Reducing dietary cation–anion difference seldom affects rumen fermentation, cellulolytic bacteria populations, and microbiota in goats

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Research

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Abstract

Background: Dietary cation–anion difference (DCAD) has been receiving increased attention in recent years; however, information on the rumen fermentation, cellulolytic bacteria populations, and microbiota of goats fed a low-DCAD diet is less. This study aimed to evaluate the feasibility of feeding a low-DCAD diet for goats with emphasis on rumen fermentation parameters, cellulolytic bacteria populations and microbiota. Growth performance, urine pH, and plasma metabolites were also analyzed as well.

Materials and method: Eighteen goats were randomly allocated to 3 treatments with six replicates of each treatment and 1 goat per replicate. Animals were fed diets with varying DCAD levels at +338 (High DCAD; HD), +152 (Control; CON), and −181 (Low DCAD; LD). This study includes 15-d experimental period and 30-d adaption period.

Results: The DCAD level did not affect the rumen fermentation parameters including pH, buffering capability, acetic acid, propionic acid, butyric acid, total volatile fatty acids, and ratio of acetic acid/propionic acid ($P > 0.05$). The 4 main ruminal cellulolytic bacteria populations including *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Butyrivibrio fibrisolvens* and *Ruminococcus albus* did not differ from DCAD treatments ($P > 0.05$). The DCAD levels did not affect bacterial richness and diversity indicated by the indices Chao, Ace and Simpson and Shannon, respectively ($P > 0.05$). Both weighted UniFrac and unweighted UniFrac showed no difference in the composition of rumen microbiota for CON, HD and LD ($P > 0.05$). At the phylum level, *Bacteroidetes* was the predominant phylum followed by *Firmicutes, Synergistetes, Proteobacteria, Spirochaetae, and Tenericutes*, and they showed no difference ($P > 0.05$) in relative abundances except for *Firmicutes*, which was higher in HD and LD compared to CON ($P < 0.05$). At the genus level, relative abundance of 11 genera were not affected by DCAD treatments ($P > 0.05$). Level of DCAD had no effect ($P > 0.05$) on growth performance including dry matter intake, average net gain, average daily gain, and feed conversion ratio; and nutrients digestibility of crude protein, neutral detergent fiber, acid detergent fiber, and organic matter ($P > 0.05$). Urine pH in LD was lower than HD and CON ($P < 0.05$). LD resulted in higher plasma calcium than HD and CON ($P < 0.05$) but not for other plasma metabolites ($P > 0.05$).

Conclusion: We conclude that, with regard to the great importance of rumen fermentation, these results suggest that reducing DCAD is unharmful for rumen status and provide the feasibility of feeding a low-DCAD to goats.

Background

Dietary cation–anion difference (DCAD; mmol/kg dry matter [DM]) refers to the difference between the number of millimoles of major cations (Na^+ and K^+) and major anions (Cl^- and S^{2-}) of per kg of DM in the diet [1]. Variations in DCAD enables maintenance of electrolyte balance and osmotic pressure, improving the health status of animals. The initial benefit of feeding a low-DCAD diet was reported by Block [2], who observed that treatment with − 172.3 (mmol/kg DM) DCAD could prevent hypocalcemia

compared to a control DCAD of + 448.6 (mmol/kg DM). The DCAD level has been an important parameter in the process of formulating diets in recent years [3–7]. Studies have shown that diets with a lower DCAD could enhance the health and extend the economic life of transition mammary animals [8–10]. Recently, attention was given to the interaction of DCAD with calcium (Ca) concentration[11], cholecalciferol/calcidiol[12], 5-hydroxy-l-tryptophan [13], vitamin D [14–16], and duration [17, 18] of blood Ca level increases. Results of these studies indicated that lower DCAD in association with the above factors was effective in improving homeostasis of peripheral blood Ca. Most recently, Santos et al. [19] and Lean et al. [20] used meta-analyses to determine the effects of varying DCAD on the performance, production, and health of cows.

Ruminant diets usually include up to 70% roughage and so are fiber-rich. As the most important digestive organ, the rumen is the main site for digestion and utilization of nutrient, especially for degradation of cellulose by cellulolytic bacteria. Among rumen measurements, pH is the key parameter determining rumen fermentation status and is often lowered when ruminants are fed high levels of concentrate, resulting in ruminal acidosis [21–23]. Bacteria account for 95% of the total amount of rumen microorganisms [24], and thus are the main factor to modulate digestive and metabolic activity of the rumen[25]. *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* are reported the four most important cellulolytic bacteria for digestion and utilization of fiber in the rumen [26–29].

However, to our knowledge, there is less information on the rumen fermentation, cellulolytic bacteria populations and microbiota for goats fed a low-DCAD diet, therefore, the present study was conducted to evaluate the effect of a low-DCAD on the above-mentioned parameters of rumen pH, buffering capability, volatile fatty acids of acid, propionic acid, butyric acid, total volatile fatty acids, and acetic acid/propionic acid profiles; ruminal cellulolytic bacteria populations of *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, *Butyrivibrio fibrisolvens* and *Ruminococcus albus*, and microbiota. Growth performance, acid-base balance, plasma calcium level and metabolites were also measured. The results should provide a comprehensive evaluation of the feasibility of feeding a low-DCAD diet to goats.

Materials And Methods

Experimental design and animal management

The animal treatment procedures were approved by the local animal care and use committee. Using a randomized block design, 18 Qianbei miscellaneous goats (a native goat breed in the southwest of China; 30.07 kg initial weight and aged 13 months) were allotted to 3 treatments of six replicates of 1 goat per replicate. Animals were fed one of three diets with different DCAD levels (mmol/kg DM): +350 (HD), +100 (CON), and −150 (LD). Diets consisted of peanut straw (*Arachis hypogaea*), faba bean straw (*Vicia faba*) and concentrate which were mixed with $NaHCO_3$ (HD) or NH_4Cl (LD) and were pelleted (4 mm diameter), with a concentrate: roughage ratio of 30:70.

Goats were fed in separate metabolic cages. The experiment duration was 45 d including a 30-d adaption period and 15-d trial period. The adaption period was divided into three stages. In the first stage (1–12 d), goats were observed for health condition and treated for parasites and disinfected. During the second stage (13–18 d), goats were allowed to adjust to their respective diets. In the third stage (19–30 d), steady DM intake (DMI) was determined for individual goats. After that, in the trial period (31–45 d), goats were fed the treatment diets strictly according to the established DMI at 09:00 and 18:00. All goats had free access to water during the whole experiment. Ingredients and nutrient levels of diets for goats are shown in Table 1. The actual DCAD levels were measured as +338, +152, and −181, showing slight differences from the designed values of +350, +100, and −150 for HD, CON, and LD, respectively.

Table 1 Ingredients and nutrient compositions of diet for goat

Items	DCAD		
	HD	CON	LD
Ingredients			
Concentrate (%)[1]	30	30	30
Peanut straw (%)	50	50	50
Faba bean straw (%)	20	20	20
$NaHCO_3$ (g/d)	12	–	–
NH_4Cl (g/d)	–	–	15
Nutrient levels[2]			
DM (%)	93.70	93.77	93.90
CP (% DM)	13.40	13.64	14.21
NDF (% DM)	39.40	39.64	39.82
ADF (% DM)	30.35	30.62	30.64
OM (% DM)	88.02	87.03	87.71
Ca (% DM)	0.67	0.64	0.68
P (% DM)	0.48	0.49	0.49
Na (% DM)	0.52	0.19	0.14
K (% DM)	1.08	0.98	0.87
Cl (% DM)	0.23	0.27	1.25
S (% DM)	0.16	0.17	0.18
DCAD (mmol/kg DM)	+338	+152	-181

[1] Composition and proportion (%): corn 51.07, soybean meal 24.82, wheat bran 11.19, rapeseed meal 8.07, $CaHPO_4$ 0.45, lysine 0.27, methionine 0.29, NaCl 0.77, and premix 3.06.

[2] Actually measured values.

Samples and measurements

Nutrient levels and growth performance

Diet samples were collected daily during 19–45 d and stored at −20°C. At the end of the feeding experiment, dietary samples were composited and dried at 65°C and were ground to pass a 1-mm screen for proximate chemical composition determination of DM, crude protein (CP), organic matter (OM), Ca and P [30]; and neutral detergent fiber (NDF) and acid detergent fiber (ADF) [31]. An atomic absorption spectrophotometer (iCE 3000 SERIES, Thermo Fisher Scientific, USA) was used to measure Na and K contents. Silver nitrate titration was used to determine Cl concentration. The S level was determined using the magnesium nitrate method as described by Wang and Beede [32]. The DCAD was calculated using the following equation:

DCAD = Na (%)/0.0023 + K (%)/0.0039 − Cl (%)/0.00355 − S (%)/0.0016

All goats were weighed on d 32 as the initial weight and on 46 d as the final weight. The DMI was recorded daily for each goat calculated by allowance of refusals. The average net gain (ANG) was determined by subtraction of initial weight from final weight. The average daily gain (ADG) was determined by dividing ANG with the trial period (15 d). The feed conversion ratio (FCR) was the ratio of DMI to ADG. Total feces was collected during the last five consecutive days for nutrients digestibility analysis. Feces samples from individual goats were dried in an oven at 65°C for 48 h and ground to pass a 1-mm screen for approximate nutrient measurements: CP and OM [30] and NDF and ADF [31].

