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Effects of dietary supplementation of inulin on rumen fermentation and bacterial microbiota, inflammatory response and growth performance in finishing beef steers fed high or low-concentrate diet

Ke Tian[1], Junhui Liu[1], Yawang Sun, Yongjiang Wu, Juncai Chen, Ruiming Zhang, Tianle He, Guozhong Dong*

College of Animal Science and Technology, Southwest University, Beibei, Chongqing, 400715, PR China

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ABSTRACT

The objective of this study was to evaluate the effects of dietary inulin supplementation on rumen fermentation and bacterial microbiota, inflammatory response, and growth performance in finishing beef steers fed high or low-concentrate diet. Twenty-four Simmental × Luxi crossbred steers were used under a 2 × 2 treatment with 2 diet types and 2 inulin levels. The 2 diet types consisted of a low-concentrate diet (LCD; concentrate:roughage = 40:60) and a high-concentrate diet (HCD; concentrate:roughage = 60:40) and inulin was supplemented into the diets at 0 or 2% (wt/wt). The trial lasted for 42 days. Rumen fluid and plasma samples were collected at the end of the animal trial. Rumen fluid samples were analyzed for pH, short-chain fatty acids, endotoxin (lipopolysaccharide, LPS), and bacterial microbiota. Illumina Miseq sequencing of the 16S rRNA gene was applied to investigate ruminal bacterial microbiota. Plasma samples were analyzed for concentrations of pro-inflammatory cytokines including interleukin (IL)-1β, IL-6, IL-8 and tumor necrosis factor alpha as well as acute phase proteins including LPS-binding protein, haptoglobin, serum amyloid A, and C-reactive protein. Results showed that the high dietary concentrate level shifted the ruminal fermentation pattern from acetate towards propionate, butyrate and valerate, and improved growth performance. However, the high dietary concentrate level promoted ruminal pH reduction and LPS release, decreased bacterial diversity and richness, modified bacterial composition, and increased systemic inflammatory response. Inulin supplementation into the LCD increased ruminal concentrations of propionate, butyrate and iso-butyrate, and improved growth performance. Inulin supplementation into the HCD increased bacterial alpha indices (1628 *vs*. 1364, 1574 *vs*. 1329, and 0.985 *vs*. 0.975 for ACE, Chao1, and Simpson indices, respectively) and operational taxonomic units (1243 *vs*. 1080), increased *Bacteroidetes* to *Firmicutes* ratio (0.84 *vs*. 0.59), and enhanced final body weight and feed utilization. In conclusion, dietary supplementation with inulin, regardless of the dietary concentrate levels, improved

Abbreviations: ACE, abundance-based coverage estimator; ADF, acid detergent fiber; ADFI, average daily feed intake; ADG, average daily gain; AIA, acid-insoluble ash; APP, acute phase protein; Ash, crude ash; BW, body weight; Ca, calcium; CP, crude protein; CRP, C-reactive protein; DM, dry matter; EE, ether extract; HCD, high concentrate diet; HCIN, high concentrate diet + 2% inulin; Hp, haptoglobin; IL, interleukin; LBP, lipopolysaccharide binding protein; LCD, low concentrate diet; LCIN, low concentrate diet + 2% inulin; LPS, lipopolysaccharide; NDF, neutral detergent fiber; OM, organic matter; OTU, operational taxonomic units; PCoA, principal coordinate analysis; SAA, serum amyloid A; SCFA, short-chain fatty acid; TNF-α, tumor necrosis factor-α; TP, total phosphorus

* Corresponding author.
E-mail address: gzdong@swu.edu.cn (G. Dong).
[1] These authors contributed equally to this study and share the first authorship.

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growth performance of finishing beef steers probably through either altering the fermentation pattern or improving ruminal bacterial microbiota, depending on the diet type. Nonetheless, inulin supplementation did not suppress inflammatory response resulting from feeding high-concentrate diet in beef steers.

1. Introduction

In practical beef production, finishing steers are often fed high-concentrate diets to meet high energy requirement for rapid growth. Compared with low-concentrate diets, high-concentrate diets can result in more short-chain fatty acids (SCFAs), especially propionate, in the rumen and thus improve feed efficiency (Plaizier et al., 2009, 2018). However, feeding high-concentrate diets would promote rapid ruminal pH reduction and microbiota dysbiosis (Plaizier et al., 2009, 2018; Mccann et al., 2016). Moreover, a prolonged low ruminal pH will lead to increased release of endotoxin (or lipopolysaccharide, LPS) in the rumen. Lipopolysaccharide is a component of gram-negative bacterial cell wall. When LPS is translocated from the gastrointestinal tract into the blood circulation, it can stimulate the release of pro-inflammatory cytokines (Dong et al., 2011; Plaizier et al., 2018), and induce acute phase protein (APP) production (Gabay and Kushner, 1999).

Inulin has been widely used as a prebiotic, which consists of fructans in the roots and tubers of many plants and in some species of algae (Apolinário et al., 2014). These polysaccharides contain 20–30 D-fructofuranose units exclusively in β-2,1 glycosidic linkages, and the C-2 position of their terminal D-fructofuranose units is linked with D-glucopyranose. Inulin is extracted mostly from roots of *Compositae* plants, among which chicory is rich (approximately 15–20%) in inulin and is the primary source of inulin (Samanta et al., 2013). According to Zou et al. (2017) inulin could increase beneficial microorganisms like *Bifidobacteriaceae* and decrease harmful ones like *Streptococcus*, *Clostridium* and *Enterococcaceae* to restore gut health in mice. Dietary inulin supplementation can also reduce mice plasma LPS, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (Li et al., 2019). It was observed that adding 2% inulin into calf milk replacer improved growth performance, decreased fecal *E. coli* and increased fecal *Bifidobacteria* (Samanta et al., 2013). However, it should be noticed that calves can't accurately represent 'ruminants' because of their 'mono-gastric' characteristics during early life (Yanez-Ruiz et al., 2015). Actually, the rumen of adult ruminants is an anaerobic, bacteria-rich organ that provides energy to the body mainly through microbial fermentation. Previous studies on the beneficial effects of dietary inulin were all conducted with mono-gastric animals. Thus, whether inulin could work on adult ruminants is still unknown. Particularly, it remains unclear whether dietary inulin supplementation could alleviate the adverse effects resulting from feeding high-concentrate diets.

Therefore, the objective of this study was to evaluate the effects of dietary supplementation of inulin on rumen fermentation and bacterial microbiota, inflammatory response and growth performance in finishing beef steers fed high or low-concentrate diet.

Table 1
Ingredients and nutrient composition of the basal experimental diets (dry matter basis).

	LCD[1]	HCD
Ingredients (g/kg of dry matter)		
Rice straw	305	105
Sorghum distiller's grains	295	295
Corn grain	298	498
Rapeseed meal	86.1	86.1
Salt	3.0	3.0
Limestone	10.5	10.5
Premix[2]	2.8	2.8
Nutrients (g/kg of dry matter)		
Metabolizable energy (MJ/kg)	9.62	10.25
Net energy for maintenance (MJ/kg)	6.51	6.90
Net energy for growth (MJ/kg)	3.85	4.38
Crude protein	126	134
Ether extract	51	55
Crude fiber	132	90
Neutral detergent fiber	438	364
Acid detergent fiber	246	161
Calcium	5.4	5.3
Total phosphorus	3.5	3.8

[1] LCD, low concentrate diet; HCD, high concentrate diet.

