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Original Research Article

Dietary saccharin sodium supplementation improves the production performance of dairy goats without residue in milk in summer

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ABSTRACT

The purpose of this study was to investigate the effects of dietary saccharin sodium supplementation on production performance, serum biochemical indicators, and rumen fermentation of dairy goats in summer. Twelve Guanzhong dairy goats with similar body weight, days in milk, and milk yield were randomly divided into two dietary treatments: (1) CON: basal diet; (2) SS: basal diet + 150 mg/kg saccharin sodium on the basis of dry matter. The experiment lasted 35 d, including 7 d for adaptation and 28 d for dietary treatments, sampling and data collection. Each dairy goat was housed individually in a clean separate pen with ad libitum access to diet and water. The goats fed SS diet had increased dry matter intake (DMI; $P = 0.037$), 4% fat corrected milk yield ($P = 0.049$), energy corrected milk yield ($P = 0.037$), milk protein yield ($P = 0.031$), and total solids yield ($P = 0.036$). Serum activity of aspartate aminotransferase ($P = 0.047$) and concentrations of 70-kDa heat shock protein ($P = 0.090$), malondialdehyde ($P = 0.092$), and total protein ($P = 0.057$) were lower in goats fed SS diet than those fed CON diet. Supplementation of saccharin sodium tended to increase activity of glutathione peroxidase in serum ($P = 0.079$). The concentrations of rumen total volatile fatty acid ($P = 0.042$) and butyrate ($P = 0.038$) were increased by saccharin sodium supplementation. Dietary supplementation of saccharin sodium increased the relative abundance of *Lachnobacterium* ($P = 0.022$), *Pseudoramibacter* ($P = 0.022$), *Shuttleworthia* ($P = 0.025$), and *Syntrophococcus* ($P = 0.037$), but reduced the relative abundance of *Prevotella_1* ($P = 0.037$) and *Lachnospiraceae_UCG_008* ($P = 0.037$) in rumen. Saccharin sodium was observed in feces and urine of goats fed diet supplemented with saccharin sodium, but saccharin sodium was undetectable in the milk of goats receiving SS diet. In conclusion, administration of saccharin sodium was effective in increasing fat and energy corrected milk yield by increasing DMI and improving rumen fermentation and antioxidant capacity of dairy goats in summer. In addition, saccharin sodium residue was undetectable in the milk.

1. Introduction

Animals' performance and physiological function are negatively impacted by heat stress, a non-specific reaction to high temperatures and humidity (Rojas-Downing et al., 2017; Yang et al., 2022). According to general consensus, goats are more tolerant to heat stress than dairy cows because they have relative larger body surface area per unit body weight and higher sweating rate (Salama et al., 2014). However, goats are also susceptible to heat stress as global warming progresses. Heat stress not only reduces production performance (Li et al., 2021), but also has negative impacts on

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rumen fermentation (Li et al., 2022), liver functions (Fan et al., 2018), and antioxidant status of dairy animals (Safa et al., 2019).

During summer, nutrient intake becomes the most limiting factor for optimal production. Previous study has indicated that increasing dry matter intake (DMI) is a useful strategy to improve milk performance under heat stress (Perdomo et al., 2020). The DMI of ruminants can be affected by physical (appearance, texture, etc.) and chemical (taste, odor, etc.) characteristics of diet (Favreau-Peigné et al., 2013). Goatcher and Church (1970) indicated that goats were sensitive to sweetness. Moreover, dietary supplementation of sweeteners has been observed to increase DMI of ruminants (Chavez and Huntington, 2013; Han et al., 2019b). As one of the most well-known low-calorie sweeteners, saccharin sodium (also known as benzoic acid sulfimide) is 300 to 500 times sweeter than sugar (Mora and Dando, 2021). Wilk et al. (2022) found that saccharin sodium could regulate the appetite and gastrointestinal function by activating T1R2/T1R3 subunits, stimulating G proteins, and evoking neuroendocrine and neurohormonal responses. Biggs et al. (2020) reported that dietary supplementation of sodium saccharide improved the growth performance of heat stressed pigs. The successful application of saccharin sodium in monogastric animals to improve feed intake has aroused great focus on its potential role in improving production performance and relieving heat stress in ruminants. We hypothesized that saccharin sodium could improve production performance of dairy goats by increasing DMI and improving physiological conditions in summer. Thus, the purpose of this study was to investigate the effects of saccharin sodium supplementation on the production performance, nutrient digestibility, antioxidant capacity, heat shock protein, and ruminal and fecal fermentation of dairy goats in summer.

2. Materials and methods

2.1. Animal ethics statement

The Animal Ethical and Welfare Committee of Northwest A&F University (DK2021027, Yangling, China) approved the experimental animals, designs and animal management in the present study. The experiment complied with the ARRIVE guidelines.

2.2. Animals, experimental design, and diets

This study was carried out at a local farm (Yangling, China) during summer (July to September 2022). Twelve Guanzhong dairy goats (first parity; 19 months of age) with similar body weight (BW, 51.2 ± 1.2 kg), days in milk (DIM, 150 ± 15 d), and milk yield (1.60 ± 0.10 kg/d) were randomly divided into following dietary treatments: (1) CON: basal diet with a forage to concentrate ratio of 45:55 on the basis of dry matter (DM); (2) SS: basal diet + 150 mg/kg saccharin sodium on the basis of DM. The dose of saccharin sodium used in this study was modified from the results of our previous study (unpublished data) and Rychen et al. (2018). The saccharin sodium (98% purity) was obtained from DadHank Biotechnology Corporation (Chengdu, China). Experimental diets were fed as a total mixed ration formulated to meet the dietary requirement of lactating dairy goats according to NRC (2007) and the respective ingredients and nutritional compositions are presented in Table 1. The saccharin sodium was progressively mixed with 5%, 20%, 50%, and 100% of concentrate. Then, the concentrate, corn silage, and alfalfa hay were thoroughly mixed by a total mixed ration mixer (9-TMR5, Ruier, Baoding, China). This feeding experiment lasted 35 d, including 7 d for adaptation and 28 d for dietary treatment, sampling, and data collection. Each dairy goat was housed individually in a clean separate pen (approximately 2 m^2) with ad libitum access to feed and fresh water and the separate pen was regularly cleaned. All goats were fed two times (07:30 and 16:00) and milked twice (08:00 and 16:30) daily. Feed was provided in excess to goats to allow 5% to 10% of daily feed refusal.

Table 1
Ingredients and nutrient compositions of the diets[1] (DM basis, %).

Item	Content
Ingredients	
Corn silage	30.99
Alfalfa hay	14.66
Corn	38.62
Soybean meal	13.23
Limestone meal	0.50
$CaHPO_4$	0.16
NaCl	0.32
$NaHCO_3$	0.60
Rumen protected fats	0.60
Premix[2]	0.32
Total	100.00
Analyzed nutrient compositions	
Gross energy, MJ/kg	18.22
Crude protein	14.74
Neutral detergent fiber	27.60
Acid detergent fiber	15.76
Calcium	0.66
Total phosphorous	0.37

[1] Fed as total mixed ration.
[2] Formulated to provide (per kilogram of basal diet) 14.96 mg Cu, 39.6 mg Mn, 66 mg Zn, 0.88 mg Co, 4840 IU vitamin A, 1584 IU vitamin D_3, and 66 IU vitamin E.