Rumen status

At 9:00, 13:00, and 17:00 on d 44, rumen fluid was collected through the esophageal cannula via a vacuum pump (VP30, Labtech Instrument Co. Ltd, Beijing, China) and was measured pH using a pH meter (PHS-3C, Youke Instrument Co. Ltd, Shanghai, China). Sample collected at 17:00 was used to assess rumen buffering capability (BC) as described by Tucker et al. [33], and the operational procedure was titrating a 10-mL aliquot of the sample from the original pH dropping to pH 5 using 1 N HCl.

Rumen fluid (20 mL) was used to detect volatile fatty acids (VFA), and the remaining sample was used for microbial high-throughput sequencing analysis. To determine the VFA, acidified samples were centrifuged at 4°C for 10 min (Thermo Fisher-ST 16R), and the supernatant fraction filtered through a 0.45-μm filter. The 1280 μL of filtrate was mixed with 600 μL of 20% metaphosphoric acid and 120 μL of crotonic acid (internal standard). The VFA concentrations in filtered samples were determined by gas chromatography (GC-2010-plus, Shimadzu, Japan) equipped with a flame ionization detector and a capillary column (SH-Rtx-Wax, Shimadzu), and nitrogen was used as the carrier gas. Rumen microbial high-throughput sequencing analysis was performed by TinyGen Bio-Tech (Shanghai) Co. Ltd.

PCR amplification of 16S rRNA genes and Miseq sequencing

Ruminal fluid samples were stored at −80°C and the DNA extracted from 200-mg samples using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The DNA concentration and purity were checked by running samples on 1.0% agarose gels.

The PCR amplification of 16S rRNA genes was performed using general bacterial primers: 515F 5′-GTGCCAGCMGCCGCGGTAA-3′ and 926R 5′-CCGTCAATTCMTTTGAGTTT-3′. The primers also contained the Illumina 5′-overhang adapter sequences for two-step amplicon library building, following the manufacturer's instructions for the overhang sequences. The initial PCR reactions were carried out in 25-μL reaction volumes with 1–2 μL of DNA template, 250 mM dNTPs, 0.25 mM of each primer, 1× reaction buffer, and 0.5 U of Phusion DNA Polymerase (New England Biolabs, USA). The PCR conditions consisted of initial denaturation at 94°C for 2 min, followed by 25 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s, with a final extension of 72°C for 5 min. The second step of PCR with dual eight-base barcodes was used for multiplexing. Eight-cycle PCR reactions were used to incorporate two unique barcodes to either end of the 16S amplicons. Cycling conditions consisted of one cycle of 94°C for 3 min, followed by eight cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 30 s, and a final extension of 72°C for 5 min. Prior to library pooling, the barcoded PCR products were purified using a DNA gel extraction kit (Axygen, China) and quantified using FTC-3000 TM real-time PCR. The libraries were sequenced by 2 × 300 bp paired-end sequencing on the MiSeq platform using MiSeq v3 Reagent Kit (Illumina) at Tiny Gene Bio-Tech (Shanghai) Co. Ltd.

Bioinformatic analysis

The raw fastq files were demultiplexed based on the barcode. The PE reads for all samples were run through Trimmomatic (version 0.35) to remove low-quality base pairs using parameters SLIDINGWINDOW: 50:20 and MINLEN: 50. Trimmed reads were then further merged using the FLASH program (version 1.2.11) with default parameters. The low-quality contigs were removed based on screen.seqs command using the following filtering parameters, maxambig = 0, minlength = 200, maxlength = 580, and maxhomop = 8. The 16S sequences were analyzed using a combination of software mothur (version 1.33.3), UPARSE (usearch version v8.1.1756, http://drive5.com/uparse/), and R (version 3.2.3). The demultiplexed reads were clustered at 97% sequence identity into operational taxonomic units (OTUs) using the UPARSE pipeline (http://drive5.com/usearch/manual/uparse cmds.html). The OTU representative sequences were used for taxonomic assignment against the Silva 128 database with a confidence score ≥0.6 by the classify.seqs command in mothur. The OTU taxonomies (from phylum to species) were determined based on NCBI. The four members of the rumen cellulolytic bacteria community (*B. fibrisolvens*, *F. succinogenes*, *R. flavefaciens*, and *R. albus*, % of total bacterial 16S rDNA) were selected from "Species" for statistical analysis.

Urine pH

For precise observation of the effect of DCAD level on acid–base balance *in vivo*, urine pH was measured once every 3 d during the first and second adaption periods (1–12 and 13–18 d), once every 2 days during the third stage (19–30 d), and daily for the trial period (31–45 d). Urine was immediately dipped with special indicator paper (5.4–7.0, SSSreagent Co. Ltd, Shanghai, China; 6.4–9.0, Fuyang Special Paper Co. Ltd, Hangzhou, China) when goats urinated.

Plasma metabolites

Blood samples (10-mL) of every goat were collected from the jugular vein into heparinized plastic syringes at 45 d. Samples were centrifuged at 805×g for 15 min to harvest plasma. Plasma was subsequently analyzed for levels of Ca, glucose (Glu), urea nitrogen (UN), alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (AKP), total protein (TP), albumin (Alb), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), and catalase (CAT).

Statistical analysis

The MIXED module in SAS 9.4 (SAS Institute Inc, Cary, NC, USA) was applied for analysis of experimental data. The DCAD levels (+338, +152, and −181) were designated as fixed effects, and goats as the random effect, then Tukey's method was adopted to determine differences among means of the three DCAD treatments. The experiment results were expressed as mean ± standard deviation. Statistical significance was defined as $P < 0.05$.

Results

Rumen fermentation

There were no significant differences in rumen pH for the same sampling intervals (0, 4, and 8 h; $P > 0.05$; Table 2) and all the collected mean among the HD, CON, and LD treatments (0, 4, and 8 h; $P > 0.05$). The variation of DCAD had no effect on ruminal BC and levels of acetic acid, propionic acid, butyric acid, total VFA (TVFA), and acetic acid/propionic acid (A/P) in the goats ($P > 0.05$).

Table 2 Effect of dietary cation–anion difference on the rumen pH of goats

Items		DCAD			SEM[1]	*P*-value
		HD (+338)	CON +152)	LD (-181)		
pH						
Sampling time interval (h)	Sampling time					
0	09:00	7.22 ± 0.19	7.21 ± 0.41	7.17 ± 0.36	0.14	0.97
	13:00	7.10 ± 0.22	7.17 ± 0.57	6.99 ± 0.49	0.23	0.86
	17:00	7.07 ± 0.25	7.20 ± 0.66	6.98 ± 0.54	0.26	0.83
4	13:00	7.05 ± 0.31	6.96 ± 0.36	7.04 ± 0.34	0.18	0.93
	17:00	7.08 ± 0.38	6.96 ± 0.52	7.14 ± 0.47	0.24	0.87
8	17:00	7.15 ± 0.23	6.99 ± 0.38	7.08 ± 0.46	0.19	0.86
Mean		7.20 ± 0.22	7.18 ± 0.41	7.17 ± 0.34	0.14	0.98
BC[2] (mL/L)		44.75 ± 0.88	43.35 ± 3.65	43.10 ± 3.81	1.38	0.67
Acetic acid (mmol/L)		42.22 ± 4.86	43.45 ± 5.71	44.81 ± 6.36	2.32	0.74
Propionic acid (mmol/L)		13.00 ± 1.96	13.07 ± 1.75	12.71 ± 2.37	0.84	0.95
Butyric acid (mmol/L)		8.00 ± 1.78	8.48 ± 2.28	8.11 ± 2.02	0.83	0.91
TVFA[3] (mmol/L)		63.21 ± 8.08	65.01 ± 8.89	65.63 ± 8.10	3.41	0.87
A/P[4]		3.27 ± 0.34	3.34 ± 0.36	3.67 ± 1.13	0.29	0.60

[1]SEM=standard error of mean. The same as below.

[2]BC=Buffering capability.

[3]TVFA = acetic acid + propionic acid + butyric acid.

[4]A/P = acetic acid/propionic acid.

Rumen cellulolytic bacteria

The relative contents of *F. succinogenes*, *R. flavefaciens*, *B. fibrisolvens*, *and R. albus* were not significantly affected among goats fed HD, CON, and LD diets ($P > 0.05$; Table 3). The proportions of *F. succinogenes* and *R. flavefaciens* were markedly increased with lower DCAD compared to *B. fibrisolvens* and *R. albus*.

Table 3 Effect of dietary cation–anion difference on the rumen cellulolytic bacteria communities of goats

Items	DCAD			SEM	*P*-value
	HD (+338)	CON (+152)	LD (-181)		
Fibrobacter succinogenes (%)	0.443±0.003 (48.63 %)	0.362±0.003 (41.18 %)	0.324±0.001 (62.91%)	0.098	0.70
Ruminococcus flavefaciens (%)	0.343±0.21 (37.65 %)	0.414±0.86 (47.10 %)	0.119±0.09 (23.11 %)	0.210	0.51
Butyrivibrio fibrisolvens (%)	0.12±0.12 (13.17 %)	0.085±0.07 (9.67 %)	0.065±0.03 (12.62 %)	0.033	0.59
Ruminococcus albus (%)	0.005±0.01 (0.55 %)	0.018±0.03 (2.05 %)	0.007±0.01 (1.36 %)	0.008	0.50

Sequencing and diversity of ruminal microbiota

After Illumina Miseq high-throughput sequencing, a total of 698,626 valid reads were obtained with an average length of 410 bp. The VENN graph showed that there were 1075 of total 1261 OTU in 3 groups shared. There were 22, 25, 5 individual OTUs, accounting for 1.89%, 2.33%, and 0.43% for LD, HD, CON, respectively (Fig. 1). Rarefaction curves were established to quantify the OUT coverage of sampling and each rarefaction tended to be gentle with the increase of sequence number, and meanwhile, the OTU rank abundance in the 3 groups exhibited a gentler slope and wider distribution on the horizontal axis (Fig. 2).