[2] Premix provided the following per kg diet: Fe (as ferrous sulfate) 50 mg, Cu (as copper sulfate) 10 mg, Zn (as zinc sulfate) 30 mg, Mn (as sulfate) 20 mg, Co (as chloride) 0.1 mg, I (as potassium iodate) 0.5 mg, Se (as selenite) 0.1 mg, vitamin A 2240 IU, vitamin D_3 280 IU, and vitamin E 30 IU.

2. Materials and methods

2.1. Ethical statement

The study was approved by Southwest University Animal Ethics Committee. The experimental procedures, including animal care, were in strict accordance with the "Guidelines on Ethical Treatment of Experimental Animals (2006, No. 398)" issued by the Ministry of Science and Technology of China and the "Regulations on the Management of Experimental Animals (2006, No. 195)" issued by Chongqing Municipal People's Government.

2.2. Animals, diets and experimental design

Twenty-four Simmental × Luxi crossbred finishing steers with an initial body weight (BW) of 318.94 ± 8.36 kg and similar body condition were used in this study, and all the animals were fed the same diet before the start of the study. The steers were randomly divided into four groups and the baseline rumen microbiome was determined. The results showed a similar ($P > 0.05$) rumen bacterial microbiota among the animals (Tables S1 and S2). The four groups of steers were randomly allotted to four treatments (n = 6/treatment) in a 2 × 2 arrangement with 2 diet types and 2 inulin levels: the low concentrate diet (LCD) with a concentrate to roughage ratio of 40:60, the high concentrate diet (HCD) with a concentrate to roughage ratio of 60:40, the LCD + 2% (wt/wt) inulin (Frutafit® HD, Roosendaal, the Netherlands; LCIN), and the HCD + 2% (wt/wt) inulin (HCIN). The ingredients and chemical composition of the LCD and HCD are showed in Table 1. The animal trial continued for 42 days. Steers were housed in individual tie stalls and offered diet twice daily at 07:00 and 17:00, with free access to diet and water. Daily orts were collected for feed intake measurement. After the animal trial, all steers were taken care by stockpersons of the university experimentation station.

2.3. Sample collection

Total mixed ration samples of the 4 experimental diets were collected once per 10 days and stored at −20 °C to determine nutrient compositions. Fecal samples were collected from the rectum from day 36 through day 42 during the trial. For each steer, 100 *g* fecal sample was mixed with 20 mL of 10% H_2SO_4 and immediately stored at −20 °C for digestibility determination. Rumen fluid samples were collected 3 h after the morning feeding on day 0 and 42 by an esophageal tube equipped with a strainer and a syringe, as described by Zhou et al. (2014) and Paz et al. (2016). To avoid contamination by saliva, the initial rumen fluid collected was discarded. Next, about 150 mL rumen fluid was collected. About 50 mL of the collected rumen fluid of each steer was transferred into sterile tubes and stored in the liquid nitrogen for later microbial analysis. The rest of the rumen fluid (about 100 mL) was squeezed through four layers of cheesecloth to obtain filtrate, and a portion of which was immediately used to determine pH values by using a mobile pH meter (Rex PHS-3E, Shanghai INESA Scientific Instrument Co., Ltd., Shanghai, China). Another portion of the filtrate was transferred into sterile tubes for SCFA analyses. The last portion of the filtrate was transferred into sterile and pyrogen-free centrifuge tubes for LPS analysis. All filtrate samples were stored at −20 °C. For plasma sample collection, steers were fasted for 10 h to collect blood from the jugular vein in the early morning on day 35, and the blood samples were collected into 10-mL vacuum tubes containing Na heparin, then centrifuged immediately at ×3000 *g* and 4 °C for 15 min to harvest plasma, which was stored at −20 °C for determining LPS, inflammatory cytokines, and APPs. Sampling involving animals was skillfully conducted in a gentle and quick manner to minimize pain of the animals or disturbance to the animals.

2.4. Growth performance measurement

The BW of each steer was measured for two consecutive days at the start and the end of the trial, respectively. Dry matter (DM) intake was recorded daily for each steer. Data were subsequently used for calculation of average daily gain (ADG), average daily DM intake (ADMI), and feed conversion ratio (ADMI/ADG).

2.5. Apparent digestibility determination

Apparent digestibility was determined by indirect method using acid-insoluble ash (AIA) as the indicator. Collected feed and fecal samples were first dried at 65 °C for 72 h in an oven with forced air. Next, the samples were ground through a 1-mm screen for analyses of dry matter (DM; 934.01), ether extract (EE; 920.39), crude protein (CP; 984.13), ash (942.05), calcium (Ca; 935.13), and total phosphorus (TP; 946.06) according to the AOAC methods (Horwitz and Latimer, 2006). The organic matter (OM) was calculated as the difference between DM and ash. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed following the methods of Van Soest et al. (1991). AIA was assayed using the method described by Van Keulen and Young (1977). The equation used for digestibility calculation was: $DC\ (\%) = 100 - (I_F/I_f \times N_f/N_F) \times 100$, where DC = nutrient digestibility, I_F = AIA content in feed, I_f = AIA content in feces, N_f = nutrient content in feces, and N_F = nutrient content in feed.

2.6. SCFAs analysis

The rumen fluid samples were analyzed for SCFAs using gas chromatograph (GC-2010, Shimadzu, Japan) with SH-RTX-WAX capillary columns (30 m × 0.25 μm × 0.25 mm, Shimadzu, Japan) as described in our previous study (Yang et al., 2018). Briefly,

rumen fluid was thawed at (25 °C), centrifuged at 10,000 × g and 4 °C for 45 min, the supernatant was aspirated and mixed with metaphosphoric acid (25%) in a ratio of 5:1 and cooled at 5 °C for 5 h, then centrifuged at 14,500 × g and 4 °C for 15 min. The supernatant was transferred into a 1-mL glass vial and 1 μL aliquot was injected in split mode (50:1). The aliquots were run at a programmed temperature gradient (100 °C initial temperature for 1 min, with a 5 °C rise per min until 190 °C, and 190 °C for 15 min). The gas used consisted of O_2 (0.4 Mpa), H_2 (0.3 Mpa), and N_2 (0.7 Mpa). The column flow rate was 1 mL per minute. The temperature was 230 °C for the injector and 240 °C for the detector.

2.7. LPS assay

The determination of LPS concentration in this study using *tachypleus amebocyte lysate* (TAL) assay was as described by Gozho et al. (2005) and Zhou et al. (2014). The assay kit was purchased from Xiamen Bioendo Technology Co., Ltd (Xiamen, China), with the reference endotoxin of *Escherichia coli* O111:B4. Rumen fluid samples were thawed at room temperature and centrifuged for 45 min at 10,000 × *g* and 4 °C, and the supernatant was aspirated gently to prevent its mixing with the pellet and was passed through a disposable 0.22 μm sterile, pyrogen-free filter (Millex, Millipore Corporation, Bedford, MA). Then the filtrate was collected into a sterile glass tube (previously depyrogenated through heating at 100 °C for 30 min and cooled at room temperature) before being diluted to acquire an LPS concentration range of 0.1–1 endotoxin units (EU)/mL (1 EU = 0.1 ng). Plasma samples used for LPS analysis were unfrozen at 4 °C and diluted according to the manufacturer's protocols, and the diluted plasma samples were incubated at 70 °C for 10 min and cooled at 0 °C for 3 min. After the pretreatment, assay was performed on a 64-well microplate, with optical density of the plate being read by a microplate reader (Bio-Rad, xMark™, USA) at 405 nm.