2.3. Sample collection

The daily ambient temperature (°C) and relative humidity (%) of the experimental goats shed were measured three times (07:00, 13:00, and 20:00) with a hygrothermograph (8845, Deli group Co., Ltd, Ningbo, China). The effective temperature and humidity scales were as follows: ambient temperature from 0 to 60 °C and relative humidity from 0% to 99%. The following equation (Li et al., 2022) was used to calculate the temperature humidity index (THI):

THI = 0.81 × ambient temperature + (0.99 × ambient temperature −14.37) × relative humidity + 46.3.

To obtain individual feed intake, the offered experimental diet and refusal of each goat were accurately recorded by an electronic weighing system throughout the experiment (ACS-30, Jinxuan, Jinhua, China). The samples of fresh experimental diets and refusals were collected biweekly and kept under −20 °C for later analysis of nutrient compositions to calculate DMI and apparent digestibility of nutrients. The BW of each goat was measured at the beginning and end of the experiment by an electronic weighing system (HY-602; Jinxuan Jinhua, China). In order to reduce the measurement error, the BW was measured for two consecutive days before the morning feeding after milking.

The individual goat milk yield was recorded throughout the experiment via a portable milking machine. Milk samples were collected on d 7, 14, 21, and 28 of the experiment. On the milk sampling days, around 10% of the milk from individual dairy goat was collected after mixing uniformly in each milking. Then, the morning and evening milk samples collected from each goat within the day were mixed at the ratio of 6:4. After that, the fully mixed milk sample was divided into two parts. One part of milk sample was mixed in bottles with potassium dichromate solution and stored at 4 °C for further milk composition analysis. The other part of milk sample was kept at −20 °C for further analysis of saccharin sodium content in milk. Fecal samples were collected via rectal massage on the last 3 d of the experiment in order to estimate nutrients digestibility, fermentation parameters, and sodium

saccharin content. Fecal sampling times were scheduled over 3 d so that the samples represented every 3 h in a 24-h feeding cycle (Ren et al., 2020). The fecal sample pH was immediately recorded by a portable pH logger (HI 90s24C, HANNA Instruments, Woonsocket, USA) according to the method described by Chen et al. (2022). The fecal samples from each goat were mixed and divided into three parts. One part of fecal sample was mixed with 10% sulfuric acid and stored at −20 °C for further nutrient analysis. The other two parts of fecal samples were stored at −20 °C for fermentation parameters and saccharin sodium content determination. Rumen fluid samples were obtained by a gastric tube-type rumen fluid sampler at 2 h after morning feeding on the last day of this experiment using the methods described by Chen et al. (2022). To avoid saliva contamination, the initial 20 to 30 mL of rumen fluid was discarded and 40 mL rumen fluid was then sampled from each goat using 50-mL screw-cap centrifuge tubes. Rumen fluid pH was immediately measured by a portable pH logger (HI 90s24C, HANNA Instruments, Woonsocket, USA). The rumen fluid samples were divided into three parts and stored at −80 °C until further determination of volatile fatty acids (VFA), ammonia nitrogen (NH_3—N), and microorganisms. On the last day of the experiment, blood samples were collected from each goat through the jugular vein at 0.5 h before morning feeding. Blood samples were centrifuged at 3000 × *g* for 15 min to obtain serum samples by a centrifuge (Sorvall-ST8, Thermo Fisher Scientific, Massachusetts, USA). The serum samples were stored at −20 °C for further analysis of serum biochemical indices. All goats' urine samples were collected during spontaneous urination in 50-mL urine collectors at 5 h after feeding the morning diet on the last day of this experiment. The urine samples were stored at − 20 °C for saccharin sodium content determination.

2.4. Sample measurements

The DM (method 930.15), crude protein (CP; method 976.05), and organic matter (OM; method 942.05) in dietary and fecal samples were analyzed according to AOAC (2000) methods. The neutral detergent fiber (NDF) in dietary and fecal samples was determined with heat-stable amylase and sodium sulfite using a fiber analyzer (A200i, ANKOM, NY, USA) by the method of Van Soest et al. (1991). The dietary and fecal acid detergent fiber (ADF; method 973.18) was measured based on the NDF residue using AOAC (2000) methods. The acid detergent insoluble ash in dietary and fecal samples was analyzed according to the methods described by Liu (2022). The gross energy of experimental diet was determined using an adiabatic oxygen bomb calorimeter (Parr 6100, Parr Instrument Co., Moline, IL, USA). The dietary calcium (method 977.29) and total phosphorus (method 995.11) were measured based on AOAC (2000) methods. The following equation was used to calculate the apparent digestibility of nutrients (Burakowska et al., 2021): apparent total tract digestibility of nutrients (%) = [1 − (A × D)/(B × C)] × 100. In this equation, A is the hydrochloric acid insoluble ash content of in diet, B is the hydrochloric acid insoluble ash content in feces, C is the nutrient content of diet, and D is the nutrient content in feces.

Milk fat, protein, lactose, and total solids were measured with a milk composition analyzer (Combi 500, Bentley, Minnesota, USA). The following equations were used to calculate 4% fat corrected milk and energy corrected milk yields (Yang et al., 2022): 4% fat corrected milk yield (kg/d) = 0.4 × milk yield (kg/d) + 15 × milk fat yield (kg/d); Energy corrected milk yield (kg/d) = 0.327 × milk yield (kg/d) + 12.95 × milk fat yield (kg/d) + 7.20 × milk protein yield (kg/d).

The NH_3—N and VFA concentrations in rumen fluid and fecal samples were measured using the methods described by Wang et al. (2016). The light absorbance at 700 nm was recorded using a spectrophotometer (UV-2300, Shimadzu, Kyoto, Japan) to determine the NH_3—N concentration. The gas chromatograph (GC7890A, Agilent, Palo Alto, USA) was used to determine the VFA concentrations. Gas chromatographic conditions were as follows: column temperature 100 °C, increased by 2 °C/min to 120 °C; inlet temperature 250 °C; detector temperature 280 °C; constant voltage 21.8 kPa; separation ratio 1:50, and injection volume 2 μL.