Fig. 1 The VENN graph of rumen bacterial of goats fed diets with varying cation-anion difference levels, which displaying that the disposition of operational taxonomic units (OTUs) among the control group (CON, n=6), high dietary cation-anion difference (HD, n=6) and low dietary cation-anion difference (LD, n=6) in rumen fluid microbiota.

Fig. 2 Rarefaction curves (A) and the OTU rank abundance (B) of rumen bacterial of goats fed diets with varying cation-anion difference levels. The CON group: CON1, CON2, CON3, CON4, CON5, CON6. The HD group: HD1, HD2, HD3, HD4, HD5, HD6. The LD group: LD1, LD2, LD3, LD4, LD5, LD6.

Alpha diversity results showed that DCAD levels did not affect Chao, Ace, Simpson, and Shannon as listed in Table 4 ($P > 0.05$). The Chao, Ace, Shanno and Simpson chart of each group was also tended to be gentle corresponding to the increase of sequence number (Fig. 3).

Table 4 Effect of varying dietary cation-anion difference levels on rumen bacterial community richness and diversity of goats

Items		Dietary treatments[1]			SEM	*P*-value
		HD (+338)	CON (+152)	LD (−181)		
Bacterial Richness	Chao	906±89	843±129	811±116	4.28	0.333
	Ace[2]	900±82	834±117	815±96	4.33	0.356
Bacterial Diversity	Simpson	0.027±0.02	0.033±0.01	0.028±0.01	0.04	0.760
	Shannon	4.87±0.31	4.45±0.21	4.69±0.29	0.21	0.048

[1]Dietary treatments: HD: High dietary cation-anion difference; CON:Control group; LD: Low dietary cation-anion difference.

[2]Ace=abundance-based coverage estimator.

Fig. 3 Rarefaction curves (A) and the OTU rank abundance (B) of rumen bacterial of goats fed diets with varying cation-anion difference levels. The CON group: CON1, CON2, CON3, CON4, CON5, CON6. The HD group: HD1, HD2, HD3, HD4, HD5, HD6. The LD group: LD1, LD2, LD3, LD4, LD5, LD6.

According to Fig. 4, both weighted UniFrac (axis 1 + axis 2 = 66.7%, Fig. 4a) and unweighted UniFrac (axis 1 + axis 2 = 38.37%, Fig. 4b) were observed no difference in the composition of rumen microbiota for CON, HD and LD ($P > 0.05$).

Fig. 4 Principle coordinate analysis (PCoA) based on weighted UniFrac and unweighted UniFrac on rumen bacterial of goats fed diets with varying cation-anion difference levels. (a) PCoA based on weighted UniFrac of rumen bacterial of goats fed diets with varying cation-anion difference levels. (b) PCoA based on unweighted UniFrac of rumen bacterial of goats fed diets with varying dietary cation-anion difference levels. The percentage of variation explained by PC1 (first principal component) and PC2 (second principal component) was indicated in the axis. CON = the control group (n = 6); HD= high dietary cation-anion difference (n = 6); LD = low dietary cation-anion difference (n = 6).

Taxonomic classification summary indicated that 16 phyla were tested in all samples (Fig. 5A). At the phylum level, Bacteroidetes (61.60%) was the predominant phylum followed by Firmicutes, Synergistetes, Proteobacteria, Spirochaetae, Tenericutes with average relative abundances of 25.32%, 5.84%, 1.82%, 2.08%, 1.2%, respectively, but there was no difference ($P > 0.05$) among the groups on the above phylum levels except for Firmicutes, which was significantly higher in HD and LD compared to CON ($P=0.008$, Table 5).

At the genus level, taxon displayed that the relative abundance of 11 genera were not affected by DCAD among all samples ($P > 0.05$; Fig. 5B). At the same time, *Prevotella, Paraprevotella, Selenomonas, Ruminococcus, Ruminococcus, Butyrivibrio, Quinella, Fretibacterium and Treponema* showed no grouping difference of the genera across treatments ($P > 0.05$). Among the genera with relative abundance exceeded 0.1%, prevotella was the dominant genus in each group with the highest proportion (Table 5).

Fig. 5 Distributions of microbiota at phyla and genus level. (a) Relative abundances of phyla levels are depicted as mean values for the CON, the LD (Low dietary cation-anion difference) group and HD (High dietary cation-anion difference) group. (b) Distributions of genera in rumen fluid of the CON, the LD (Low dietary cation-anion difference) group and HD (High dietary cation-anion difference) group.

Table 5 Effect of varying dietary cation-cation difference on relative abundance (%) of bacteria taxa > 0.1% of average abundance in the rumen fluid of goats

Phylum	Genus	Dietary treatments[1]			SEM	*P*-value
		HD	CON	LD		
Bacteroidetes		60.40±6.24	65.08±4.41	59.33±9.13	2.81	0.328
	Prevotella	18.92±3.66	24.16±2.72	16.09±2.68	3.05	0.223
	Paraprevotella	3.58±1.23	2.76±0.89	3.51±1.52	1.24	0.877
Firmicutes		28.43±4.90[a]	18.71±5.30[b]	28.81±6.08[a]	2.23	0.008
	Selenomonas	1.59±0.97	0.56±0.29	1.32±0.89	0.78	0.629
	Ruminococcus	1.07±0.16	1.54±0.74	1.29±0.39	0.49	0.786
	Succiniclasticum	1.34±0.36	0.69±0.09	0.68±0.24	0.25	0.143
	Butyrivibrio	1.02±0.32	0.46±0.13	0.48±0.01	0.20	0.118
	Quinella	2.29±0.37	0.71±0.30	3.73±2.15	1.27	0.277
Synergistetes		5.20±1.87	6.34±1.91	5.99±2.29	2.03	0.923
	Fretibacterium	5.18±1.88	6.31±1.92	5.97±2.30	2.04	0.923
Spirochaetae		1.44±0.25	2.13±0.74	1.89±0.35	0.49	0.610
	Treponema	0.72±0.16	0.98±0.52	1.39±0.35	0.37	0.458
Proteobacteria		1.10±0.42	4.49±2.38	0.67±0.07	1.40	0.140
Tenericutes		1.07±0.17	1.20±0.16	1.33±0.44	0.29	0.817

Growth performance

Levels of DMI were unaffected by DCAD variations (P > 0.05; Table 6). Lower DCAD had no effect (P > 0.05) on gwowth performance of final weight, ANG, ADG, and FCR and digestibility of crude protein, NDF, ADF, and OM for goats.

Table 6 Effect of dietary cation–anion difference on growth performance of goats

Items	DCAD			SEM	*P*-value
	HD (+338)	CON (+152)	LD (-181)		
Performance					
Initial weight (kg)	30.35 ± 3.4	30.43 ± 2.6	29.44 ± 3.7	1.36	0.86
DMI (g/d)	899.0 ± 213.6	857.5 ± 120.9	864.0 ± 124.3	64.85	0.89
Final weight (kg)	31.33 ± 3.4	31.50 ± 3.8	30.41 ± 3.3	1.48	0.82
ANG (kg)	0.98 ± 0.3	1.07 ± 0.4	0.97 ± 0.3	0.21	0.89
ADG (g/d)	65.3 ± 12.2	71.3 ± 22.7	64.7 ± 18.8	8.28	0.63
FCR	13.76 ± 6.6	12.02 ± 3.5	13.36 ± 5.9	3.00	0.81
Digestibility (%)					
CP (%)	56.37 ± 2.41	58.66 ± 5.91	57.68 ± 5.42	1.97	0.75
NDF (%)	50.26 ± 6.01	45.84 ± 8.39	45.58 ± 7.65	3.12	0.50
ADF (%)	51.19 ± 6.84	48.35 ± 7.74	47.59 ± 8.27	3.20	0.71
OM (%)	62.34 ± 3.59	57.73 ± 5.66	58.59 ± 3.92	2.11	0.36

Urine pH

There was no difference ($P > 0.05$) in urine pH for HD, CON, and LD during the observation period of 1–12 d, with pH values of 8.48, 8.43, and 8.46, respectively (Fig. 6a). Urine pH decreased slightly (8.45, 8.50, and 8.13 for HD, CON, and LD, respectively) during the dietary replacement period (13–18 d). Urine pH decreased significantly (8.43, 8.36, and 7.40 for HD, CON, and LD, respectively) with LD obviously lower than both HD and CON. During the trial period (31–45 d), compared with HD and CON, LD reduced urine pH over HD and CON (8.43, 8.42, and 6.75 for HD, CON, and LD, respectively; $P < 0.05$). Urine pH values were unaffected by DCAD variation between HD and CON ($P > 0.05$). Furthermore, urine pH was closely related to DCAD levels within the trial period (31–45 d; $R^2 = 0.9066$, $P < 0.05$; Fig. 6b).