2.8. Plasma cytokines and APPs assay

The bovine-exclusive ELISA kit was used to measure the concentrations of plasma IL-1β, IL-6, IL-8 and TNF-α. The bovine ELISA kits were purchased from Shanghai Sinobest Biotechnology Co., Ltd. (Shanghai, China). APPs including LPS-binding protein (LBP), haptoglobin (Hp), serum amyloid A (SAA) and C-reactive protein (CRP) were determined using the bovine ELISA kits purchased from Shanghai Jinma Biotechnology Co., Ltd. (Shanghai, China). The determination was conducted according to the manufacturer's instructions. All samples including the standards were tested in duplicate, and the optical density values were read on a microplate reader (Bio-Rad, xMarkTM, USA) at 450 nm.

2.9. DNA isolation and PCR amplification

After bead-beating (homogenizing) and incubating at 90 °C for 10 min, ruminal fluid DNAs were extracted using the DNeasy PowerSoil Kit (QIAGEN, Inc., Netherlands) according to the manufacturer's instructions, and extracted DNAs were quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The V3-V4 regions of rumen fluid bacterial 16S rRNA genes were amplified by PCR using primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGT-WTCTAAT-3′) as described by Caporaso et al. (2012) and Wang et al. (2017) and were duplicately processed in 25 μL mixture containing ddH2O 8.75 μL, 5 × reaction buffer 5 μL, 5 × GC buffer 5 μL, dNTP (2.5 mM) 2 μL, DNA template 2 μL, Forwardprimer (10 uM) 1 μL, Reverseprimer (10 uM) 1 μL, and Q5 DNA polymerase 0.25 μL. The PCR amplification was performed as follows: initial denaturation at 98 °C for 2 min; followed by 25–30 cycles of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; with final extension of 5 min at 72 °C. PCR products were selected by 2% agarose gel electrophoresis, retrieved using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences; Union City, CA, USA), quantified using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, USA) and read on the microplate reader (BioTek, FLx800, USA).

2.10. Construction and quality control of sequencing libraries

Sequencing libraries were constructed using the TruSeq Nano DNA LT Library Prep Kit (Illumina, San Diego, CA, USA). The End Repair Mix 2 of the kit was used to remove the bulged base at the 5′ end of the DNA sequence, and the phosphate group was added to fill the missing base at the 3′ end. Adenine was added at the 3′ end of the DNA sequence to prevent DNA fragments from self-linking and to ensure that the target sequence could be connected to the sequencing connector. At the 5′ end of the sequence, a sequencing connector containing a library specific label (index sequence) was added to enable DNA molecules to be fixed on the Flow Cell, and the BECKMAN AMPure XP Beads (Beckman Coulter, Brea, CA, USA) were used to remove the self-connecting fragments for purifying the library. Again, PCR was performed and BECKMAN AMPure XP Beads were used for fragments amplification and purification. Next, these fragments were selected and purified by 2% agarose gel electrophoresis to complete the bank. Before sequencing on the Illumina MiSeq platform, the Agilent High Sensitivity DNA Kit (Agilent, Santa Clara, CA, USA) was employed to carry out quality control of the library on Agilent Bioanalyzer (Agilent, Santa Clara, CA, USA). Subsequently, the Quant-it PicoGreen dsDNA Assay Kit (Invitrogen, Thermo Fisher Scientific, Dublin, Ireland) was used to quantify the library on the Promega QuantiFluor Fluorescent Quantitative System (Promega, Madison, WI, USA), and sequencing libraries with > 2 nM concentration were considered qualified.

2.11. Illumina Miseq sequencing and raw data processing

Libraries from each sample were pooled in equal amounts, and denatured to single chains using 0.1 N NaOH for paired-end

2 × 300 bp sequencing on the Illumina MiSeq platform using the MiSeq Reagent Kit V3 (Illumina, San Diego, CA, USA). To control the quality, the raw sequencing data in Fastq format were quality-filtered using the sliding window method (window size 10 bp, step length 1 bp). The window slided from the 5′ end at the first base, and the average quality score of the bases in the window should be ≥Q20. The sequence was cut off immediately at the window if the quality score is < Q20, and the remaining sequences which containing ambiguous bases or < 150 bp were discarded. FLASH (v1.2.7) was used to match and connect the sequences (Magoc and Salzberg, 2011). And based on the index sequence, connected sequences were assigned to the relevant samples to obtain the valid sequence of each sample. Valid sequences were further selected using the QIIME (Quantitative Insights Into Microbial Ecology, v1.8.0) (Caporaso et al., 2010), and the valid sequences should be ≥160 bp, containing no ambiguous bases, < 1 of the primer mismatch base at the 5′ end, and < 8 of the same base in a row. Also, USEARCH (v5.2) was used to remove the chimera sequence. The obtained sequences were clustered and divided into operational taxonomic units (OTUs) on a basis of 97% similarity using the UCLUST (Edgar, 2010). The sequence with the highest abundance in each OTU was selected as the representative sequence of this OTU. Finally, the taxonomic information of these OTUs was identified by comparing with the existing data in Greengenes Database v13.8 (DeSantis et al., 2006). Alpha diversity of Shannon, Simpson, ACE and Chao1 was evaluated using the QIIME (v1.8). Principle coordinate analysis (PCoA) was conducted using the R software. Sequence data of the present study were submitted to the Sequence Read Archive (SRA) of the NCBI under the project number of PRJNA545177.

2.12. Statistical analysis

Data of the present study were analyzed using MIXED procedure in SPSS software v20.0 (IBM, Armonk, NY, United States). A two (diet types) by two (inulin supplementation levels) factorial arrangement of treatments in a randomized complete block design was employed in the analysis of variance for data of ruminal fermentation, growth performance, nutrient digestibility, microbial microbiota, and inflammatory response. Fixed effects included diet type, inulin supplementation level, and their interaction. The animals within the same diet type and inulin supplementation level were considered as the random effect. The model used in the statistical analysis is as follows: $Yijk = \mu + Di + Ij + Ck + Tij + eijk$, where Yijk is the observation of dependent variables; μ is the overall mean; Di represents the fixed effect of diet type; Ij is the fixed effect of inulin; Ck is the random cattle effect; Tij is the fixed effect of the diet × inulin interaction; and eijk is the residual error for the observation. Differences among treatment means were classified using Duncan's multiple range test and significant level and statistical tendency were set at $P < 0.05$ and $0.05 < P < 0.10$, respectively. Results were presented as means ± standard deviation (SD).

3. Result

3.1. Growth performance

The results showed that final BW, ADG and ADFI were elevated by feeding high-concentrate diet ($P < 0.01$) while feed conversion ratio was decreased ($P < 0.01$, Table 2). Dietary supplementation of inulin did not affect ADFI ($P > 0.1$), but significantly ($P < 0.05$) increased final BW, and decreased feed conversion ratio ($P = 0.005$). The diet × inulin interaction was significant for feed conversion ratio ($P = 0.017$). Inulin supplementation into both the LCD and HCD resulted in lower feed conversion ratio ($P < 0.05$).