The E.Z.N.A. soil DNA Kit (Omega BioTek, Norcross, USA) was used to extract total DNA from each goat rumen fluid sample according to the manufacturer's protocol. The quality and concentration of all DNA samples were detected by a FLX800T Microplate reader (Omega BioT-tek, Norcross, USA). Bacterial 16S rRNA gene fragments (V3—V4) were amplified from the extracted DNA using forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and reverse primer 806R (5′- GGACTACHVGGGTWTCTAAT-3′), with the following PCR conditions: initial denaturation 98 °C 2 min, denaturation 98 °C 15 s, annealing 55 °C 30 s, extension 72 °C 30 s, final extension 72 °C 5 min, 10 °C hold 25 to 30 cycles. The PCR system included: 5 × reaction buffer 5 μL, 5 × GC buffer 5 μL, dNTP (2.5 mmol/L) 2 μL, forward primer (10 μmol/L) 1 μL, reverse primer (10 μmol/L) 1 μL, DNA template 2 μL, ddH_2O 8.75 μL, and Q5 DNA Polymerase 0.25 μL. Amplicon size verification was performed by agarose gel electrophoresis. Paired-end sequencing was performed on the Illumina MiSeq/NovaSeqPE250 sequencing platform by Personal Biotechnology Co., Ltd. (Shanghai, China).

Microbiome bioinformatics were performed following the official tutorial (https://docs.qiime2.org/2019.4/tutorials/), with slight modifications, using QIIME 2 2019.4 (Bolyen et al., 2019). The raw sequence data were demultiplexed by demux plugin following by primers cutting using the cut adapt plugin. The DADA2 plugin (Callahan et al., 2016) was then used to filter, denoise, merge, and remove chimeras from the sequences. Taxonomic assignment of amplicon sequence variants (ASV) was performed using the naive Bayes consensus taxonomy classifier implemented in Qiime2 and the SILVA 16S rRNA database (v138). The online genes cloud platform (www.genescloud.cn) was used to analyze the 16S rRNA microbiome sequencing data.

The total bilirubin (TBIL), γ-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose (GLU), triglycerides (TG), cholesterol (CHOL), creatinine (CRE), serum urea nitrogen (BUN), albumin (ALB), total protein (TP), and globulin (GLO) in serum were determined with an automatic biochemical analyzer (Hitachi 7600, Hitachi Group, Tokyo, Japan). The enzymatic activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), as well as malondialdehyde (MDA) content and total antioxidant capacity (T-AOC) were determined by commercial assay kits from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The serum concentrations of neuropeptide Y (NPY), ghrelin, γ-aminobutyric acid (GABA), and 70-kDa heat shock proteins (HSP70) were determined with goat-specific ELISA kits (Angle Gene Biotechnology Co., Ltd., Nanjing, China) according to the instructions provided by manufacturer.

An high performance liquid chromatography (HPLC) system (LC-30A, Shimadzu, Kyoto, Japan) coupled with an ultraviolet (UV) detector was employed to determine the content of saccharin sodium in urine, milk, and feces. The chromatographic separation for saccharin sodium was performed using the Sapphiresil™ (C18) column (250 mm × 4.6 mm, 5 μm) with isocratic mobile phase flow consisting of methyl alcohol and ammonium acetate buffer (0.02 mol/L, pH 6.44) in a ratio of 5:95 (v/v) at 1 mL/min. Additionally, the eluents were monitored for saccharin sodium using a UV detector at a wavelength of 230 nm. Samples and standards solutions were injected in duplicate using a full-loop injection method with 1 μL loop volume. Shimadzu LC-30A data software

was used for data collection and analysis. The detection limit for saccharin sodium residue was 10 ng/mL.

2.5. Statistical analysis

Data were presented as means ± standard error and were analyzed by independent sample t-tests production performance, apparent total tract digestibility of nutrients, serum biochemical parameters, HSP70, antioxidant status, factors influencing feed intake, rumen and fecal fermentation parameters, and alpha diversity were calculated using independent sample *T*-tests. SPSS 28.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Statistical significance was declared at $P < 0.05$. Significant trend was defined as a value of $0.05 \leq P < 0.10$. Principal Coordinate Analysis (PCoA) plots were based on Bray–Curtis dissimilarity. Statistical significance of differences was tested using permutational multivariate analysis of variance (PERMANOVA). Linear discriminant analysis effect size (LEfSe) was used to analyze the differential microorganism.

3. Results

3.1. Environmental THI

During the experiment, the THI ranged from 73.33 to 81.84 and was higher than 70, indicating that the goats were in a heat stress environment (Fig. 1).

3.2. Production performance

Production performance for dairy goats fed diets with or without saccharin sodium are summarized in Table 2. Goats fed SS diet had increased DMI ($P = 0.037$), energy corrected milk yield ($P = 0.037$), 4% fat corrected milk yield ($P = 0.049$), milk protein yield ($P = 0.031$), and total solids yield ($P = 0.036$) than those fed CON diet. Milk yield ($P = 0.085$), milk fat yield ($P = 0.073$), and milk lactose yield ($P = 0.099$) were tended to be increased by saccharin sodium supplementation. However, average daily gain (ADG), feed conversion ratio, milk fat rate, milk protein rate, lactose rate, and total solids were not affected by dietary treatment ($P > 0.10$).

Table 2
Effects of dietary saccharin sodium supplementation on production performance of lactating dairy goats.[1,2]

Item	CON	SS	*P*-value
DMI, kg/d	1.33 ± 0.04	1.43 ± 0.02	0.037
ADG, g/d	−20.2 ± 19.0	15.5 ± 26.3	0.297
Milk yield, kg/d	1.40 ± 0.07	1.60 ± 0.08	0.085
4% fat corrected milk yield[3], kg/d	1.25 ± 0.05	1.43 ± 0.06	0.049
Energy corrected milk yield[4], kg/d	1.38 ± 0.06	1.58 ± 0.06	0.037
Feed conversion rate[5]	0.94 ± 0.02	1.00 ± 0.05	0.264
Milk composition, %			
Fat	3.30 ± 0.14	3.33 ± 0.18	0.896
Protein	3.24 ± 0.07	3.25 ± 0.11	0.929
Lactose	4.64 ± 0.10	4.65 ± 0.10	0.907
Total solid	12.26 ± 0.25	12.29 ± 0.24	0.933
Milk composition yield, g/d			
Fat	45.9 ± 2.24	52.9 ± 2.66	0.073
Protein	45.1 ± 1.55	51.8 ± 2.17	0.031
Lactose	64.8 ± 3.41	74.5 ± 4.10	0.099
Total solid	170.9 ± 7.0	196.2 ± 7.8	0.036

DMI = dry matter intake; ADG = average daily gain.
[1] Data are shown as means ± standard errors ($n = 6$).
[2] CON: basal diet; SS: basal diet + 150 mg/kg saccharin sodium on the basis of dry matter.
[3] 4% fat corrected milk yield (kg/d) = 0.4 × milk yield (kg/d) + 15 × milk fat yield (kg/d).
[4] Energy corrected milk (kg/d) = 0.327 × milk yield (kg/d) + 12.95 × milk fat yield (kg/d) + 7.20 × milk protein yield (kg/d).
[5] Feed conversion rate = 4% fat corrected milk/DMI.

3.3. Apparent total tract digestibility of nutrients

Dietary supplementation of saccharin sodium did not affect apparent total tract digestibility of DM, CP, OM, NDF, and ADF (Table 3; $P > 0.10$).