Fig. 6a Goat urine pH for dietary cation–anion difference levels throughout the experiment

Fig. 6b Association between urine pH and dietary cation-anion difference of goats in the trial period

Plasma Metabolites

Feeding of the LD diet resulted in the highest plasma Ca level (Table 7), which was significantly higher than both HD and CON ($P < 0.05$).There were no significant differences in plasma Glu, UN, ALT, AST, AKP, TP, Alb, GSH-Px, CAT, SOD, and MDA among the DCAD treatments ($P > 0.05$; Table 7).

Table 7 Effect of dietary cation–anion difference on the plasma metabolites of goats

Items	DCAD			SEM[1]	*P*-value
	HD (+338)	CON (+152)	LD (−181)		
Ca (mmol/L)	2.31 ± 0.21	2.38 ± 0.17	2.91 ± 0.17	0.08	<0.01
Glu (mmol/L)	4.57 ± 1.06	5.07 ± 0.91	4.60 ± 0.36	0.34	0.56
UN (mmol/L)	6.22 ± 0.66	5.63 ± 1.09	6.24 ± 1.16	0.41	0.50
ALT (IU/L)	8.61 ± 2.55	10.22 ± 3.21	10.76 ± 3.57	1.70	0.85
AST (IU/L)	11.25 ± 3.63	13.98 ± 4.77	11.91 ± 3.41	2.01	0.80
AKP (King unit/100 mL)	20.85 ± 6.90	21.73 ± 12.57	17.81 ± 6.92	3.75	0.75
TP (g/L)	99.38 ± 14.10	112.89 ± 28.72	92.62 ± 21.62	11.47	0.37
Alb (g/L)	36.38 ± 4.34	37.11 ± 6.36	35.19 ± 6.31	2.35	0.85
GSH-Px (U/mL)	670.59 ± 127.49	755.29±296.72	709.41 ± 164.46	152.50	0.59
CAT (U/mL)	2.35 ± 0.79	1.95 ± 0.60	2.47 ± 1.03	0.66	0.84
SOD (U/mL)	68.53 ± 9.27	63.59 ± 8.59	67.98 ± 6.79	3.38	0.43
MDA (nmol/mL)	37.14 ± 5.57	34.61 ± 7.06	36.79 ± 5.94	2.54	0.75

Discussion

Rumen pH has a key role in measuring rumen status and can reflect the composition and abundance of rumen microflora. Rumen health is maintained by an appropriate pH which is in range of 6.26 ~ 6.79 [34]. Rumen pH decreases when ruminants are fed high levels of concentrate. This is quite different from the acidified rumen status induced by increases in anion (Cl^- and S^{2-}) concentrations in the diet and which elevates Cl^- and S^{2-} in the urine and so reduces urine pH. In this study, rumen pH was unaffected for all DCAD treatments and ruminal fluid sampling time points. This was also shown in the study of Apper-Bossard [35].

Briggs et al. [36] found that rumen BC was closely related to rumen pH, and Church [37] argued that the rumen buffer system was controlled by pH, pCO_2, and VFA. The rumen BC can maintain a stable rumen status by keeping any sudden rise or fall in rumen pH within a certain range [38]. The rumen has a relatively stable buffer system, which is closely related to the feed, saliva, and secretion of the rumen wall and maintains the rumen internal environment. In our study, the lack of significant difference in rumen BC and pH showed that DCAD reduction did not influence the rumen internal environment. However, the rumen BC was numerically elevated with increased DCAD level on cows when adding $NaHCO_3$ [33, 39, 40]. The explanation could be attributed to the use of a different animal species and different dietary ingredients. Generally, up to now there has been insufficient information on rumen BC in goats, and further study is needed due to the importance of BC for rumen status.

The rumen VFA profile is mainly impacted by the proportion of concentrate and forage in the diet. Increasing DCAD, by adding K and Na, had no effect on rumen VFA concentration [41]. Tucker et al. [42] reported that the rumen VFA profile was unaffected by DCAD levels of − 100, 0, 100, and 200. Apper-Bossard et al. [35] found no significant difference in VFA concentration with varying DCAD. These results are consistent with the present study in which rumen VFA concentration was not significantly affected by DCAD level. Correspondingly, the VFA profiles and A/P levels were unaffected by DCAD. This indicates that the rumen fermentation pattern was unaltered by the three DCAD treatments.

In the current study, DCAD variation had no influence on the populations of *B. fibrisolvens*, *F. succinogenes*, *R. flavefaciens*, and *R. albus*. This result indicates that reducing DCAD would not affect the growth and colonization of rumen cellulolytic bacteria. This is likely associated with stable rumen pH and VFA maintained by the constant ratio of concentrate to roughage (30:70) for the three DCAD diets used in this study. Grilli et al. [43] reported that a high proportion of maize disturbed the ruminal bacterial ecosystem of goats. Wang et al. [44] and Li et al. [45] noted that the relative quantity of ruminal *B. fibrisolvens*, *F. succinogenes*, *R. flavefaciens*, and *R. albus* was improved with decreasing rumen pH. In our most recent study (unpublished data) with dairy goats as experimental animals fed 4 DCAD levels at 349, 120, and − 167, respectively, rumen BC was not influenced. This result also supports the conclusions in the present study.

The microbiota composition of the gastrointestinal tract (GIT) influence the health of animals as well as productivity [46], the structure and composition of ruminant microbial community are often devoted to evaluating the health status of the host and ensuring the healthy development of the rumen. Furthermore, the diversity and composition of the GIT microbiota can be influenced by many factors including age, diet, feeding management and feed additives [47, 48]. Microbial richness in rumen will be altered with dietary composition [49, 50]. Dietary nutrients are fermented by rumen rumen microbial, such as bacteria, fungi and protozoa, and then degraded into VFA and MCP to provide energy for ruminants [51] and to guarantee the healthy and stable rumen environment [52, 53]. In such reactions, rumen pH plays a decisive role in the composition and abundance of rumen microflora, and is an important indicator reflecting whether the composition and abundance of rumen microflora are normal [54, 55]. Similarly, rumen microbial is also influenced by animal species, diet composition and different ages [56–58].

The study of Zhang et al. [59] showed that rumen bacterial species, Chao index and Ace index were affected by rumen pH which can alter the bacterial community structure. Accordingly, Guo [60] found that decreased rumen pH would up-regulate bacterial diversity, composition and abundance of bacteria. In this experiment, rumen pH was not impacted by DCAD level, which exactly explained why the Chao index, Ace index, Simpson index and Beta diversity of the three groups were homogeneous. The Shannon index of the HD group was the highest, possibly because $NaHCO_3$ was added as a buffer to neutralize gastric acid and was essential for stomach health by creating a suitable internal environment for rumen microorganisms. Reducing DCAD had little effect on Shannon index. In addition, the dominant bacteria of the three groups in this experiment were *Bacteroidetes* followed by *Firmicutes* and *Synergistetes*, which was coincide with the results of previous studies on the basis of phylum [61–63]. In terms of genus level, the relative abundance of *Prevotella*, one of the primary protein-degrading microorganisms of Qianbei miscellaneous goats, was the highest, which was supported by the results of other ruminant studies [64]. The *Firmicutes* are a kind of intestinal bacteria related to obesity can degrade insoluble fiber and play an important role in the process of substance metabolism in rumen. The abundance of *Firmicutes* in HD and LD were 51.95% and 53.98% higher than CON, respectively in the study. The up-regulation in *Firmicutes* abundance might be due to our application of a high forage diet. In summary, neither the reduction of DCAD have a negative effect on the bacterial diversity nor changed the content of the main bacterial genus.

As described above, rumen pH, BC, VFA, cellulolytic bacteria population and rumen microflora diversity are crucial in the fermentation status of ruminants. The unaffected these parameters in the present study provide further reliable information and the feasibility on feeding a low-DCAD diet to goats.

Level of feed intake is the most important prerequisite for animal growth performance. Generally, pure anionic salt exerts some reduction on feed intake when it was simply mixed into diet, due to its bitter taste and poor palatability [65, 66]. Therefore, improving palatability of anionic salt is important for DMI and growth performance. Our previous study [67] showed that DMI of goats fed a low-DCAD did not decrease because the anionic salts were mixed with molasses and dried distillers grains with solubles. Takagi and Block [68, 69] also observed that reducing DCAD did not impact DMI containing anionic salts when feeding a total mixture ration. Diets were pelleted in our study, and therefore, they were unaffected for goats fed diet HD, CON, and LD. This indicates that DMI is unaffected by anionic salts inclusion as long as the bitter taste is concealed.

The levels of DCAD had no effect on goat final weights. This can be attributed to the similar DMI level and possibly because the goats in this experiment were a local breed, with their adult steady body weight averaging as much as 35 kg, and thus there was limited potential for body weight gain. Accordingly, ADG and FCR did not show difference among goats provided HD, CON, or LD diets.

Nutrients digestibility can be used to measure the digestion and absorption degree of a diet. Correlative analysis in the study demonstrated the digestibility of CP, NDF, ANF, and OM were unaffected for goats fed the three DCAD diets. Apper-Bossard et al. [35] reached similar conclusions. Positive DCAD diet is

beneficial to the growth and reproduction of rumen cellulolytic bacteria, and can maintain the activity of digestive enzymes in the digestive tract, thus improving nutrient digestibility [70]. However, different levels of DCAD did not alter the digestibility of CP, NDF, ADF and OM with the rumen pH and rumen cellulolytic bacteria community remained unchanged in this research.