3.2. Apparent digestibility

The apparent digestibility of nutrients is presented in Table 3. High dietary concentrate level significantly increased the digestibility of DM, OM, CP and Ca ($P < 0.05$), but decreased that of NDF ($P < 0.001$) and TP ($P = 0.008$). Inulin supplementation increased EE digestibility ($P = 0.007$), but decreased NDF digestibility ($P < 0.05$). There was a significant diet × inulin interaction for the digestibility of CP, NDF and TP ($P < 0.05$). Inulin supplementation into the HCD increased the digestibility of CP ($P < 0.05$),

Table 2
Effects of different diets on growth performance of beef steers.[1]

	Low-concentrate		High-concentrate		*P*-value		
Items	LCD[2]	LCIN	HCD	HCIN	Diet	Inulin	Diet × Inulin
Initial BW[3](kg)	315 ± 10.47	321 ± 6.80	320 ± 10.84	319 ± 6.16	0.745	0.571	0.335
Final BW (kg)	330 ± 11.23^{a}	339 ± 6.58^{b}	342 ± 7.52^{b}	351 ± 6.04^{c}	0.001	0.013	0.986
ADG (kg)	0.34 ± 0.07^{a}	0.41 ± 0.06^{a}	0.53 ± 0.13^{b}	0.77 ± 0.21^{c}	< 0.001	0.009	0.128
ADFI (kg, DM)	6.43 ± 0.31^{a}	6.19 ± 0.26^{a}	6.71 ± 0.18^{b}	6.96 ± 0.12^{b}	< 0.001	0.993	0.953
Feed conversion ratio	19.43 ± 3.91^{c}	15.20 ± 1.96^{b}	13.65 ± 4.04^{b}	9.58 ± 2.23^{a}	< 0.001	0.005	0.017

$^{a-d}$Means within a row without a common superscript differ (P < 0.05).
[1] Data are shown as the mean ± SD.
[2] LCD, low concentrate diet; LCIN, LCD + 2% inulin; HCD, high concentrate diet; HCIN, HCD + 2% inulin.
[3] BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; DM, dry matter.

Table 3
The apparent digestibility of nutrients in beef steers fed different diets.[1]

	Low-concentrate		High-concentrate		*P*-value		
Nutrients	LCD[2]	LCIN	HCD	HCIN	Diet	Inulin	Diet × Inulin
DM[3]	0.61 ± 0.05[a]	0.62 ± 0.04[a]	0.64 ± 0.04[ab]	0.68 ± 0.05[b]	0.047	0.232	0.341
OM	0.61 ± 0.04[a]	0.62 ± 0.04[a]	0.66 ± 0.03[b]	0.68 ± 0.03[b]	0.002	0.635	0.658
CP	0.55 ± 0.08[a]	0.52 ± 0.07[a]	0.69 ± 0.07[b]	0.79 ± 0.06[c]	< 0.001	0.023	0.049
EE	0.62 ± 0.07[a]	0.70 ± 0.03[b]	0.59 ± 0.07[a]	0.67 ± 0.07[ab]	0.275	0.007	0.950
Ash	0.19 ± 0.06	0.21 ± 0.05	0.17 ± 0.04	0.19 ± 0.06	0.346	0.483	0.861
NDF	0.58 ± 0.06[ac]	0.61 ± 0.05[c]	0.55 ± 0.03[a]	0.49 ± 0.04[b]	< 0.001	0.049	0.027
ADF	0.64 ± 0.08	0.65 ± 0.04	0.65 ± 0.05	0.66 ± 0.06	0.608	0.748	0.870
Ca	0.41 ± 0.06[a]	0.43 ± 0.06[a]	0.52 ± 0.06[b]	0.57 ± 0.07[b]	< 0.001	0.245	0.448
TP	0.53 ± 0.07[ab]	0.60 ± 0.07[b]	0.52 ± 0.06[b]	0.45 ± 0.04[a]	0.008	0.964	0.023

[a−d]Means within a row without common superscripts are significantly different (P < 0.05).
[1] Data are shown as the mean ± SD.
[2] LCD, low concentrate diet; LCIN, LCD + 2% inulin; HCD, high concentrate diet; HCIN, HCD + 2% inulin.
[3] DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; Ash, crude ash; NDF, neutral detergent fiber; ADF, acid detergent fiber; Ca, calcium; TP, total phosphorus.

but deceased that of NDF and TP ($P < 0.05$), whereas inulin supplementation into the LCD did not affect the digestibility of CP, NDF and TP ($P > 0.05$).

3.3. Ruminal fermentation

High dietary concentrate level significantly lowered the ruminal pH value ($P < 0.001$, Table 4), whereas dietary inulin supplementation further reduced ruminal pH values ($P = 0.009$). The interaction of diet × inulin was not significant for the ruminal pH value ($P > 0.1$). With regard to ruminal SCFA concentrations, higher concentrations of propionate, butyrate and valerate with lower content of acetate were observed in high-concentrate diet groups compared with low-concentrate diet groups ($P < 0.01$). Inulin supplementation led to higher propionate, butyrate and iso-butyrate ($P < 0.05$). There was a significant diet × inulin interaction for ruminal propionate ($P = 0.011$). Inulin supplementation into the LCD rather than the HCD resulted in higher ruminal propionate content ($P < 0.05$).

3.4. Ruminal bacterial microbiota

In the present study, a total of 7,60,994 reads with an average number of 33,087 ± 5618 per sample were obtained using 16S rRNA sequencing. Based on the 97% similarity of taxonomic classification, a total of 7223 OTUs with an average number of 1187 ± 85 per sample were identified. As the rarefaction curves of Shannon index reached a plateau (Fig. 1), it indicated that the sequencing depth in the present study was enough to detect most of the bacteria.

Results showed that diet type and inulin supplementation significantly affected the OTUs of ruminal bacteria, and there existed a diet × inulin interaction ($P < 0.001$, Table 5). The LCD displayed higher OTUs than the HCD ($P < 0.05$). When steers were fed with high-concentrate diets, inulin supplementation increased OTUs ($P < 0.001$); but when given low-concentrate diets, inulin supplementation decreased OTUs ($P = 0.035$). Diet type had little effect on ACE or Chao1 indices ($P > 0.1$), but significantly affected

Table 4
The ruminal pH and short-chain fatty acids (SCFAs) concentrations (mmol/L) in beef steers fed different diets.[1]