3.4. Serum biochemical parameters

The influence of dietary saccharin sodium supplementation on serum biochemical parameters of dairy goats are shown Table 4. Goats receiving SS diet had lower activity of AST ($P = 0.047$) and decreasing trend in TP content ($P = 0.057$) than those offered CON diet. However, no significant differences were found for serum TBIL, GLU, TG, CHOL, CRE, BUN, ALB, GLO ALT, GGT, and ALP among dietary treatments ($P > 0.10$).

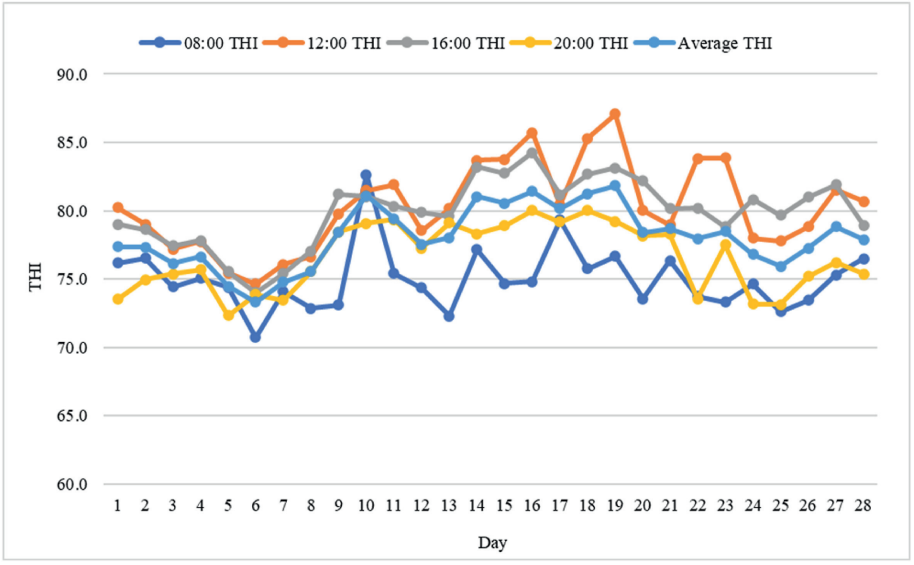


Fig. 1. Temperature and humidity index (THI) of the goats shed during the experiment.

Table 3
Effects of dietary saccharin sodium supplementation on apparent total tract digestibility of nutrients of lactating dairy goats.[1,2]

Item, %	CON	SS	*P*-value
Dry matter (DM)	74.5 ± 0.9	72.8 ± 0.7	0.143
Organic matter (OM)	75.9 ± 0.9	74.2 ± 0.6	0.175
Crude protein (CP)	70.5 ± 1.0	69.0 ± 1.4	0.388
Neutral detergent fiber (NDF)	62.5 ± 1.2	60.9 ± 0.8	0.326
Acid detergent fiber (ADF)	51.8 ± 1.2	50.3 ± 1.3	0.425

[1] Data are shown as means ± standard errors ($n = 6$).
[2] CON: basal diet; SS: basal diet + 150 mg/kg saccharin sodium on the basis of dry matter.

Table 4
Effects of dietary saccharin sodium supplementation on blood biochemical parameters of lactating dairy goats.[1,2]

Item	CON	SS	*P*-value
Total bilirubin (TBIL), μmoL/L	7.00 ± 0.71	6.76 ± 0.98	0.844
Alanine aminotransferase (ALT), U/L	25.5 ± 0.43	23.8 ± 2.06	0.446
Aspartate aminotransferase (AST), U/L	130.0 ± 11.9	99.5 ± 6.3	0.047
Gamma−glutamyl transferase (GGT), U/L	58.7 ± 2.2	55.4 ± 3.5	0.442
Alkaline phosphatase (ALP), U/L	80.3 ± 14.4	74.0 ± 7.9	0.708
Glucose (GLU), mmoL/L	3.30 ± 0.22	3.47 ± 0.25	0.616
Triglycerides (TG), mmoL/L	0.33 ± 0.07	0.22 ± 0.06	0.245
Cholesterol (CHOL), mmoL/L	2.59 ± 0.19	2.74 ± 0.09	0.491
Creatinine (CRE), μmoL/L	54.3 ± 3.6	59.5 ± 2.8	0.283
Blood urea nitrogen (BUN), mmoL/L	4.05 ± 0.46	4.02 ± 0.32	0.963
Total protein (TP), g/L	69.1 ± 0.9	65.1 ± 1.7	0.057
Albumin (ALB), g/L	30.5 ± 0.5	29.6 ± 0.7	0.336
Globulin (GLO), g/L	38.7 ± 0.7	35.5 ± 1.7	0.112

[1] Data are shown as means ± standard errors ($n = 6$).
[2] CON: basal diet; SS: basal diet + 150 mg/kg saccharin sodium on the basis of dry matter.

3.5. HSP70, antioxidant status and factors influencing feed intake

Serum concentrations of HSP70 ($P = 0.090$) and MDA ($P = 0.092$) tended to be lower in goats fed SS diet compared with those offered CON diet (Table 5). Whereas, supplementation of saccharin sodium tended to increase the activity of GSH-Px in serum ($P = 0.079$). No significant differences were observed in T-AOC, SOD, CAT, NPY, ghrelin, and GAGB between dietary treatments ($P > 0.10$).

3.6. Rumen and fecal fermentation parameters

Table 6 shows the effect of saccharin sodium supplementation on ruminal and fecal fermentation parameters. The rumen total VFA ($P = 0.042$) and butyrate ($P = 0.038$) concentrations were increased by saccharin sodium supplementation. Rumen valerate concentration showed an increasing trend with saccharin sodium supplementation ($P = 0.057$). No differences were observed on rumen acetate, propionate, isobutyrate, isovalerate, and NH_3−N concentration, acetate-to-propionate ratio, and pH ($P > 0.10$). No effects were observed on fecal fermentation with dietary saccharin sodium supplementation. ($P > 0.10$).