Urine pH is a useful indicator to monitor the effect of a reduced DCAD diet on acid–base balance in goats and sheep [71, 72], dairy cows [8, 73, 74], and buffalo [4]. This phenomenon can be explained using the strong ion difference theory of Stewart [75], who argued that with reductions of DCAD, the concentration of anions in blood would increase and cause the kidney to expel redundant H^+ in urine, resulting in lower urine pH. The recommended urine pH is 6.5–6.8, because too low a level would exert a burden on the kidneys [76–78]. Our results showed that urine pH value in goats fed LD was lower than HD and CON. This is accordant with the recommended level, and there is a strong association between DCAD and urine pH in the trial period, suggesting that the LD level is appropriate for the diet of goats.

Muscle contraction, conduction of nervous impulses, and signal transduction are closely dependent on blood Ca homeostasis. Following the study of Block [2] and subsequent results of ruminant researchers [71, 79, 80], reducing the DCAD level has been the most commonly used strategy to increase blood Ca levels in transition mammary animals [81]. Our previous study showed that reducing DCAD could increase the plasma Ca concentration of female goats [67]. In the current experiment, the LD caused higher plasma Ca level than HD and CON by 25.97% and 22.27%, respectively, indicating more stable blood Ca homeostasis. Horst et al. [82] and Goff and Horst [83] claimed this may be because LD-induced acidic status enhanced Ca absorption in the gastro-intestine and also increased Ca resorption in the bone, facilitating Ca matrix flow into blood for easier transfer from lumen to blood.

In the review of Khanal and Nemere [84] who described three steps for Ca absorption. First, Ca^{2+} influx occurs at the apical membrane via epithelial Ca^{2+} channels, which include the transient receptor potential vanilloid receptor 6 (TRPV6). This step is considered to be rate-limiting for transcellular Ca^{2+} transport [85]. Second, intracellular diffusion is facilitated by the vitamin D-dependent calcium binding protein D9k (CaBP-D9k) [86]. Last, extrusion at the basolateral membrane is achieved by either the Na^+/Ca^{2+} exchanger1 (NCX1) or the plasma membrane calcium ATPase 1b (PMCA1b) [87]. The proteins of TRPV6, CaBP-D9k, and PMCA1b are believed to be the three key factors involved in the Ca absorption process, therefore, we speculate that TRPV6, CaBP-D9k, and PMCA1b expression levels in the intestine might be upregulated for animals fed the LD diet. However, a lack of data on TRPV6, CaBP-D9k, and PMCA1b expression in the intestine limits our further discussion. Future work is needed in this area.

Blood measurements are useful to reflect the metabolic status of animals. Our results showed that all plasma levels of Glu, UN, ALT, AST, AKP, TP, Alb, SOD, GSH-Px, MDA, and CAT were unaffected by DCAD variation. This indicates that reducing DCAD had no effect on nutrient metabolite processes in the plasma. This is supported by previous study. Melendez and Poock [9] reported that lowering DCAD had little effect on blood Alb. Wu et al. [67] found that DCAD level (+ 300, + 150, 0, and − 150) had no significant effect on plasma GSH-Px and MDA content in female goats.

Conclusion

Reducing DCAD has little influence on rumen status and rumen microbiota, showing no harmful to rumen fermentation of goats. Blood Ca level is increased and urine pH is decreased by DCAD reduction. These results indicate the feasibility of feeding a low-DCAD diets to goats.

Abbreviations

DCAD
Dietary cation-anion difference; HD:High DCAD; CON:Control; LD:Low DCAD; DM:Dry matter; DMI:Dry matter intake; NDF:Neutral detergent fiber; ADF:Acid detergent fiber; AIA:Acid insoluble ash; VFA:Volatile fatty acid; TVFA:Total volatile fatty acid; A/P:Acetate/Propionate; NH_3-N:Ammonia nitrogen; CP:Crude protein; OM:Organic matter; ANG:Average net gain; ADG:Average daily gain; F/G:Feed/Gain; FCR:Feed conversion ratio; BC:Buffer capability; Glu:Glucose; UN:Urea nitrogen; ALT:Alanine aminotransferase; AST:Aspartate transaminase; SOD:Superoxide dismutase; AKP:Alkaline phosphatase; ALB:Albumin; TP:Total protein; GSH-Px:Glutathione peroxidase; MDA:Malondialdehyde; CAT:Catalase; SEM:Standard error of means

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval

All procedures with animals received prior approval from the Animal Care and Use Committee of the Guizhou University and followed the regulations and guidelines for animal care and welfare established by the committee.

Consent for publication

Not applicable.

Competing interests

All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

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Authors' contributions

K.Y., X.T., and W.W. conceived and designed the experiment. S.J., L.S., E.H., F.W., Z.G., and Z.M. carried out the farm and the collection of samples. K.Y. and S.J. gathered and analyzed the data, and both took charge for data interpretation and sequencing data (figures and tables), and K.Y. and X.T. wrote the manuscript. T.H. made feasible suggestions for the manuscript. WW acted as overall study director. All authors have read and approved the final manuscript.

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References

1. Riond JL. Animal nutrition and acid-base balance. Eur J Nutrit. 2001;40:245–54.
2. Block E. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J Dairy Sci. 1984;67:2939–48.
3. Graham-Thiers PM, Kronfeld DS. Dietary protein influences acid-base balance in sedentary horses. J Equine Vet Sci. 2005;25:434–8.
4. Shahzad MA, Sarwar M, Mahr-un-Nisa. Influence of varying dietary cation anion difference on serum minerals, mineral balance and hypocalcemia in Nili Ravi buffaloes. Livest Sci. 2008;113:52–61.
5. Edwards LN, Engle TE, Paradis MA, Correa JA, Anderson DB. Persistence of blood changes associated with alteration of the dietary electrolyte balance in commercial pigs after feed withdrawal, transportation, and lairage, and the effects on performance and carcass quality. J Anim Sci. 2010;88:4068–77.
6. Nørgaard JV, Højberg O, Sørensen KU, Eriksen J, Medina JMS, Poulsen HD. The effect of long-term acidifying feeding on digesta organic acids, mineral balance, and bone mineralization in growing pigs. Anim Feed Sci Tech. 2014;195:58–66.
7. Heer F, Dobenecker B, Kienzle E. Effect of cation-anion balance in feed on urine pH in rabbits in comparison with other species. J Anim Physiol Anim Nutr. 2017;101:1324–30.
8. Leno BM, Ryan CM, Stokol T, Kirk D, Zanzalari KP, Chapman JD, et al. Effects of prepartum dietary cation-anion difference on aspects of peripartum mineral and energy metabolism and performance

of multiparous Holstein cows. J Dairy Sci. 2017;100:4604–22.

9. Melendez P, Poock S. A dairy herd case investigation with very low dietary cation–anion difference in prepartum dairy cows. Front Nutr. 2017;4:26.
10. Al-Rabadi G, Al-Hijazeen M. Variation in dietary cation-anion differences (DCAD) of feed ingredients in relation to milk fever disease in dairy cattle. Ukr J Ecol. 2018;8:51–6.
11. Diehl AL, Bernard JK, Tao S, Smith TN, Kirk DJ, McLean DJ, et al. Effect of varying prepartum dietary cation-anion difference and calcium concentration on postpartum mineral and metabolite status and milk production of multiparous cows. J Dairy Sci. 2018;101:9915–25.
12. Rodney RM, Martinez NP, Celi P, Block E, Thomson PC, Wijffels G, et al. Associations between bone and energy metabolism in cows fed diets differing in level of dietary cation-anion difference and supplemented with cholecalciferol or calcidiol. J Dairy Sci. 2018a;101:6581–601.
13. Slater CJ, Endres EL, Weaver SR, Cheng AA, Lauber MR, Endres SF, et al. Interaction of 5-hydroxy-l-tryptophan and negative dietary cation-anion difference on calcium homeostasis in multiparous peripartum dairy cows. J Dairy Sci. 2018;101:5486–501.
14. Martinez N, Rodney RM, Block E, Hernandez LL, Nelson CD, Lean IJ, et al. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Lactation performance and energy metabolism. J Dairy Sci. 2018a;101:2544–62.
15. Martinez N, Rodney RM, Block E, Hernandez LL, Nelson CD, Lean IJ, et al. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Health and reproductive responses. J Dairy Sci. 2018b;101:2563–78.
16. Rodney RM, Martinez N, Block E, Hernandez LL, Celi P, Nelson CD, et al. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Vitamin D, mineral, and bone metabolism. J Dairy Sci. 2018b;101:2519–43.
17. Collazos C, Lopera C, Santos JEP, Laporta J. Effects of the level and duration of maternal diets with negative dietary cation-anion differences prepartum on calf growth, immunity, and mineral and energy metabolism. J Dairy Sci. 2018;100:9835–50.
18. Lopera C, Zimpel R, Vieira-Neto A, Lopes FR, Ortiz W, Poindexter M, et al. Effects of level of dietary cation-anion difference and duration of prepartum feeding on performance and metabolism of dairy cows. J Dairy Sci. 2018;101:7907–29.
19. Santos JEP, Lean IJ, Golder HM, Block E. Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. J Dairy Sci. 2019;102:2134–54.
20. Lean IJ, Santos JEP, Block E, Golder HM. Effects of prepartum dietary cation-anion difference intake on production and health of dairy cows: A meta-analysis. J Dairy Sci. 2019;102:2103–33.
21. Fanning JP, Hynd PI, Cockcroft PD. The relative roles of the ruminal fluid and epithelium in the aetiology of ruminal acidosis. Small Ruminant Res. 2018;162:57–62.
22. Giger-Reverdin S. Recent advances in the understanding of subacute ruminal acidosis (SARA) in goats, with focus on the link to feeding behaviour. Small Ruminant Res. 2008;163:24–8.

