Research Article

Influences of Rumen Fermentation and Bacterial Community Structure of Holstein and Jersey Steers by Dietary Changes

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Abstract | This study investigated the influence of diet types and breed of steers on rumen fermentation and bacterial community composition in Korea during the summer seasons. Three Holstein and 3 Jersey steers were fed a high forage (HF) and a high concentrate (HC) diet for two subsequent periods of 21 days under 2 × 2 factorial arrangements. The steers received HF diet had higher pH ($P < 0.05$). Higher acetate concentration was recorded in the Jersey steers and those received HF diet; however, the Holstein steers and those received HC diet had higher propionate concentration ($P < 0.05$). The lowest A:P ratio was observed in the Holstein steers and those received HC diet ($P < 0.05$). From Metataxonomic analysis, Bacteroidetes and Firmicutes were the most abundant bacterial phyla in both breeds and diets. Bacteroidetes was more abundant in the steers received HF diet while Firmicutes was increased with the HC diet. All four groups of steers had a distinct bacterial community with higher relative abundances. The principal component analysis (PCA) represented that the overall rumen bacterial community along with fermentation parameters varied among four groups. Overall results suggest that the rumen fermentation characteristics differ according to both diets and breeds which was influenced by a distinct bacterial community.

Keywords | Diet types, Holstein steer, Jersey steer, Volatile fatty acids, Rumen bacterial community

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INTRODUCTION

According to the third assessment report of the International Panel on Climate Change predicted that environmental temperature would rise by 1.4–5.8 °C anywhere on the earth from 1990 to 2100 (Masson-Delmotte et al., 2018). Indeed, the Korean peninsula experienced an increase by 2°C from 1992 to 2004. In addition, the number of summer days in Korea increased between 1908 and 2009 (Kim,2010). Climate changes affects animal normal metabolism and physiological activities. Furthermore, ruminant diet, a combination of forage and concentrate, remarkably regulates the rumen fermentation and the microbial population within the rumen ecosystem. Rumen harbors different microorganisms viz. bacteria, protozoa, fungi and archaea that fermented a wide variety of ingested feedstuffs and produce volatile fatty acids (VFA) viz. acetate, propionate and butyrate which subsequently absorbed by the cattle as their energy metabolism and protein synthesis (Hook et al., 2010). Several factors such as heredity, host, age, physiological state, and sampling method affect rumen microbial diversity. Also, diet (Hua et al., 2017), feed additives (Uyeno et al., 2015), forage to concentrate ratio (Olijhoek et al., 2018), and feeding cycles (Wang et al., 2020) influence VFAs production and rumen microbial diversity. Among them forage content is

of high importance to formulate an optimum ration for the improvement of animal diet. It was reported earlier that Bacteroidetes and Firmicutes were the top two abundant bacterial phyla in ruminants (Islam et al., 2021a, b; Kim et al., 2021; Ramos et al., 2021a, b). High concentrate (HC) diets decreases the relative abundance of rumen cellulotytic bacteria (Zhang et al., 2017). Furthermore, decrease in the dietary forage concentration increases the relative abundance of Firmicutes, while the opposite is true for the Bacteroidetes in ruminants (Ramos et al., 2021b). However, most previous research focused on the forage to concentrate ratio in the dairy cows and little is known about the steers. Also, the above-mentioned studies lack the information about temperature and humidity. It was reported earlier that, rumen bacterial diversity was affected by high temperature and humidity that leads to heat stress in ruminants (Zhao et al., 2019; Kim et al., 2020). Moreover, association of rumen fermentation and microbial community with dietary changes between steers especially during summer season was less documented. Therefore, the present study was designed to evaluate the influence of HF and HC diets on the rumen fermentation and bacterial community composition of Holstein and Jersey steers during summer season.

MATERIALS AND METHODS

Animals, experimental design, and diet

The animal experiment was conducted at the animal farm of Sunchon National University (SCNU), Jeonnam, South Korea. All animals used in this feeding trial were reviewed and approved by the SCNU Institutional Animal Care and Use Committee (IACUC approval number: SCNU-IACUC-2020-06). Three (3) non-cannulated Holstein (690 ± 10.50 kg) and 3 Jersey (550 ± 15.75 kg) steers were fed a HF and a HC diet for two subsequent periods of 21 days under a 2×2 factorial arrangements during the summer with recorded temperature humidity index (THI) about 85. The temperature (°C) and relative humidity (%) of the experimental period were recorded by using a Testo 174H Mini data logger (West Chester, PA, USA). The THI was calculated as THI = (0.8 × maximum ambient temperature) + [% relative humidity/100 × (mean ambient temperature-14.4)] + 46.4 (Davis et al., 2003). The particular diets (Table 1) was offered once a day at 08:00 a.m. at a rate of 5%–10% of left-over diet. Seven days washing was performed between the two feeding trials.