Table 6
Effects of dietary saccharin sodium supplementation on ruminal and fecal fermentation parameters of lactating dairy goats.[1,2]

Item	CON	SS	*P*-value
Ruminal fermentation parameters			
pH	6.63 ± 0.10	6.53 ± 0.09	0.459
Total VFA, mmol/L	101.6 ± 6.7	125.6 ± 7.7	0.042
Acetate, mmol/L	64.5 ± 4.8	76.4 ± 2.7	0.157
Propionate, mmol/L	23.2 ± 2.7	27.1 ± 2.0	0.274
Butyrate, mmol/L	10.5 ± 0.8	17.4 ± 0.3	0.038
Isobutyrate, mmol/L	0.85 ± 0.10	1.15 ± 0.19	0.191
Valerate, mmol/L	1.23 ± 0.08	1.62 ± 0.15	0.057
Isovalerate, mmol/L	1.41 ± 0.30	1.94 ± 0.34	0.272
A:P	2.91 ± 0.31	2.90 ± 0.27	0.991
NH_3−N, mg/dL	12.5 ± 1.9	12.9 ± 1.3	0.852
Fecal fermentation parameters			
pH	6.93 ± 0.11	6.86 ± 0.10	0.522
Total VFA, μmol/g	63.9 ± 8.6	65.3 ± 9.6	0.915
Acetate, μmol/g	40.2 ± 5.0	42.1 ± 7.2	0.835
Propionate, μmmol/g	7.97 ± 1.34	6.88 ± 1.32	0.576
Butyrate, μmol/g	5.96 ± 1.98	5.27 ± 1.16	0.770
Isobutyrate, μmol/g	2.88 ± 0.32	3.25 ± 0.84	0.685
Valerate, μmol/g	0.45 ± 0.09	0.43 ± 0.09	0.881
Isovalerate, μmol/g	0.44 ± 0.10	0.40 ± 0.06	0.703
A:P	5.30 ± 0.43	6.32 ± 0.66	0.225
NH_3−N, mg/dL	4.76 ± 1.38	4.97 ± 1.16	0.653

VFA = volatile fatty acid; A:P = acetate-to-propionate ratio.
[1] Data are shown as means ± standard errors ($n = 6$).
[2] CON: basal diet; SS: basal diet + 150 mg/kg saccharin sodium on the basis of dry matter.

3.7. Rumen microorganisms

Venn diagram analysis shows that 4070 ASV were from CON and 4398 ASV from SS, with 1887 ASV owned by two treatments (Fig. 2A). Alpha diversity calculations (Table 7) showed no differences in Chao1, Shannon, Goods coverage, Simpson, and observed-species indexes between the two groups ($P > 0.10$). According to PCoA (Fig. 2B), the rumen microflora showed no differences between the CON and SS groups ($P > 0.10$).

At the phylum level, Firmicutes and Bacteroidetes were the most abundant in samples (Fig. 2C). At the family level, Ruminococcaceae, Prevotellaceae, Rikenellaceae, F082, and Muribaculaceae were the dominant bacteria (Fig. 2D). *Ruminococcus_2*, *Prevotella_1*,

Table 5
Effects of dietary saccharin sodium supplementation on serum antioxidant status, factors influencing feed intake, and 70-kDa heat shock protein of lactating dairy goats.[1,2]

Item	CON	SS	*P*-value
Antioxidant status			
Total antioxidant capacity (T-AOC), U/mL	11.79 ± 0.76	11.61 ± 0.73	0.870
Glutathione peroxidase (GSH-Px), U/mL	462.3 ± 18.9	501.2 ± 5.9	0.079
Superoxide dismutase (SOD), U/mL	97.1 ± 2.9	103.3 ± 4.0	0.238
Catalase (CAT), U/mL	3.91 ± 0.39	3.75 ± 0.31	0.378
Malondialdehyde (MDA), nmol/mL	5.25 ± 0.23	4.69 ± 0.19	0.092
Factors influencing feed intake			
Neuropeptide Y (NPY), ng/mL	20.17 ± 0.32	19.56 ± 0.79	0.494
Ghrelin, ng/mL	3.46 ± 0.19	3.64 ± 0.29	0.614
Gamma-aminobutyric acid (GABA), μmoL/L	12.19 ± 0.72	12.10 ± 1.01	0.945
70-kDa heat shock protein, μg/mL	4.08 ± 0.21	3.51 ± 0.22	0.090

[1] Data are shown as means ± standard errors ($n = 6$).
[2] CON: basal diet; SS: basal diet + 150 mg/kg saccharin sodium on the basis of dry matter.