23. Khiaosa-ard R, Pourazad P, Aditya S, Humer E, Zebeli Q. Factors related to variation in the susceptibility to subacute ruminal acidosis in early lactating Simmental cows fed the same grain-rich diet. Anim Feed Sci Tech. 2018;238:111–22.
24. Mackie RI, Aminov RI, White BA, Mcsweeney CS, Cronje PB. Molecular ecology and diversity in gut microbial ecosystems. Ruminant Physiol Digest Metabol Growth Reproduct. 2000;2:61–77.
25. Shi PJ, Meng K, Zhou ZG, Wang YR, Diao QY, Yao B. The host species affects the microbial community in the goat rumen. Lett Appl Microbiol. 2008;46:132–5.
26. Russell JB, Muck RE, Weimer PJ. Quantitative analysis of cellulose degradation and growth of cellulolytic bacteria in the rumen. FEMS Microbiol Ecol. 2009;67:183–97.
27. Dai X, Tian Y, Li J, Su X, Wang X, Zhao S, et al. Metatranscriptomic analyses of plant cell wall polysaccharide degradation by microorganisms in the cow rumen. Appl Environ Microb. 2015;81:1375–86.
28. Deng Y, Huang Z, Ruan W, Zhao M, Miao H, Ren H. Co-inoculation of cellulolytic rumen bacteria with methanogenic sludge to enhance methanogenesis of rice straw. Int Biodeter Biodegr. 2017;117:224–35.
29. Sousa DO, Oliveira CA, Velasquez AV, Souza JM, Chevaux E, Mari LJ, et al. Live yeast supplementation improves rumen fibre degradation in cattle grazing tropical pastures throughout the year. Anim Feed Sci Tech. 2018;236:149–58.
30. AOAC. Official methods of analysis. 15th ed. Vol. I. Arlington: Association of Official Analtical Chemists; 1990.
31. Van Soest PJ, Robertson JB, Lewis BA. Methods for dietary fiber, neutral detergentfiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 1991;74:3583–97.
32. Wang C, Beede DK. Effects of supplemental protein on acid-base status and calcium metabolism of nonlactating Jersey cows. J Dairy Sci. 1990;73:3178–86.
33. Tucker WB, Hogue JF, Aslam M, Lema M, Martin M, Owens FN, et al. A buffer value index to evaluate effects of buffers on ruminal milieu in cows fed high or low concentrate, silage, or hay diets. J Dairy Sci. 1992;75:811–9.
34. Russell JB, Wilson DB. Why Are Ruminal Cellulolytic Bacteria Unable to Digest Cellulose at Low pH? J Dairy Sci. 1996;79:1503–9.
35. Apper-Bossard E, Faverdin P, Meschy F, Peyraud JL. Effects of dietary cation-anion difference on ruminal metabolism and blood acid-base regulation in dairy cows receiving 2 contrasting levels of concentrate in diets. J Dairy Sci. 2010;93:4196–210.
36. Briggs PK, Hogan JP, Reid RL. Effect of volatile fatty acids, lactic acid and ammonia on rumen pH in sheep. Aust J Agr Res. 1957;8:674–90.
37. Church DC. Digestive physiology and nutrition of ruminants. Vol. 1 - Digestive physiology. Portland Oregon. 1976;169:148.

38. Tucker WB, Aslam M, Lema M, Shin IS, Le Ruyet P, Hogue JF, et al. Sodium bicarbonate or multielement buffer via diet or rumen: effects on performance and acid-base status of lactating cows. J Dairy Sci. 1992;75:2409–20.
39. Aslam M, Tucker WB, Hogue JF, Vernon RK, Adams GD. Controlled ruminal infusion of sodium bicarbonate. 2. Effects of dietary and infused buffer on ruminal milieu. J Dairy Sci. 1991;74:3496–504.
40. Zali A, Nasrollahi SM, Khodabandelo S. Effects of two new formulas of dietary buffers with a high buffering capacity containing Na or K on performance and metabolism of mid-lactation dairy cows. Prev Vet Med. 2019;163:87–92.
41. Catterton TL, Erdman RA. The effect of cation source and dietary cation-anion difference on rumen ion concentrations in lactating dairy cows. J Dairy Sci. 2016;99:6274–84.
42. Tucker WB, Harrison GA, Hemken RW. Influence of dietary cation-anion balance on milk, blood, urine, and rumen fluid in lactating dairy cattle. J Dairy Sci. 1988;71:346–54.
43. Grilli DJ, Fliegerová K, Kopečný J, Lama SP, Egea V, Sohaefer N, et al. Analysis of the rumen bacterial diversity of goats during shift from forage to concentrate diet. Anaerobe. 2016;42:17–26.
44. Wang C, Liu Q, Guo G, Huo WJ, Ma L, Zhang YL, et al. Effects of rumen-protected folic acid on ruminal fermentation, microbial enzyme activity, cellulolytic bacteria and urinary excretion of purine derivatives in growing beef steers. Anim Feed Sci Tech. 2016;221:185–94.
45. Li HQ, Liu Q, Wang C, Guo G, Huo WJ, Zhang SL, et al. Effects of rumen-protected pantothenate on ruminal fermentation, microbial enzyme activity, cellulolytic bacteria and urinary excretion of purine derivatives in growing beef steers. Livest Sci. 2017;202:159–65.
46. Uyeno Y, Shigemori S, Shimosato T. Effect of Probiotics/Prebiotics on Cattle Health and Productivity. Microbes Environ. 2015;30:126–32.
47. Castro JJ, Gomez A, White BA, Mangian HJ, Loften JR, Drackley JK. Changes in the intestinal bacterial community, short-chain fatty acid profile, and intestinal development of preweaned Holstein calves. 1. Effects of prebiotic supplementation depend on site and age. J Dairy Sci. 2016;99:9682–702.
48. Malmuthuge N, Griebel PJ. Guan le L. The Gut Microbiome and Its Potential Role in the Development and Function of Newborn Calf Gastrointestinal Tract. Front Vet Sci. 2015;2:36.
49. Creevey CJ, Kelly WJ, Henderson G, Leahy SC. Determining the culturability of the rumen bacterial microbiome. Microb Biotechnol. 2014;7:467–79.
50. Henderson G, Cox F, Ganesh S, Jonker A, Young W, Collaborators GRC, et al. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci Rep-UK. 2015;5:14567.
51. Rey M, Enjalbert F, Combes S, Cauquil L, Bouchez O, Monteils V. Establishment of ruminal bacterial community in dairy calves from birth to weaning is sequential. J Appl Microbiol. 2014;116:245–57.
52. Guo W, Li Y, Wang LZ, Wang JW, Xu Q, Yan TH, et al. Evaluation of composition and individual variability of rumen microbiota in yaks by 16S rRNA high-throughput sequencing technology.

Anaerobe. 2014;34:74–9.

53. John GK, Mullin GE. The Gut Microbiome and Obesity. Curr Oncol Rep. 2016;18:45.
54. Zhang L, Chung JS, Jiang QQ, Sun R, Zhang J, Zhong YJ, et al. Characteristics of rumen microorganisms involved in anaerobic degradation of cellulose at various pH values. Rsc Adv. 2017;7:40303–10.
55. Liang YS, Li GZ, Li XY, Lü JY. Li FD, Tang DF, et al. Growth performance, rumen fermentation, bacteria composition, and gene expressions involved in intracellular pH regulation of rumen epithelium in finishing Hu lambs differing in residual feed intake phenotype. J Anim Sci. 2017;95:1727–38.
56. Avguštin G, Wallace RJ, Flint HJ. Phenotypic diversity among ruminal isolates of Prevotella ruminicola: proposal of Prevotella brevis sp.nov, Prevotella bryantii sp.nov, and Prevotella albensis sp.nov, and redefinition of Prevotella ruminicola. Int J Syst Bacteriol. 1997;47:284–8.
57. Chen Y, Penner GB, Li M, Oba M, Guan LL. Changes in bacterial diversity associated with epithelial tissue in the beef cow rumen during the transition to a high-grain diet. Appl Environ Microb. 2011;77:5770–81.
58. Jami E, Israel A, Kotser A, Mizrahi I. Exploring the bovine rumen bacterial community from birth to adulthood. ISME J. 2013;7:1069–79.
59. Zhang R, Zhang WB, Bi YL, Tu Y, Ma T, Dong LF, et al. Sanguinarine and resveratrol affected rumen fermentation parameters and bacterial community in calves. Anim Feed Sci Tech. 2019;251:64–75.
60. Guo W, Guo XJ, Zhu BC, Guo YY, Zhou X. In situ degradation, ruminal fermentation, and the rumen bacterial community of cattle fed corn stover fermented by lignocellulolytic microorganisms. Anim Feed Sci Tech. 2019;248:10–9.
61. Jami E, Mizrahi I. Composition and similarity of bovine rumen microbiota across individual animals. PLoS One. 2012;7:e33306.
62. Pope PB, Denman SE, Jones M, Tringe SG, Barry K, Malfatti SA, et al. Adaptation to herbivory by the Tammar wallaby includes bacterial and glycoside hydrolase profiles different from other herbivores. P Natl Acad Sci USA. 2010;107:14793–8.
63. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, et al. Evolution of mammals and their gut microbes. Science. 2008;320:1647–51.
64. Mickdam E, Khiaosa-Ard R, Metzler-Zebeli BU, Klevenhusen F, Chizzola R, Zebeli Q. Rumen microbial abundance and fermentation profile during severe subacute ruminal acidosis and its modulation by plant derived alkaloids *in vitro*. Anaerobe. 2016;39:4–13.
65. West JW, Mullinix BG, Sandifer TG. Changingdietary electrolyte balance for dairy cows in cool and hot environments. J Dairy Sci. 1991;74:1662–74.
66. Iwaniuk ME. Determination of optimal DCAD concentration and relative effectiveness of potassium versus sodium cation supplementation for maximal feed efficiency in lactating dairy cows. College Park: University of Maryland; 2013.