	Low-concentrate		High-concentrate		*P*-value		
Items	LCD[2]	LCIN	HCD	HCIN	Diet	Inulin	Diet × Inulin
Ruminal pH	7.40 ± 0.17[c]	7.26 ± 0.09[c]	6.84 ± 0.20[a]	6.59 ± 0.15[b]	< 0.001	0.009	0.421
Total SCFA[3]	52.54 ± 2.12	54.33 ± 1.82	54.84 ± 3.25	55.40 ± 1.19	0.091	0.229	0.525
Acetate	36.22 ± 1.95[c]	31.52 ± 1.94[c]	26.72 ± 2.98[a]	26.32 ± 1.09[a]	< 0.001	0.100	0.270
Propionate	9.59 ± 1.17[a]	13.16 ± 1.96[b]	17.80 ± 1.94[c]	17.35 ± 1.69[c]	< 0.001	0.043	0.011
Butyrate	5.51 ± 1.04[a]	8.16 ± 1.07[ab]	8.33 ± 0.83[a]	9.81 ± 0.94[d]	< 0.001	< 0.001	0.172
Iso-butyrate	0.21 ± 0.03[ac]	0.37 ± 0.06[d]	0.17 ± 0.02[a]	0.28 ± 0.04[b]	0.001	< 0.001	0.162
Valerate	0.49 ± 0.07[a]	0.47 ± 0.12[a]	1.07 ± 0.16[b]	0.96 ± 0.13[b]	< 0.001	0.239	0.401
Iso-valerate	0.53 ± 0.12	0.65 ± 0.17	0.76 ± 0.20	0.68 ± 0.15	0.059	0.759	0.136

[a−d]Means within a row without common superscripts are significantly different (P < 0.05).
[1] Data are shown as the mean ± SD.
[2] LCD, low concentrate diet; LCIN, LCD + 2% inulin; HCD, high concentrate diet; HCIN, HCD + 2% inulin.
[3] SCFA, short-chain fatty acid.

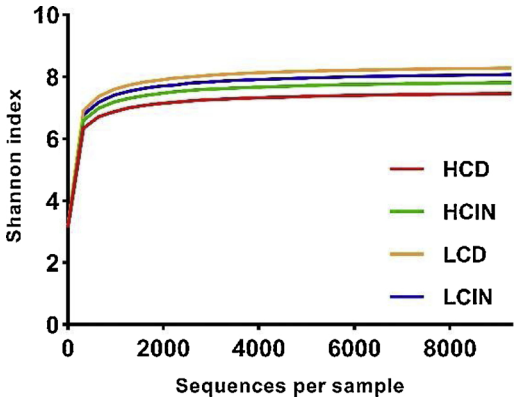

Fig. 1. Rarefaction curves of Shannon index of ruminal bacteria in beef steers fed different diets. LCD = low concentrate diet, LCIN = LCD + 2% inulin, HCD = high concentrate diet, and HCIN = HCD + 2% inulin.

Table 5
Operational taxonomic units (OTUs) and alpha indices of ruminal bacteria in beef steers fed different diets.[1]

	Low-concentrate		High-concentrate		*P*-value		
Items	LCD[2]	LCIN	HCD	HCIN	Diet	Inulin	Diet × Inulin
OTUs[3]	1250 ± 51^{c}	1183 ± 72^{b}	1080 ± 45^{a}	1243 ± 16^{bc}	0.020	0.040	< 0.001
ACE	1522 ± 128^{bc}	1429 ± 97^{ab}	1365 ± 96^{a}	1628 ± 30^{c}	0.611	0.050	< 0.001
Chao1	1511 ± 121^{bc}	1417 ± 56^{ab}	1329 ± 92^{a}	1574 ± 69^{c}	0.755	0.046	< 0.001
Shannon	8.48 ± 0.28^{c}	7.81 ± 0.33^{ab}	7.43 ± 0.66^{a}	8.08 ± 0.11^{c}	0.033	0.949	0.001
Simpson	0.988 ± 0.005^{b}	0.985 ± 0.002^{b}	0.975 ± 0.002^{a}	0.985 ± 0.001^{b}	< 0.001	0.017	< 0.001

$^{a-d}$Means within a row without common superscripts are significantly different (P < 0.05).
[1] Data are shown as the mean ± SD.
[2] LCD, low concentrate diet; LCIN, LCD + 2% inulin; HCD, high concentrate diet; HCIN, HCD + 2% inulin.
[3] OTUs, operational taxonomic units; ACE, abundance-based coverage estimator.

Shannon index ($P = 0.033$) and Simpson index ($P < 0.001$). The diet × inulin interaction was significant for all alpha diversity indices ($P < 0.01$). Inulin supplementation into the LCD did not improve alpha diversity indices, whereas inulin supplementation into the HCD increased alpha diversity indices ($P < 0.05$).

The differences of OTUs across the treatments acquired by PCoA (principal coordinate analysis) are presented in Fig. 2. The distance between points indicates the degree of similarity and variation of samples, and the PC1 (44.66%) and PC2 (17.21%) represent variation percentages between samples. Result showed there were clear separations between HCD and LCD groups. HCIN group had a clear separation with LCIN group, but couldn't be clearly separated from HCD and LCD groups.

Results of rumen bacterial microbiota revealed shifts in relative abundance at phylum and genus levels among experimental groups (Table 6). High dietary concentrate level increased *Firmicutes*, *Proteobacteria* and *Actinobacteria* ($P < 0.05$), but decreased

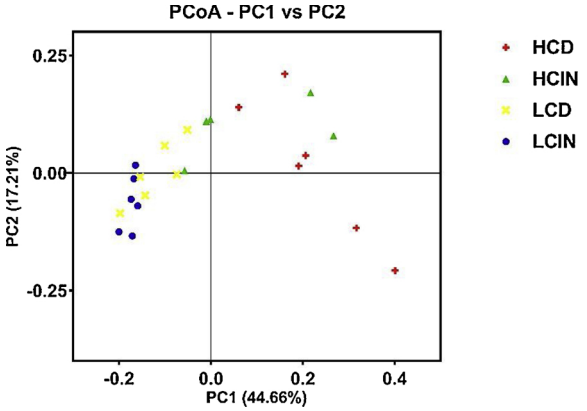

Fig. 2. Principal coordinate analysis (PCoA) of ruminal bacteria in beef steers fed different diets. PC1, PCoA axis 1; PC2, PCoA axis 2; LCD, low concentrate diet; LCIN, LCD + 2% inulin; HCD, high concentrate diet; and HCIN, HCD + 2% inulin.

Table 6
Phylum level composition (% of total sequences) of ruminal bacteria in beef steers fed different diets.[1]

	Low-concentrate		High-concentrate		*P*-value		
Phyla	LCD[2]	LCIN	HCD	HCIN	Diet	Inulin	Diet × Inulin
Bacteroidetes	51.50 ± 2.35^{c}	57.83 ± 3.60^{d}	34.83 ± 3.19^{a}	41.40 ± 1.14^{b}	< 0.001	< 0.001	0.922
Firmicutes	43.17 ± 2.93^{b}	35.67 ± 4.23^{a}	58.83 ± 4.36^{d}	49.20 ± 2.28^{c}	< 0.001	< 0.001	0.489
Proteobacteria	0.83 ± 0.41^{a}	1.33 ± 0.52^{a}	1.33 ± 0.82^{a}	3.00 ± 2.00^{b}	0.025	0.025	0.206
Actinobacteria	0.33 ± 0.52^{b}	< 0.01^{a}	2.17 ± 2.04^{c}	0.40 ± 0.55^{b}	0.026	0.036	0.139
Spirochaetes	1.17 ± 0.41^{a}	2.33 ± 0.51^{b}	0.83 ± 1.67^{a}	2.00 ± 1.41^{ab}	0.516	0.017	0.755
Tenericutes	0.83 ± 0.41	0.83 ± 0.41	0.67 ± 0.52	0.80 ± 0.45	0.599	0.726	0.726
Fibrobacteres	0.01 ± 0.63	0.33 ± 0.52	0.67 ± 0.82	0.40 ± 0.55	0.626	0.099	0.467
TM7	0.33 ± 0.52	< 0.01	0.67 ± 0.82	0.60 ± 0.89	0.099	0.467	0.626
Verrucomicrobia	0.50 ± 0.55	0.17 ± 0.41	0.33 ± 0.82	0.20 ± 0.45	0.788	0.351	0.687
SR1	< 0.01	< 0.01	< 0.01	1.20 ± 1.79	0.097	0.097	0.097
WPS-2	< 0.01	0.17 ± 0.41	< 0.01	0.2 ± 0.45	0.893	0.151	0.893
Cyanobacteria	< 0.01	< 0.01	< 0.01	< 0.01	0.200	0.667	0.393
Synergistetes	< 0.01	< 0.01	< 0.01	< 0.01	0.094	0.923	0.417
Lentisphaerae	< 0.01	< 0.01	< 0.01	< 0.01	0.718	0.682	0.68
Elusimicrobia	< 0.01	< 0.01	< 0.01	< 0.01	0.867	0.470	0.921
Fusobacteria	< 0.01	< 0.01	< 0.01	< 0.01	0.068	0.409	0.165