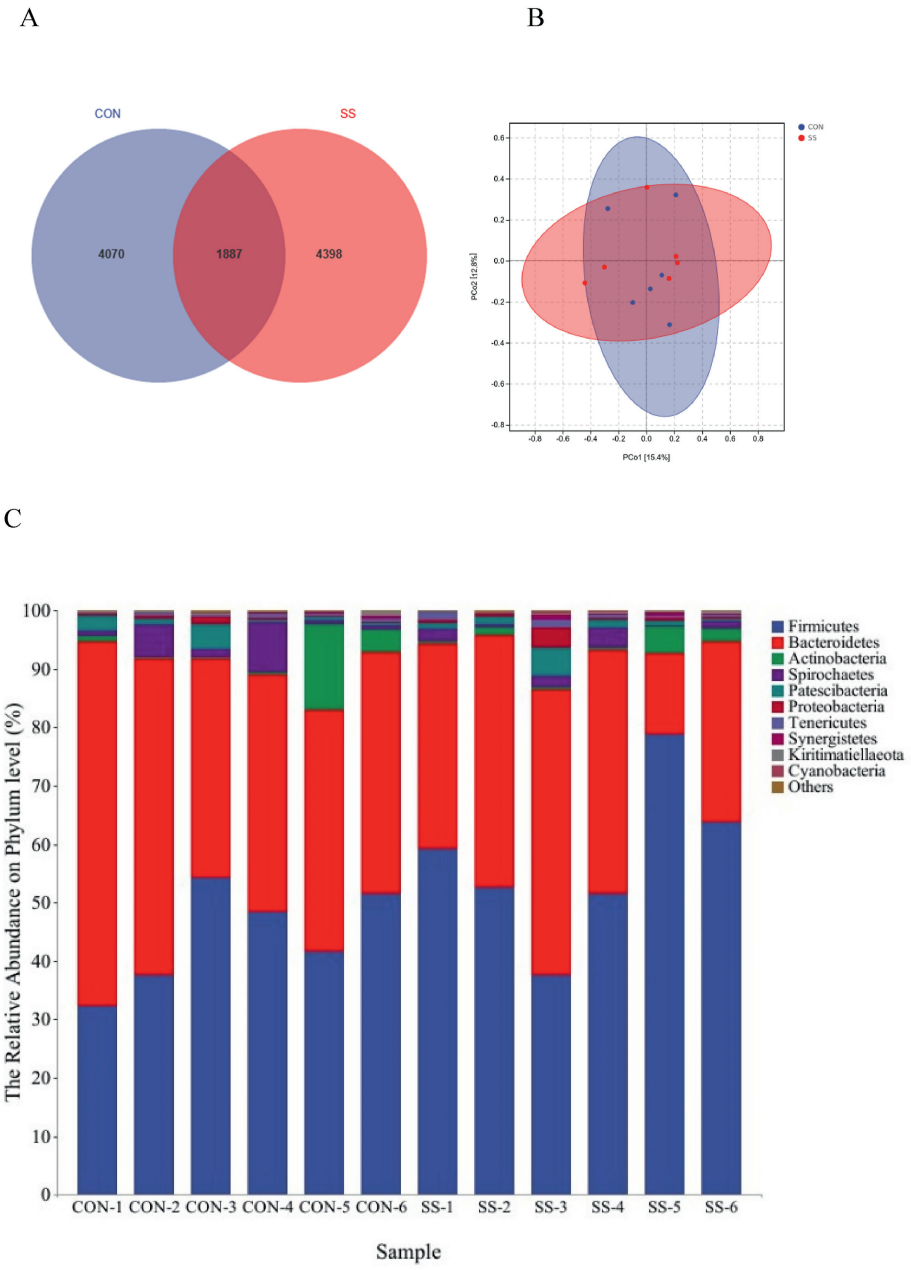


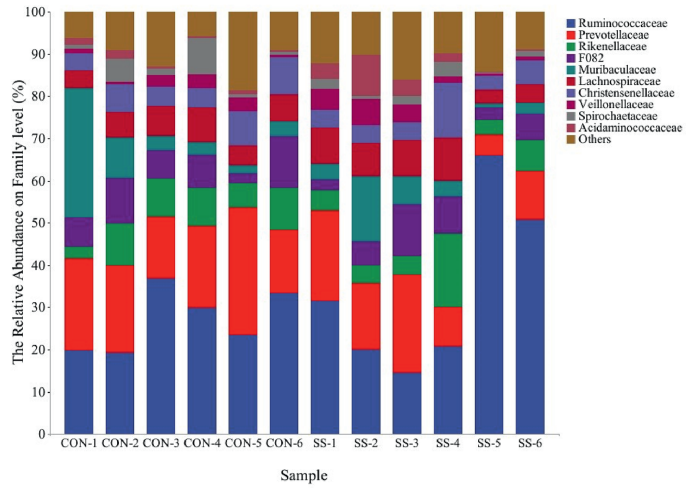
Fig. 2. Effects of dietary saccharin sodium supplementation rumen microorganisms of lactating dairy goats. (A) The Venn diagram is used to count the common and unique species of the two groups on the amplicon sequence variants (ASV) level. (B) Principal coordinate analysis (PCoA) analysis is used to study the similarities in group community composition. Plotted points represent rumen fluid samples. Bray Curtis and PERMANOVA were used for distance algorithms and difference tests between treatments. (C-E) Community structure composition at phylum (others < 0.001), family (others < 0.01), and genus (others < 0.01) levels. (F) Linear discriminant analysis (LDA) effect size analysis of differential microorganisms between two groups. The LDA discriminant histogram counts the microbial groups that have significant effects on multiple groups. The LDA score is obtained through LDA analysis (linear regression analysis). The larger the LDA score, the greater the influence of the species abundance on the effect. The levels of taxonomy are phylum, family and genus; all-against-all was selected for multiple group comparison strategies and LDA > 2. CON: basal diet; SS: basal diet + 150 mg/kg saccharin sodium on the basis of dry matter.

Rikenellaceae_RC9_gut_group, *F082*, and *Muribaculaceae* were the dominant bacteria at the genus level (Fig. 2E). LEfSe was employed to observe the differential bacteria that best explain the differences between treatments and the degree of influence of these characteristics (Fig. 2F). The relative abundance of *Lachnobacterium* ($P = 0.022$), *Pseudoramibacter* ($P = 0.022$), *Shuttleworthia* ($P = 0.025$), and *Syntrophococcus* ($P = 0.037$) were higher in rumen of goats on SS diet than those of goats fed CON diet. Dairy goats fed saccharin sodium had lower relative abundance of *Prevotella*_1 ($P = 0.037$), *[Eubacterium__xylanophilum]* ($P = 0.049$), Betaproteobacteriales ($P = 0.037$), *U29_B03* ($P = 0.039$), and *Lachnospiraceae_UCG_008* ($P = 0.037$).

3.8. Residue of saccharin sodium in milk, urine and feces

As shown in Table 8, saccharin sodium was undetectable in the milk, feces, and urine of lactating goats fed CON diet. Residue of saccharin sodium was observed in the feces as well as urine of goats

D



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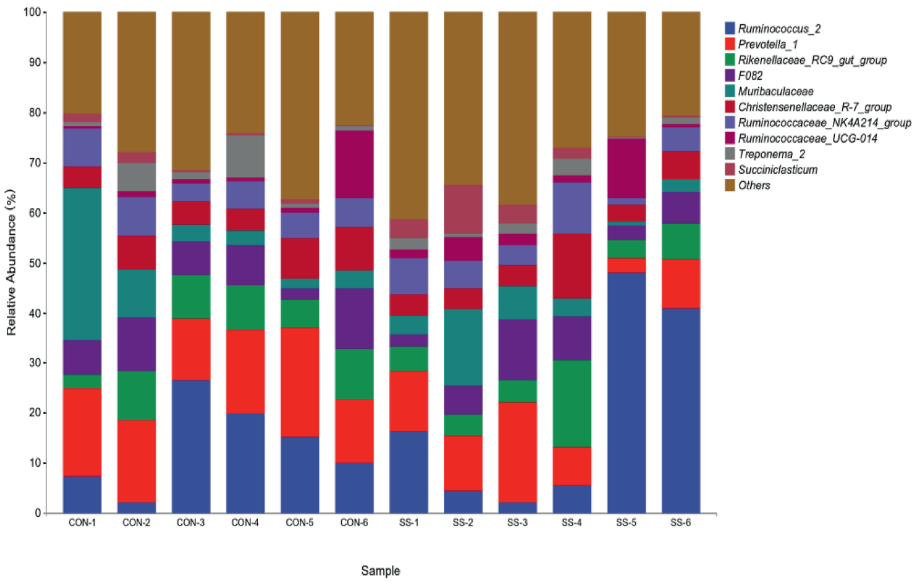

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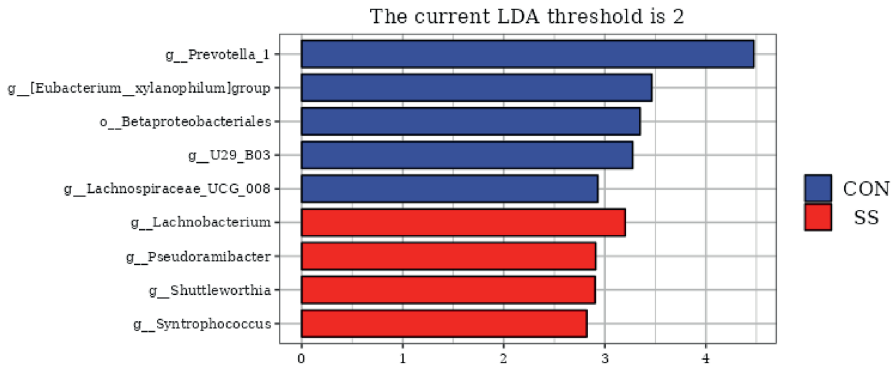

Fig. 2. (*continued*).