Sample collection, processing and analysis of rumen fermentation characteristics

On the last day of each period, rumen fluids were collected from each of the steers 2 h before feeding by stomach tubing. The first 300 mL of rumen fluids were discarded in order to minimize contamination with saliva. Immediate after collection, the pH was measured using a pH meter (Seven Compact TM pH/Ion meter S220, Mettler Toledo, Switzerland immediately after collection). Rumen fluids were then transported to the laboratory and three separate aliquots for the analysis of ammonia nitrogen (NH_3-N), volatile fatty acid (VFA), and rumen metagenomes were stored at -80 °C until analysis. The NH_3-N concentration was measured by using a Libra S22 spectrophotometer (CB40FJ; Biochrom Ltd., Cambourne, UK) (Chaney and Marbach, 1962). The VFA concentration was measured by using high-performance liquid chromatography (HPLC; Agilent Technologies 1200 series, Waldbronn, Germany) (Tabaru et al., 1988; Han et al., 2005).

Table 1: Feed ingredients and chemical compositions of different diets used in this experiment.

Ingredients	Compositions (% of DM)	
	High forage diet	High concentrate diet
Corn grain	8.34	39.18
Corn gluten feed	4.17	16.64
Wheat bran	2.48	12.15
Lupin	13.86	11.06
Oat hay	4.97	19.58
Rice straw	19.96	-
Tall fescue	44.98	-
Limestone	0.79	1.00
Vitamin premix	0.09	0.09
Mineral premix	0.10	0.10
Salt	0.27	0.30
Total	100.00	100.00
Chemical composition (% of DM)		
DM (% as fed basis)	78.94	88.66
CP	16.66	22.35
CF	21.06	9.58
Crude fat	2.27	4.37
Ash	7.28	7.17
Calcium	0.73	0.60
Phosphorus	0.35	0.38
NDF	52.57	23.44
ADF	26.88	13.64
NFC	21.22	42.67

DM: dry matter; CP: crude protein; CF: crude fiber; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: non-fibrous carbohydrates.

Metataxonomic analysis

DNA from 12 rumen fluid samples were extracted by using a DNA Isolation Kit (PowerSoil®; Cat. No. 12888, MO BIO) following the manufacturer's protocol. After extraction, 4 pooled DNA samples were made from each group and sent

to Macrogen Inc. (Seoul, South Korea) for metataxonomic analysis of the rumen bacteria. The amplicon library of each pooled sample, using two-step PCR amplification of the V3-V4 region of the 16S rRNA genes with the primers Bakt_341F (5-AGATGTGTATAAGAGACAG-3) and Bakt_805R (5-GATGTGTATAAGAGACAGG-3) by Illumina 16S Metagenomic Sequencing Library protocols (Illumina, San Diego, CA, USA). Raw sequence data were trimmed using Trimmomatic (v0.38), and paired reads were merged using FLASH (1.2.11) software. Sequences shorter than 400 bp were discarded. In order to identify and remove chimeric sequences rDnaTools (https://github.com/PacificBiosciences/rDnaTools) was used. To avoid bias generated at different sequencing depths, samples were subsampled to an even depth of 10,000 sequences per sample. The filtered sequences were then clustered into operational taxonomic units (OTUs) at 97% sequence similarity using CD-HIT-OTU. For taxonomic assignment, the representative sequence of each OTU was compared against the 16S Microbial DB of NCBI (https://www.ncbi.nlm.nih.gov/r efseq/targetedloci/16S_process/) using BLASTN (v2.9.0+). The Shannon diversity index and Chao1 richness estimate were determined by using QIIME (v1.8).

Statistical analysis

All data of rumen fermentation characteristics were analyzed using the Mixed procedure in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) (SAS, 2013). Statistical significance is reported at ($P < 0.05$). The relative abundance of rumen bacteria at different taxa level was numerically compared among groups. Principal component analysis (PCA) score plot was made by using Minitab17 program (Minitab Ltd., UK) (Minitab Inc, 2010).