$^{a-d}$Means within a row without common superscripts are significantly different (P < 0.05).
[1] Data are shown as the mean ± SD.
[2] LCD, low concentrate diet; LCIN, LCD + 2% inulin; HCD, high concentrate diet; HCIN, HCD + 2% inulin.

Bacteroidetes ($P < 0.001$). Inulin supplementation significantly increased the relative abundance of *Bacteroidetes*, *Proteobacteria* and *Spirochaetes* ($P < 0.05$), but decrease that of *Firmicutes* and *Actinobacteria* ($P < 0.05$). The *Bacteroidetes* to *Firmicutes* ratios were 0.84 and 0.59 for the HCIN and HCD groups, respectively. The diet × inulin interaction was not significant ($P > 0.1$) for any of the phyla.

At the genus level (Table 7), high dietary concentrate level increased relative abundance of *Succiniclasticum*, *Butyrivibrio*, and

Table 7
Genus level composition (% of total sequences) of ruminal bacteria in beef steers fed different diets.[1]

	Low-concentrate		High-concentrate		*P*-value		
Genera	LCD[2]	LCIN	HCD	HCIN	Diet	Inulin	Diet × Inulin
Prevotella	30.33 ± 1.97^{c}	20.00 ± 2.97^{b}	17.83 ± 2.48^{ab}	15.60 ± 4.62^{a}	< 0.001	< 0.001	0.005
Unclassified_Bacteroidales	11.00 ± 3.52^{a}	21.50 ± 2.81^{b}	10.00 ± 3.69^{a}	10.60 ± 3.85^{a}	0.001	0.001	0.003
Unclassified_Clostridiales	10.17 ± 2.04	12.83 ± 2.04	10.00 ± 4.47	11.40 ± 3.85	0.563	0.151	0.647
Unclassified_Ruminococcaceae	9.67 ± 2.42	8.67 ± 2.50	9.50 ± 6.41	11.40 ± 5.94	0.516	0.819	0.463
Succiniclasticum	7.83 ± 1.94^{b}	5.50 ± 1.05^{a}	15.00 ± 5.66^{c}	8.00 ± 1.87^{b}	0.002	0.003	0.101
Ruminococcus	7.17 ± 2.79^{b}	3.33 ± 1.03^{a}	10.00 ± 4.86^{b}	4.00 ± 2.74^{a}	0.204	0.002	0.426
Unclassified_RF16	2.33 ± 1.97^{a}	11.50 ± 3.89^{b}	1.17 ± 1.33^{a}	9.20 ± 0.84^{b}	0.096	< 0.001	0.574
Unclassified_S24-7	4.50 ± 1.52^{b}	1.00 ± 0.63^{a}	3.50 ± 2.74^{b}	3.00 ± 1.87^{ab}	0.526	0.018	0.068
Unclassified_Lachnospiraceae	2.00 ± 0.63	2.00 ± 1.10	4.00 ± 2.83	2.40 ± 1.45	0.109	0.276	0.276
Unclassified_BS11	1.17 ± 0.98^{a}	1.00 ± 1.27^{a}	0.50 ± 0.84^{a}	3.20 ± 3.63^{b}	0.349	0.029	0.088
Butyrivibrio	0.67 ± 0.82^{a}	0.50 ± 0.55^{a}	3.50 ± 3.62^{b}	2.20 ± 0.84^{ab}	0.012	0.383	0.498
Unclassified_Succinivibrionaceae	0.17 ± 0.41^{b}	0.17 ± 0.41^{b}	< 0.01^{a}	2.80 ± 0.84^{b}	< 0.001	< 0.001	< 0.001
Treponema	1.50 ± 0.84	1.83 ± 0.98	0.67 ± 0.82	1.20 ± 1.10	0.075	0.280	0.800
Unclassified_Coriobacteriaceae	< 0.01	< 0.01	2.00 ± 3.10	< 0.01	0.149	0.149	0.149
Sharpea	< 0.01^{a}	< 0.01^{a}	0.50 ± 0.24^{a}	1.20 ± 0.45^{b}	< 0.001	0.045	0.095
CF231	1.33 ± 0.82^{b}	0.67 ± 0.52^{ab}	< 0.01^{a}	0.60 ± 0.55^{a}	0.007	0.887	0.013
Moryella	0.67 ± 0.52	0.50 ± 0.55	0.67 ± 0.52	1.00 ± 0.01	0.217	0.675	0.217
Unclassified_RF39	1.00 ± 1.55	0.50 ± 0.55	0.67 ± 0.52	0.20 ± 0.45	0.414	0.218	0.965
Fibrobacter	0.83 ± 0.75	0.50 ± 0.84	0.67 ± 0.82	0.40 ± 0.55	0.678	0.355	0.917
F16	0.50 ± 0.84	< 0.01	0.67 ± 0.82	0.60 ± 0.89	0.223	0.363	0.485
Clostridium	< 0.01	< 0.01	< 0.01	< 0.01	0.508	0.287	0.162
Bulleidia	< 0.01	< 0.01	1.83 ± 3.06	< 0.01	0.179	0.179	0.179
Asteroleplasma	1.00 ± 1.55	< 0.01	< 0.01	0.80 ± 1.30	0.813	0.813	0.054
Selenomonas	0.33 ± 0.52	0.33 ± 0.52	0.17 ± 0.41	< 0.01	0.180	0.648	0.648
Unclassified_SR1	< 0.01	< 0.01	< 0.01	< 0.01	0.097	0.097	0.097
Unclassified_Bifidobacteriaceae	< 0.01	0.33 ± 0.82	0.83 ± 1.33	< 0.01	0.464	0.464	0.098
Unclassified_Christensenellaceae	0.27 ± 0.29	< 0.01	< 0.01	0.20 ± 0.45	0.112	0.112	0.116

$^{a-d}$Means within a row without common superscripts are significantly different (P < 0.05).
[1] Data are shown as the mean ± SD.
[2] LCD, low concentrate diet; LCIN, LCD + 2% inulin; HCD, high concentrate diet; HCIN, HCD + 2% inulin.