Table 7
Effects of dietary saccharin sodium supplementation on alpha-diversity of rumen microbiota in lactating dairy goats.[1,2]

Item	CON	SS	*P*-value
Chao1	1419 ± 75	1467 ± 182	0.630
Shannon index	7.84 ± 0.22	7.85 ± 0.43	0.633
Goods coverage index	0.997 ± 0.005	0.997 ± 0.006	0.341
Simpson index	0.976 ± 0.061	0.977 ± 0.061	0.870
Observed-species index	1361 ± 71	1428 ± 174	0.872

[1] Data are shown as means ± standard errors ($n = 6$).
[2] CON: basal diet; SS: basal diet + 150 mg/kg saccharin sodium on the basis of dry matter.

fed diet supplemented with saccharin sodium. However, residue of saccharin sodium was undetectable in the milk of goats receiving SS diet.

4. Discussion

As a comprehensive indicator combining ambient temperature and relative humidity, THI is widely used for estimating heat stress in animal production (Li et al., 2022). Heat stress in dairy goats is generally recognized to occur when THI exceeds 70 (Hamzaoui et al., 2013). In this study, the mean daily minimum and maximum THI were 73.33 and 81.84, indicating that the goats were under heat stress condition for the entire period of the experiment.

4.1. Production performance

Adequate nutrient intake is essential to optimize performance of animals. However, heat stress has been reported to decrease DMI and nutrient digestibility in goats, which results in inadequate nutrient intake and negative influences on overall body health (Rojas-Downing et al., 2017; Tao et al., 2018). Han et al. (2019b) reported that dietary inclusion of sweetener (stevioside) increased forage intake in goats. Ponce et al. (2014) demonstrated that beef cattle fed diet with a saccharin-based artificial sweetener (Sucram) at 200 mg/kg (DM basis) had a numerically higher ADG than those fed basal diet under heat stress environment. In accordance with these findings, in this study dietary supplementation with saccharin sodium resulted in increased DMI in dairy goats suffering from heat stress. Goats are sensitive to sweetness, the improvement in the sweetness of diet may enhance their appetite (Goatcher and Church, 1970). Therefore, the increased DMI with saccharin sodium supplementation may be related to the improved palatability of diet through sweetness enhancement in present study. However, dietary saccharin sodium supplementation had no effect on the NPY, ghrelin, and GABA in serum of heat-stressed dairy goats. Further studies are required to investigate the regulation mechanisms of saccharin sodium on feed intake. Interestingly, goats fed diet with saccharin sodium had numerically higher ADG than those fed basal diet, although no statistical difference was observed due to the relatively high within-treatment variation. Considering the limited number of animals used in the present study, a larger sample size would be required to provide sufficient replication to investigate the potential effects of saccharin sodium supplementation on ADG.

In dairy goats, extensive studies have demonstrated that DMI decreases are generally accompanied by impaired lactation performance under heat stress (Henry et al., 2012; Silanikovea and Koluman, 2015; Rojas-Downing et al., 2017). As expected in the present study, goats receiving diet with saccharin sodium had improved lactation performance, showing as increased milk yield, 4% fat corrected milk, and energy corrected milk. Sufficient nutrient intake is crucial for maximizing milk yield in lactating dairy goats. Thus, the increased milk yield by dietary saccharin sodium supplementation probably resulted from the observed increase in DMI (Silanikovea and Koluman, 2015). In addition, the increased production performance by dietary addition of saccharin sodium can be attributed to improved general body health of goats, such as relieved oxidative stress and liver damage (Song et al., 2021). Thus, the influence of dietary saccharin sodium on oxidative stress and liver damage were further investigated in this study.

Several investigators (Chanda et al., 2017; Gao et al., 2017; Hooper et al., 2020) have reported that heat stress can reduce milk quality through influencing the content of milk protein, fat, lactose, solids-not-fat, and total solid. In this study, supplementation of saccharin sodium had no effect on milk composition, whereas the yield of milk composition was increased in goats fed SS diet compared with those offered CON diet. These results indicate that milk yield of heat stressed dairy goats was increased by saccharin sodium supplementation without interfering with milk composition. Heat shock proteins are produced by breast epithelial cells to resist the effects of heat stress (Yang et al., 2022). Heat shock protein synthesis can reduce amino acid availability for milk protein synthesis, resulting in lower milk protein rates (Cowley et al., 2015; Hu et al., 2016). Therefore, the increase in milk composition yield with dietary saccharin sodium supplementation may be explained by reduced HSP70.

4.2. Apparent total tract digestibility of nutrients

Nutrient digestibility can be impaired by heat stress in dairy animals (Gao et al., 2017). Han et al. (2019b) observed that dietary stevioside inclusion improved the apparent total tract digestibility of NDF and ADF in male Xiangdong Black goats, but no differences were observed in the apparent total tract digestibility of DM, OM and CP. Xu et al. (2021) reported that apparent total tract digestibility of DM and OM tended to be increased with dietary rebaudioside A supplementation in goats in summer. However, the addition of saccharin sodium did not affect apparent total tract digestibility of nutrients in lactating dairy goats during heat stress in this study. The inconsistencies might be due to the effect of sweeteners on nutrient digestibility varying with the dosages and species of sweeteners, dietary composition, and environmental conditions.

4.3. Serum biochemical parameters

Liver is the main site of biological metabolism (Han et al., 2019a). The degree of liver damage can be reflected by serum ALT or AST activity (Ben et al., 2011; Hu et al., 2014). The reduced serum AST activity in goats fed SS diet indicate that saccharin sodium supplementation may relieve liver damage in heat-stressed dairy goats. The level of TP in serum is often used as an indicator of protein utilization (Donsbough et al., 2010). Heat stress may cause decreased serum TP levels in animals (Gudev et al., 2007). Xu et al.

Table 8
Effects of dietary saccharin sodium supplementation on residue in milk, urine, and feces of lactating dairy goats.[1,2]

Item	CON	SS
Milk, mg/L	UD	UD
Urine, mg/L	UD	14.48 ± 0.21
Feces, mg/kg	UD	1.62 ± 0.27

UD = undetectable.
[1] Data are shown as means ± standard errors ($n = 6$).
[2] CON: basal diet; SS: basal diet + 150 mg/kg saccharin sodium on the basis of dry matter.

(2021) found that serum concentration of TP was increased by rebaudioside A supplementation in adult goats during summer. However, Han et al. (2019b) and Jiang et al. (2020) reported that serum concentration of TP was not influenced by dietary sweetener (stevioside or saccharin sodium) supplementation. In this study, the addition of saccharin sodium decreased the content of TP in serum. The inconsistencies about serum TP changes between this and other studies might be due to the effect of sweeteners on serum TP levels varying with the dosages and species of sweeteners, species of animals, physiological status of animals, and conditions of rearing environment. Further studies are warranted to figure out the exact mechanism of reduced serum TP content in dairy goats by saccharin sodium supplementation.

