, indicating that the cows were under moderate heat stress. In the region where this study was conducted, humidity in summer was usually very high, in conjunction with high temperature. High humidity can affect moisture evaporation from the body surface for cooling so that cows cannot emit enough body heat to prevent the increase of the body temperature [29]. As resultant responses to heat stress, respiratory frequency and rectal temperature of animals may increase. In non-heat stress conditions, the respiratory rate of dairy cows ranges from 15 to 35 breaths/minute, and rectal temperature ranges from 38.0°C to 39.0°C [30,31]. We observed high respiratory frequency in the cows in the present study, but the rectal temperature fell in the normal range, indicating that the cows were under heat stress, but the body temperature continued under the control. The change in the DCAD level did not result in any differences in both respiratory rate and rectal temperature.

The changes in DCAD in diet for dairy cows have been found to affect milk production and reduce the productivity loss caused by heat stress. Mallonée et al.[32] reported that milk yield of dairy cows increased with an addition of K$^+$ in diet. Schneider et al.[33] found when Na$^+$ in diet increased from 0.18% to 0.55%, DMI and milk yield in dairy cows increased, and the DMI increased linearly with the DCAD level increased from 120 to 460 meq/kg DM. In hot climate conditions, K$^+$ is lost in sweat of cows, because K$^+$ is the main cation in sweat [34]. Delaquis and Block [35] enlarged the dietary anion-cation difference, which resulted in an increase in milk production, milk protein and milk fat in early and middle lactating cows in hot weather. However, there is a research report on the dietary anion-cation difference in late lactation dairy cows. The present study showed that when DCAD increased from 335 mEq/kg DM to 507 mEq/kg DM, the milk yield and milk composition had no significant changes. The reason may be that there was adequate K$^+$ in the feed to compensate the K$^+$ loss in sweat. West et al.[17] observed that DCAD ranged from 120 to 456 mEq/kg DM with supplementation of KHCO$_3$ in the diet for lactating dairy cows. Chan et al.[36] observed that the DCAD levels at 200, 350, and 500 mEq/kg DM had no significant effect on milk protein and milk fat in early lactating cows. These results are in consistent with our findings in the current study. Although there was no significant difference in dietary intake, we found that the digestibility for the high DCAD diet was greater than those for the basal diet DCAD diet. Wang et al.[37] found that the higher DCAD content significantly enriched the phylum *Fibrobacteres* and genus *Fibrobacter* in the microflora of rumen fluid and elevated the total volatile fatty acid production. This may be responsible for the greater nutrient digestion in the high DCAD group.

Milk is rich in various nutrients, including lactose, protein, fat, and phospholipids. Generally speaking, the concentrations of these nutrients are stable, but could vary among individual animals and with environmental changes, such as ambient temperature. Milk fat is more viable in relevance to milk protein. We did not find any significant change in milk composition in the current study. Solorzan et al.[38] found that an addition of buffers in the diet could increase milk fat, but under heat stress conditions, an increase in dietary DCAD from 220 mEq/kg DM to 470 mEq/kg had no influence on milk yield and milk fat. No significant difference in the milk yield in the present study may be due to the cows in the late stage of lactation, and usually the milk yield is low during this stage. So lactating stage could influence the effect of DCAD on milk production. For example, Wildman et al.[39] reported that high DCAD increased milk yield in cows in the early and middle lactating stages, alleviated the symptoms of heat stress, and reduced the respiratory rate.

Blood pH value is mainly related to the buffer system of HCO_3^- & CO_2. The actual bicarbonate (HCO_3^-) level in blood is mainly dissociated from bicarbonate, and its concentration can directly affect the change in blood pH value. Partial pressure of CO_2 refers to the amount of dissolved CO_2 in plasma, which is an important indicator to the respiratory acid-base balance. When other anions are deficient, an increase in HCO_3^- can replace other anions and keep the balance with cations. The present study showed that there was no significant difference in the concentrations of different ions and pH value, which indicate that lactating cows maintained the blood acid-base balance through the homeostasis regulation mechanism in the body. Martins et al.[40] found that pCO_2 and tCO_2 in blood increased with an increase of DCAD, while pO_2 decreased, and proposed that the change in the respiratory rate in cows maintained the acid-base balance in blood. The respiratory rate increases in a hot environment, but the concentration of Na^+ and K^+ affect the concentration of HCO_3^- and maintain the stability of pH value in blood.

We found in the current study that the high DCAD was associated with lower SCC. Bouraoui et al.[11] found that under a heat stress condition, SCC could be doubled. An increase in DCAD in diet may reduce SCC in milk of cows, but its mechanism remains unclear. We also noted that most of the immune cells, except for monocytes, in blood were lower in cows fed the high DCAD, indicating the high DCAD may alleviate the innate immune response. Roland et al.[41] assessed the white blood cell populations in healthy dairy cows, and reported that leukocytosis was often associated with stress, excitement, fear and calving. We proposed that high DCAD may relief the symptoms of heat stress in dairy cows by comforting their innate immune systems.

The effect of DCAD on postpartum energy metabolism in dairy cows has been reported [40,42]. In the present study, lipid concentrations were not affected by the DCAD treatments. However, plasma glucose was greater in the high DCAD group, which may be attributed to an improvement of dietary digestion in those cows.

In the present study, an increase in DCAD in diet from 335 to 507 mEq/kg DM for lactating cows subject to moderate heat stress had no significant effect on feed intake, milk yield, and milk composition; However, the apparent digestibility of diet was increased, and SCC in milk was significantly reduced. The respiratory frequency of cows was greater under heat stress but the frequency and rectal temperature were not affected by the difference in DCAD. The blood gas indexes and blood pH value were not affected. An increase in DCAD in diet could stabilize milk production and feed intake in cows under heat stress, improve milk quality, and alleviate heat stress to some extent.

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CONFLICT OF INTEREST

The authors have declared that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

PZ designed the study. XL drafted and wrote the manuscript. XL and ST collected and analyzed the data. XL and BF performed the animal trial and laboratory analysis. ZW, LY and ZT revised the manuscript. All authors gave intellectual input to the study and approved the final version of the manuscript.

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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