Sharpea ($P < 0.05$), but decreased that of *Prevotella*, *CF231*, Unclassified_Succinivibrionaceae and Unclassified_Bacteroidales ($P < 0.05$). Inulin supplementation increased the relative abundance of Unclassified_RF16 ($P < 0.05$), but decreased that of *Prevotella*, *Succiniclasticum*, and *Ruminococcus* ($P < 0.05$). When steers were fed with low-concentrate diets, inulin supplementation increased the relative abundance of Unclassified_Bacteroidales ($P < 0.05$), but decreased that of Unclassified_S24-7 ($P < 0.05$). When steers were fed with high-concentrate diets, inulin supplementation increased the relative abundance of Unclassified_BS11, Unclassified_Succinivibrionaceae, and *Sharpea* ($P < 0.05$). The diet × inulin interaction was significant for *Prevotella*, Unclassified_Bacteroidales, and Unclassified_Succinivibrionaceae, and *CF231* ($P < 0.05$). Inulin supplementation into the LCD reduced ($P < 0.05$) the relative abundance of *Prevotella* and *CF231*, increased ($P < 0.05$) that of Unclassified_Bacteroidales, but did not affect ($P > 0.05$) that of Unclassified_Succinivibrionaceae, whereas inulin supplementation into the HCD increased ($P < 0.05$) the relative abundance of Unclassified_Succinivibrionaceae, but did not affect ($P > 0.05$) that of Prevotella, Unclassified_Bacteroidales, and CF231.

3.5. LPS concentrations and inflammatory response

High dietary concentrate level certainly had higher LPS concentrations in rumen fluid ($P < 0.001$; Fig. 3A). Inulin supplementation increased LPS concentration in rumen fluid ($P = 0.014$; Fig. 3A). The LPS concentration in plasma also increased in high concentrate diet groups ($P < 0.001$; Fig. 3B). Inulin supplementation did not affect plasma LPS concentration ($P = 0.158$; Fig. 3B). The diet × inulin interaction was not significant for rumen fluid ($P = 0.075$, Fig. 3A) and plasma LPS concentrations ($P = 0.179$, Fig. 3B).

With respect to plasma cytokines, IL-1β, IL-6, IL-8 and TNF-α were increased by high dietary concentrate level ($P < 0.01$, Table 8). Supplementation of inulin had no significant effects on all the cytokines ($P > 0.1$), and diet × inulin interaction was not significant for plasma cytokines ($P > 0.1$).

High dietary concentrate level resulted in higher plasma APPs including SAA, CRP, Hp and LBP ($P < 0.01$, Table 9). Supplementation of inulin had no significant effects on plasma APPs ($P > 0.1$). There was no diet × inulin interaction for plasma APPs ($P > 0.1$).

4. Discussion

Beef steers generally are given high concentrate diet when they reach the fattening stage to achieve higher growth performance. Dietary concentrate is rapidly fermented by ruminal microorganism to produce SCFAs (Kim et al., 2018; Ma et al., 2018). The present study observed that feeding the HCD shifted the fermentation pattern from acetate to propionate, butyrate, and valerate. This could explain the higher growth performance observed in the present study, since propionate and butyrate as well as valerate are conducive to enhancing growth performance (Bergman, 1990).

However, in the present study, feeding the HCD promoted ruminal pH reduction and LPS release, as compared with feeding the LCD. LPS originates from cell wall of gram-negative bacteria, and is released due to cell lysis (Dong et al., 2011). In the present study, high dietary concentrate level induced lower ruminal pH, which is consistent with the results of other study (Khafipour et al., 2016). The lower ruminal pH can result in more deaths of gram-negative bacteria and excessive release of LPS (Khafipour et al., 2016). When LPS was translocated from the rumen epithelium into the blood, it ultimately induced body inflammatory responses (Humer et al., 2018; Li et al., 2012; Chang et al., 2018; Eckel and Ametaj, 2016). Dong et al. (2011) previously concluded that translocation of LPS from the rumen into blood could increase pro-inflammatory cytokines release and APPs production. Similarly, we observed that compared with the LCD, the HCD significantly increased plasma LPS concentration, and accordingly the higher concentrations of pro-inflammatory cytokines including IL-1β, IL-6, IL-8 and TNF-α as well as APPs including LBP, CRP, SAA and HP were observed in our present study.

It is noted that the ruminal pH values in our present study were higher than in other studies (Plaizier et al., 2009; Khafipour et al.,

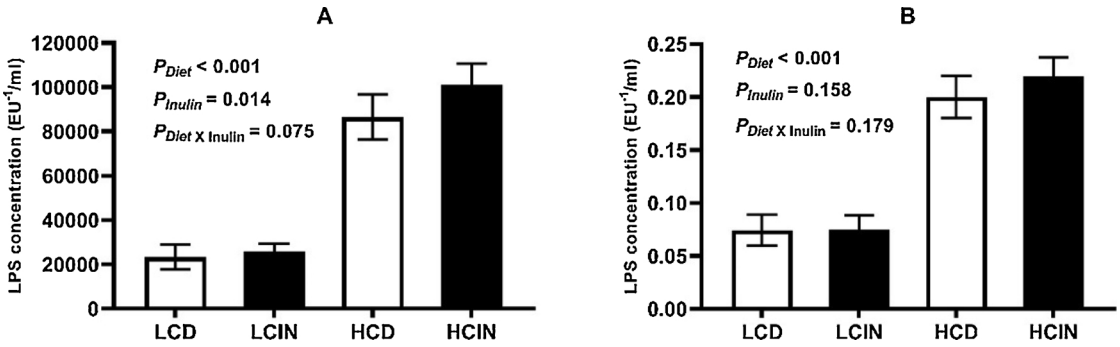

Fig. 3. The lipopolysaccharide (LPS) concentrations in ruminal fluid (A) and plasma (B) of beef steers fed different diets. LCD = low concentrate diet, LCIN = LCD + 2% inulin, HCD = high concentrate diet, and HCIN = HCD + 2% inulin. P_{Diet} represents the significance level of the main effect of diet type; P_{Inulin} represents that of the main effect of inulin; $P_{Diet \times Inulin}$ represents that of diet × inulin interaction.

Table 8
Concentrations of plasma pro-inflammatory cytokines in beef steers fed different diets.[1]

	Low-concentrate		High-concentrate		*P*-value		
Items[3]	LCD[2]	LCIN	HCD	HCIN	Diet	Inulin	Diet × Inulin
TNF-α (pg/mL)[3]	1209 ± 121^{a}	1217 ± 124^{a}	1430 ± 119^{b}	1552 ± 88^{b}	< 0.001	0.180	0.235
IL-1β (pg/mL)	135 ± 6^{a}	136 ± 5^{a}	159 ± 21^{b}	155 ± 18^{b}	0.002	0.869	0.647
IL-6 (pg/mL)	298 ± 15^{a}	298 ± 10^{a}	320 ± 15^{b}	323 ± 11^{b}	< 0.001	0.796	0.790
IL-8 (pg/mL)	291 ± 28^{a}	301 ± 11^{a}	325 ± 11^{b}	345 ± 11^{b}	< 0.001	0.145	0.541

$^{a-d}$Means within a row without common superscripts are significantly different (P < 0.05).
[1] Data are shown as the mean ± SD.
[2] LCD, low concentrate diet; LCIN, LCD + 2% inulin; HCD, high concentrate diet; HCIN, HCD + 2% inulin.
[3] IL-1β, interleukin 1β; IL-6, interleukin 6; IL-8, interleukin 8; TNF-α, tumor necrosis factor alpha.