4.4. Antioxidant status and HSP70

Under suitable environment, the oxidant and antioxidant systems are maintained at a balanced level to prevent the body from becoming damaged (Kurokawa et al., 2016). However, heat stress can cause hypoxia in body, indicating as the reduced activity of antioxidant enzymes and impaired the ability of cells to remove free radicals (Han et al., 2019a). MDA is the final product of lipid peroxidation, and high levels of MDA production may cause some degree of cytotoxicity induced by free radical attack on biofilms (Li et al., 2021). Conversely, GSH-Px as key scavengers of reactive oxygen species can protect animals from oxidative injury (Min et al., 2018). Safa et al. (2019) observed that decreased T-AOC, SOD, and GSH-Px levels and increased MDA content in serum under heat stress. In the present study, supplementation with saccharin sodium resulted in an improvement in GSH-Px activity and a reduction MDA concentration in serum, indicating enhanced antioxidant capacity by saccharin sodium supplementation. As an important member of the heat shock protein family, HSP70 is widely considered as a cell thermometer due to its role in heat thermotolerance (Mishra and Palai, 2014; Hassan et al., 2019; Kaushik et al., 2022). It has been observed that the expression of HSP70 is increased during heat stress in goats (Dangi et al., 2014) and sheep (Romero et al., 2013). In this study, it was found that the consumption of sodium saccharin could decrease the level of HSP70 in serum, suggesting that dietary saccharin sodium had the potential to alleviate heat stress in dairy goats. The reduction of HSP70 can be attributed to the improved antioxidant capacity by dietary sodium saccharin supplementation (Szyller et al., 2022).

4.5. Rumen and fecal fermentation parameters

Rumen pH, VFA and NH_3–N are important indicators used to illustrate the ruminal environment and digestive function (Carpinelli et al., 2021). Rumen microorganisms can convert plant polysaccharides into VFA and provide energy for the body (Newbold and Ramos-Morales, 2020). Around 70% of energy for adult ruminants is provided by VFA (Wei et al., 2021). In rumen, NH_3–N is the main source of nitrogen for microbial protein synthesis (Nasrollahi et al., 2019). In the present study, although NH_3–N was not affected, the addition of saccharin sodium increased the total VFA, butyrate, and valerate contents in the rumen of heat stressed dairy goats. The increased levels of total VFA, butyrate, and valerate may be attributed to the effect of saccharin sodium on rumen microorganisms. Besides, the improved rumen fermentation may partially explain the improvement of milk yield of goats. However, supplementation with saccharin sodium did not affect fecal pH, VFA, and NH_3–N, which may be explained by the fact that limited saccharin sodium and nutrients arrived in the hindgut to influence fecal fermentation.

4.6. Rumen microorganisms

Analysis of rumen microorganisms can help to explain the changes in rumen fermentation parameters. The results from the current study support the findings of Koester et al. (2023) showing that feeding saccharin sodium did not affect alpha and beta diversities of microorganisms in rumen of dairy cows suffering from heat stress. However, previous studies have indicated the changes in microbial communities by saccharin sodium supplementation (Daly et al., 2016; Kelly et al., 2017). In the present study, the relative abundances of *Shuttleworthia* and *Pseudoramibacter* were higher in the rumen of goats on SS diets than those of goats on CON diet, and dairy goats fed saccharin sodium had lower relative abundance of *Prevotella*. It was found that saccharin increased the transfer ability of heat-resistant gene binding plasmid, which may change the resistance of some microorganisms to heat stress (Yu et al., 2021). This may be one of the reasons for the changes of rumen microbial abundance in heat stressed dairy goats. Besides, previous research indicated that rumen microbes of dairy cows with different DMI were significantly different (Huang et al., 2021). Therefore, the change in rumen microbes resulting from dietary saccharin sodium supplementation may be partially explained by the increased DMI. *Shuttleworthia* and *Pseudoramibacter* are important butyric acid-producing bacteria (O'Hara et al., 2018; Kong et al., 2020). *Shuttleworthia* was found to be highly positively correlated with total VFA and butyrate (Hao et al., 2021). Kong et al. (2020) indicated a positive correlation between the butyrate molar ratio and *Pseudoramibacter* relative abundance. Consistent with these results, dietary saccharin sodium supplementation increased rumen butyrate and total VFA concentrations as well as the relative abundance of *Shuttleworthia* and *Pseudoramibacter* in this study. Therefore, the increase of the relative abundance of *Shuttleworthia* and *Pseudoramibacter* is one of the main reasons for the increase of the concentration of butyrate and total VFA in the rumen. *Prevotella* are hemicellulolytic and proteolytic bacteria that mainly produce acetate and propionate rather than butyrate (Matsui et al., 2000). Previous studies reported that the concentrations of butyrate and total VFA in the rumen were negatively correlated with the abundance of *Prevotella* (Xiao et al., 2016; Liu et al., 2019). Supportively, in this study, dietary supplementation with saccharin sodium increased rumen butyrate content but decreased the relative abundance of *Prevotella*.

4.7. Saccharin sodium excretion

Previous studies have indicated that about 85% to 95% of ingested saccharin is absorbed and excreted in the urine, and the remainder is eliminated in feces (Rychen et al., 2018; Moriconi et al., 2020). In this study, saccharin sodium was detected in the feces and urine of dairy goats fed diet with saccharin sodium, but undetectable in the milk. These results indicate that saccharin sodium was absorbed by the gastrointestinal tract into the serum and excreted through urine, and the unabsorbed part was excreted through feces in dairy goats. Magnuson et al. (2016) reported that saccharin was rapidly eliminated from the general circulation when it was directly injected intravenously, with a plasma elimination half-life about 40 min in rats and 70 min in humans. Therefore, one of the reasons of undetectable residue in milk may be the quick excretion of absorbed saccharin sodium by urine. Saturation of renal excretion and excessive accumulation of saccharin will occur when high dosages (>3% in the diet) are given (Magnuson et al., 2016). Rychen et al. (2018) suggested that the maximum safe intake of saccharin sodium for calves weighing 100 kg was 500 mg per day. Based on this data, the dose of saccharin sodium per kilogram of BW used in the present study was in the safety range.

5. Conclusions

In conclusion, these findings suggest that supplementation with saccharin sodium (150 mg/kg DM) was effective in increasing fat and energy corrected milk yield by increasing DMI and improving rumen fermentation and antioxidant capacity of dairy goats in summer. In addition, residual saccharin sodium was undetectable in milk of dairy goats fed saccharin sodium at 150 mg/kg DM. However, long-term consequences of saccharin sodium supplementation in dairy goats and its potential contribution of residue in tissues need further investigations.

Author contributions

Xiongfei Zhang: Investigation, Data curation, Formal analysis, Writing-original draft, Project administration. **Jirong Lv:** Conceptualization, Methodology, Project administration. **Jingtao Hui:** Investigation. **Ao Wu:** Investigation. **Lichao Zhao:** Formal analysis. **Linyu Feng:** Editing and revising. **Lu Deng:** Editing and revising. **Miao Yu:** Project administration. **Feng Liu:** Project administration. **Junhu Yao:** Conceptualization, Methodology, Supervision, Project administration, Editing and revising. **Xinjian Lei:** Conceptualization, Methodology, Formal analysis, Supervision, Project administration, Funding, Editing and revising. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interests

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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