67. Wu WX, Yang Y, Zhang JK, Li SL. Reducing dietary cation-anion difference on acid-base balance, plasma minerals level and anti-oxidative stress of female goats. J Integr Agr. 2013;12:1620–8.
68. Takagi H, Block E. Effects of various dietary cation-anion balances on response to experimentally induced hypocalcemia in sheep. J Dairy Sci. 1991b;74:4215–24.
69. Takagi H, Block E. Effects of reducing dietary cation-anion balance on calcium kinetics in sheep. J Dairy Sci. 1991c;74:4225–37.
70. Sharif M, Shahzad MA, Mahr-un-Nisa, Sarwar M. Influence of varying levels of dietary cation anion difference on ruminal characteristics, nitrogen metabolism and in situ digestion kinetics in buffalo bulls. Anim Sci J. 2010;81:657–65.
71. Takagi H, Block E. Effects of manipulating dietary cation-anion balance on macromineral balance in sheep. J Dairy Sci. 1991a;74:4202–14.
72. Liesegang A. Influence of anionic salts on bone metabolism in periparturient dairy goats and sheep. J Dairy Sci. 2008;91:2449–60.
73. Wu WX, Liu JX, Xu GZ, Ye JA. Calcium homeostasis, acid–base balance, and health status in periparturient Holstein cows fed diets with low cation–anion difference. Livest Sci. 2008;117:7–14.
74. Rodrigues RO, Cooke RF, Rodrigues SMB, Bastos LN, de Camargo VFS, Gomes KS, et al. Reducing prepartum urine pH by supplementing anionic feed ingredients: Effects on physiological and productive responses of Holstein × Gir cows. J Dairy Sci. 2018;101:9296–308.
75. Stewart PA. Modern quantitative acid-base chemistry. Can J Physiol Pharm. 1983;61:1444–61.
76. DeGaris PJ, Lean IJ. Milk fever in dairy cows: A review of pathophysiology and control principles. Vet J. 2008;176:58–69.
77. Seifi HA, Mohri M, Farzaneh N, Nemati H, Nejhad SV. Effects of anionic salts supplementation on blood pH and mineral status, energy metabolism, reproduction and production in transition dairy cows. Res Vet Sci. 2010;89:72–7.
78. Grünberg W, Donkin SS, Constable PD. Periparturient effects of feeding a low dietary cation-anion difference diet on acid-base, calcium, and phosphorus homeostasis and on intravenous glucose tolerance test in high-producing dairy cows. J Dairy Sci. 2011;94:727–45.
79. Espino L, Guerrero F, Suarez ML, Santamarina G, Goicoa A, Fidalgo LE. Long-term effects of dietary anion-cation balance on acid-base status and bone morphology in reproducing ewes. J Vet Med A Physiol Pathol Clin Med. 2003;50:488–95.
80. Roche JR, Petch S, Kay JK. Manipulating the dietary cation-anion difference via drenching to early-lactation dairy cows grazing pasture. J Dairy Sci. 2005;88:264–76.
81. USDA. Changes in the U.S. dairy cattle industry, 1991–2007, USDA, APHIS: VS CEAH, National Animal Health Monitoring System, Fort Collins, CO. 2007, p. 480.
82. Horst RL, Goff JP, Reinhardt TA, Buxton DR. Strategies for preventing milk fever in dairy cattle. J Dairy Sci. 1997;80:1269–80.

83. Goff JP, Horst RL. Role of acid-base physiology on the pathogenesis of parturient hypocalcemia (milk fever)-The DCAD theory in principle and practice. Acta Vet Scand. 2003;97:1–66.
84. Khanal RC, Nemere I. Regulation of intestinal calcium transport. Annu Rev Nutr. 2008;28:179–96.
85. Hoenderop JG, Nilius B, Bindels RJ. Calcium absorption across epithelia. Physiol Rev. 2005;85:373–422.
86. Ko SH, Choi KC, Oh GT, Jeung EB. Effect of dietary calcium and 1,25-$(OH)_2D_3$ on the expression of calcium transport genes in calbindin-D9k and-D28k double knockout mice. Biochem Bioph Res Co. 2009;379:227–32.
87. Choi KC, An BS, Yang H, Jeung EB. Regulation and molecular mechanisms of calcium transport genes: do they play a role in calcium transport in the uterine endometrium. J Physiol Pharmacol. 2011;62:499–504.

Figures

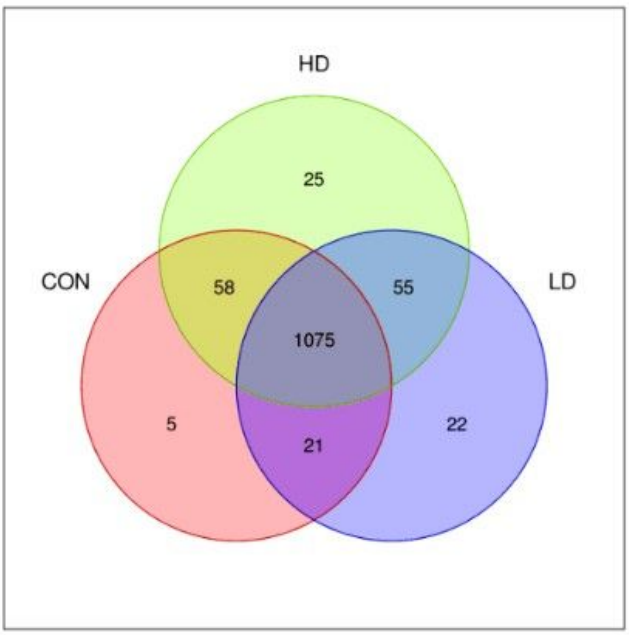

Figure 1

The VENN graph of rumen bacterial of goats fed diets with varying cation-anion difference levels, which displaying that the disposition of operational taxonomic units (OTUs) among the control group (CON,

n=6), high dietary cation-anion difference (HD, n=6) and low dietary cation-anion difference (LD, n=6) in rumen fluid microbiota.

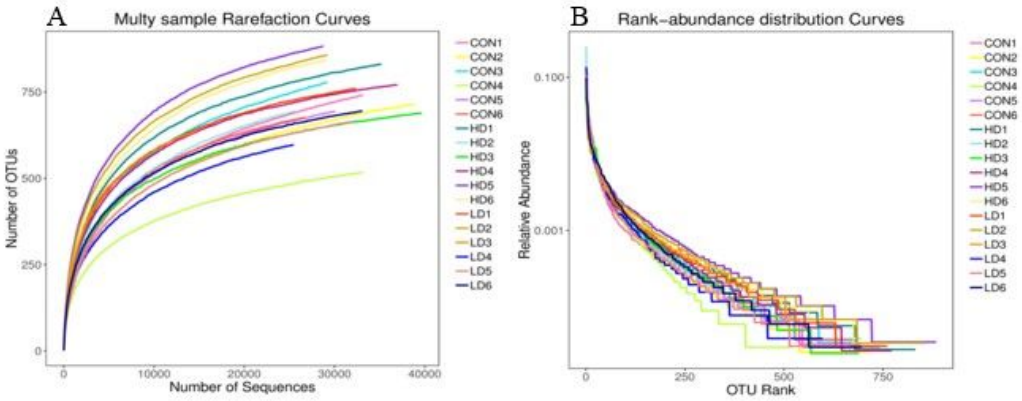

Figure 2

Rarefaction curves (A) and the OTU rank abundance (B) of rumen bacterial of goats fed diets with varying cation-anion difference levels. The CON group: CON1, CON2, CON3, CON4, CON5, CON6. The HD group: HD1, HD2, HD3, HD4, HD5, HD6. The LD group: LD1, LD2, LD3, LD4, LD5, LD6.