Table 9
Concentrations of plasma acute phase proteins in beef steers fed different diets.[1]

	Low-concentrate		High-concentrate		*P*-value		
Items	LCD[2]	LCIN	HCD	HCIN	Diet	Inulin	Diet × Inulin
LBP (μg/mL)[3]	298 ± 13^{a}	301 ± 28^{ab}	324 ± 25^{bc}	344 ± 12^{c}	0.001	0.186	0.284
SAA (μg/mL)	13.73 ± 0.46^{a}	14.44 ± 0.86^{ab}	15.27 ± 0.65^{bc}	16.31 ± 1.52^{c}	< 0.001	0.058	0.675
CRP (mg/L)	9.33 ± 0.99^{a}	9.79 ± 1.82^{a}	11.31 ± 0.80^{bc}	12.96 ± 0.95^{c}	< 0.001	0.087	0.389
Hp (μg/mL)	1753 ± 370^{a}	1765 ± 210^{a}	2108 ± 215^{b}	2357 ± 270^{b}	< 0.001	0.197	0.234

$^{a-d}$Means within a row without common superscripts are significantly different (P < 0.05).
[1] Data are shown as the mean ± SD.
[2] LCD, low concentrate diet; LCIN, LCD + 2% inulin; HCD, high concentrate diet; HCIN, HCD + 2% inulin.
[3] LBP, lipopolysaccharide-binding protein; Hp, haptoglobin; SAA, serum amyloid A; CRP, C reactive protein.

2016; Kim et al., 2018), which could be due to the rice straw used in our study. Rice straw is of poor quality due to plentiful and slow-degradable fiber, thereby probably resulting in lower SCFA production in the rumen and precluding a sharp decline of ruminal pH.

Plaizier et al. (2009) and Kim et al. (2018) reported that high-grain diet led to a significant decrease in richness and diversity of ruminal bacterial microbiota under the pH value of 5.6–6.0. Though the pH value of the present study was well above 6.0, the HCD still reduced the richness and diversity of ruminal bacterial microbiota compared with the LCD. Studies also revealed that compared with low-concentrate diet, high-concentrate diet decreased the relative abundance of *Bacteroidetes* and increased that of *Firmicutes* at the phylum level and that of *Prevotella* at the genus level (Khafipour et al., 2016; Hua et al., 2017; Liu et al., 2014). In accordance with these studies, *Firmicutes* had replaced *Bacteroidetes* to become the most abundant phylum in the rumen fluid of the HCD group in this present study. Our results showed that the abundance of the genus *Prevotella* decreased when beef steers were fed the HCD compared with the LCD group, which concords with the results of Mao et al. (2013) and Kim et al. (2018) Our results showed that although the *Prevotella* abundance was reduced in the HCD group, but still remained one of the predominant genera.

In the present study, inulin supplementation into the HCD improved the ruminal bacterial diversity, richness and composition, which might contribute to the improved performance such as ADG and feed conversion ratio when beef steers were fed the HCIN rather than the HCD. Based on 16S rRNA gene sequencing, we found the OTU numbers and the indices of Chao1, ACE and Simpson were significantly increased in the HCIN group compared with the HCD group. Furthermore, inulin supplementation significantly increased the relative abundance of *Bacteroidetes*, *Proteobacteria* and *Spirochaetes*, but decrease that of *Firmicutes* and *Actinobacteria*. Inulin is a dietary fiber and prebiotic, which may selectively favor some bacteria and meanwhile inhibit others. Inulin has been extensively reported to benefit health by regulating gut microbiota in mono-gastric animals like humans, pigs and mice (Chambers et al., 2019; Passlack et al., 2015; Li et al., 2018; Bjoern et al., 2018). Pervious study reported dietary inulin improved the relative abundance of *Bacteroidetes* and reduced that of *Firmicutes* in the mice gut microbiota to alleviate microbiota dysbiosis caused by type 2 diabetes (Li et al., 2019). Khafipour et al. (2016) concluded that the increase in the *Firmicutes* to *Bacteroidetes* ratio as a result of high concentrate diet was an undesirable result, which would interfere with the degradation and digestion of dietary fiber. Our study showed that compared with the HCD, the HCIN led to an increase in *Bacteroidetes* and decrease in *Firmicutes*, indicating that inulin has the ability to optimize rumen bacterial microbiota. At the genus level, inulin supplementation into the HCD increased the relative abundance of Unclassified_RF16, Unclassified_BS11, Unclassified_Succinivibrionaceae and *Sharpea*, but decreased that of *Succiniclasticum* and *Ruminococcus*.

It was observed in the present study that compared with LCD, inulin supplementation into the LCD improved growth performance, especially the final BW and feed conversion, significantly increased the digestibility of EE and tented to increase the digestibility of NDF in the beef steers. Our results also showed that when inulin was supplemented into the LCD, it increased the propionate to acetate ratio. Moreover, compared with the LCD, LCIN resulted in a modified bacterial composition although the bacterial diversity and richness were not improved. Inulin supplementation into LCD significantly decreased the relative abundance of *Firmicutes*, increased that of *Bacteroidetes* and *Spirochaetes*. At the genus level, *Prevotella* was the most predominant bacteria,

followed by Unclassified_Bacteroidales in the LCD group. Adding inulin into the LCD significantly decreased the relative abundance of *Prevotella* and increased that of Unclassified_Bacteroidales. This result is in agreement with that of Kleessen et al. (2007) who reported that inulin significantly decreased the relative abundance of *Prevotella* in human feces.

Dietary Inulin supplementation was reported to be able to decrease plasma LPS concentration (Li et al., 2019) and to reduce *E. coli* in calve feces (Samanta et al., 2013). These results prompted us to hypothesize that inulin supplementation might decrease LPS produced in the rumen and in turn reduce plasma LPS. Unexpectedly, inulin supplementation into either the LCD or the HCD failed to minimize the LPS concentrations in rumen fluid and plasma. There exist arguments as to whether inulin could manipulate systemic inflammatory response. Several studies had reported that inulin could ease inflammatory conditions by reducing plasma LPS, IL-6 and TNF-α (Li et al., 2019), but Taranu et al. (2012) found inulin could increase IL-8 and TNF-α release. In the present study, no significance was observed in both plasma pro-inflammatory cytokines and APPs after inulin supplementation, which indicates that the inflammatory response wasn't affected by inulin supplementation.

5. Conclusions

In conclusion, dietary supplementation with inulin, regardless of the dietary concentrate levels, improved final body weight and feed conversion ratio in beef steers. Inulin supplementation into the LCD increased the propionate to acetate ratio and modified the ruminal bacterial composition, whereas inulin supplementation into the HCD increased ruminal bacterial diversity and richness as well as improved the ruminal bacterial microbiota. Nonetheless, inulin supplementation did not suppress inflammatory response resulting from feeding high-concentrate diet in beef steers.

Declaration of Competing Interest

The authors declare no competing financial interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anifeedsci.2019.114299.

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