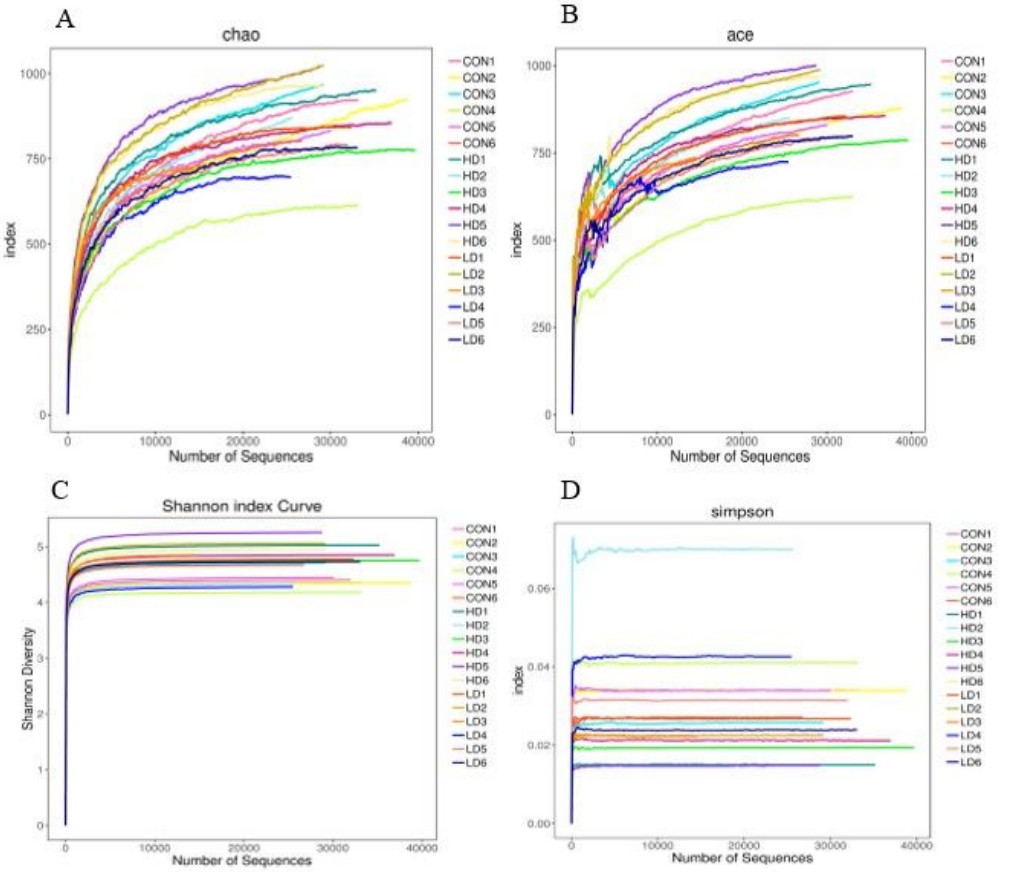

Figure 3

Rarefaction curves (A) and the OTU rank abundance (B) of rumen bacterial of goats fed diets with varying cation-anion difference levels. The CON group: CON1, CON2, CON3, CON4, CON5, CON6. The HD group: HD1, HD2, HD3, HD4, HD5, HD6. The LD group: LD1, LD2, LD3, LD4, LD5, LD6.

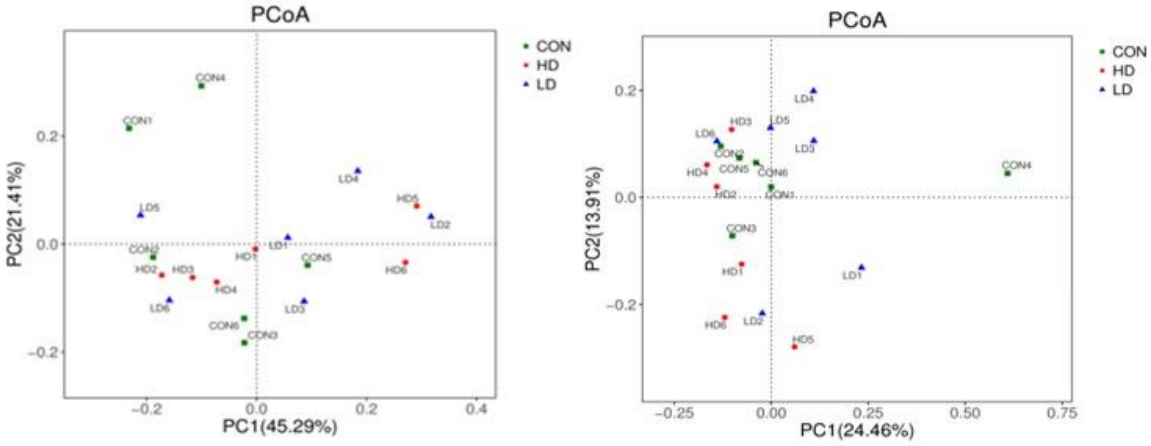

Figure 4

Principle coordinate analysis (PCoA) based on weighted UniFrac and unweighted UniFrac on rumen bacterial of goats fed diets with varying cation-anion difference levels. (a) PCoA based on weighted UniFrac of rumen bacterial of goats fed diets with varying cation-anion difference levels. (b) PCoA based on unweighted UniFrac of rumen bacterial of goats fed diets with varying dietary cation-anion difference levels. The percentage of variation explained by PC1 (first principal component) and PC2 (second principal component) was indicated in the axis. CON = the control group (n = 6); HD= high dietary cation-anion difference (n = 6); LD = low dietary cation-anion difference (n = 6).

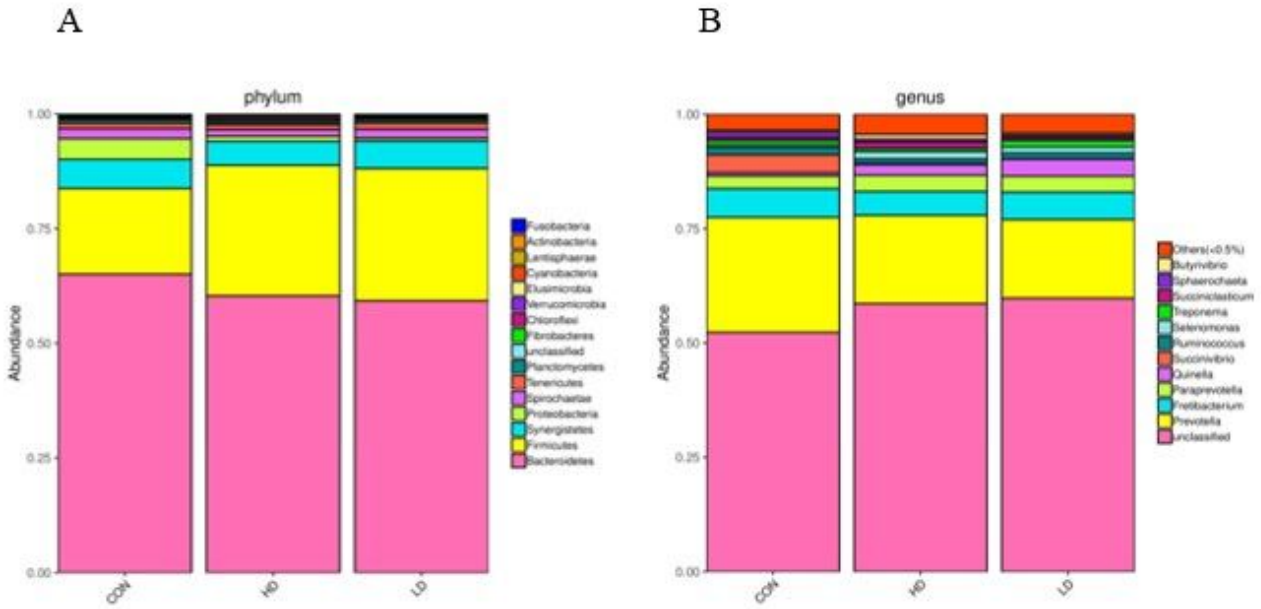

Figure 5

Distributions of microbiota at phyla and genus level. (a) Relative abundances of phyla levels are depicted as mean values for the CON, the LD (Low dietary cation-anion difference) group and HD (High dietary cation-anion difference) group. (b) Distributions of genera in rumen fluid of the CON, the LD (Low dietary cation-anion difference) group and HD (High dietary cation-anion difference) group.

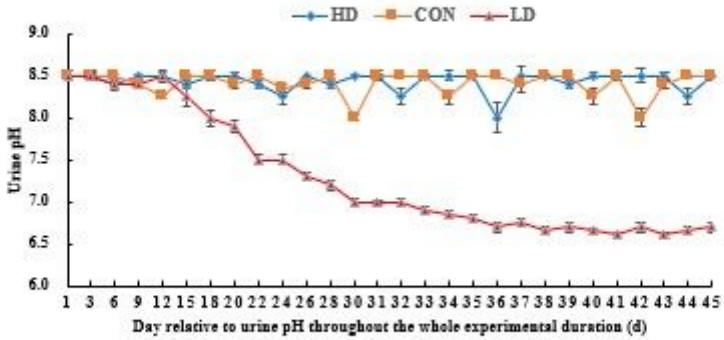

Fig. 6a Goat urine pH for dietary cation–anion difference levels throughout the experiment

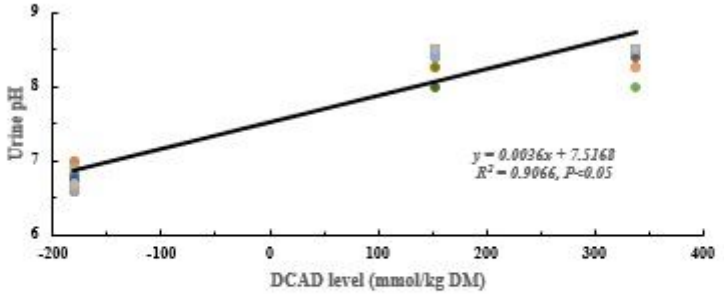

Fig. 6b Association between urine pH and dietary cation-anion difference of goats in the trial period

Figure 6

6a Goat urine pH for dietary cation–anion difference levels throughout the experiment 6b Association between urine pH and dietary cation-anion difference of goats in the trial period

Supplementary Files

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