RESULTS

Rumen fermentation characteristics

The variation in the rumen fermentation parameters between Holstein and Jersey steers at different dietary conditions were analyzed and the results are presented in Table 2. The rumen pH was significantly higher in the HF diet compared to HC diet in both the Holstein (6.66 vs 6.25) and Jersey (6.64 vs 6.31) steers ($P < 0.05$). The highest NH_3-N (mg/dl) concentration was observed in the Jersey steers received HC diet (8.82); however, not significant ($P > 0.05$). The acetate and propionate concentration (mM), and A:P ratio were significantly influenced by the diet types, and breeds. Significantly higher acetate was recorded in the Jersey steers and those steers received HF diet ($P < 0.05$). However, the Holstein steers and those steers received HC diet had significantly higher propionate concentration ($P < 0.05$). The lowest A:P ratio was observed in the Holstein steers and those steers received HC diet ($P < 0.05$). The butyrate and total VFA concentration (mM) were significantly increased while steers received HC diet ($P < 0.05$).

Rumen bacterial community compositions

The richness and diversity of rumen microbes of Holstein and Jersey steers with HF and HC diets were analyzed and the results are presented in Table 3. The observed OTUs, Chao 1, and Shannon index were numerically higher in the Holstein steers regardless of diet while the lowest value was observed in the Jersey steers received HC diet. At phylum level, the Bacteroidetes and Firmicutes were the most abundant bacterial phyla in both breeds and diets (Figure 1); however, their relative abundance varies according to dietary groups and breeds. The relative abundance of Bacteroidetes was higher in those steers received HF diet (72.37 vs 38.38% in Holstein and 75.15 vs 54.43% in Jersey steers) compared to HC diet. In contrast, the relative abundance of Firmicutes was increased when the steers received HC diet (from 25.36 to 59.80% and 23.40 to 45.00% in Holstein and Jersey steers, respectively).

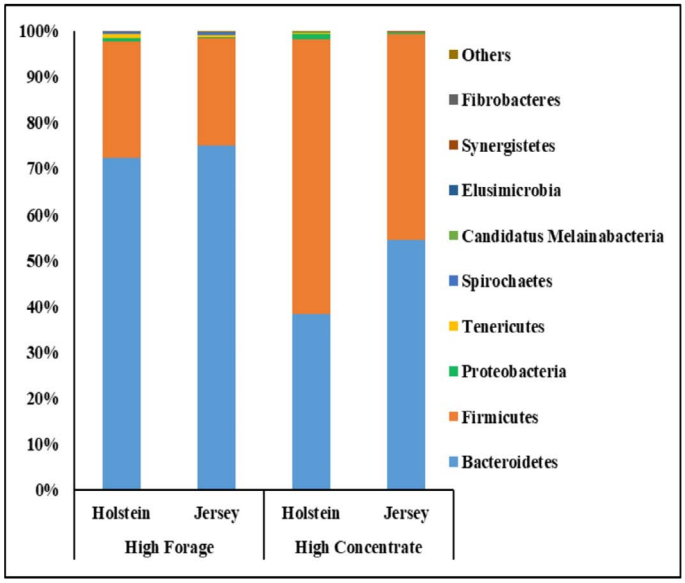

Figure 1: Phylum level relative abundance of rumen bacteria of Holstein and Jersey steers with high forage and concentrate diets.

At the species level, a total of 15 bacterial species, out of 248 species, were identified having relative abundance >2% at least in one group (Figure 2). The *Prevotella ruminicola* was the most abundant bacterial species in both breeds and diets; however, the highest relative abundance of it was recorded in the Jersey steer when received HC diet (49.59%). In contrast, the highest relative abundance of *Ruminococcus bromii, Holdemania massiliensis, Barnesiella intestinihominis,* and *Vallitalea pronyensis* were found in Holstein steer received HC diet. The highest relative

abundance of *Paludibacter propionicigenes* and *Prevotella brevis* were observed in the Holstein steers received HF diet, while the highest relative abundance of *Olivibacter sitiensis* (23.56%) was recorded in the Jersey steer received HF diet. The relative abundance of *Flintibacter butyricus*, *Anaerobacterium chartisolvens*, *Intestinimonas butyriciproducens*, and *Eubacterium ventriosum* were increased, while the relative abundance of *Succiniclasticum ruminis* was decreased with the HC diet regardless of breed. Also, the lowest relative abundance of *Capnocytophaga cynodegmi* was observed in the Jersey steers received HC diet. The PCA score plot represented that the overall rumen bacterial community along with the rumen fermentation parameters were different among the four groups (in both breeds and diets) (Figure 3).

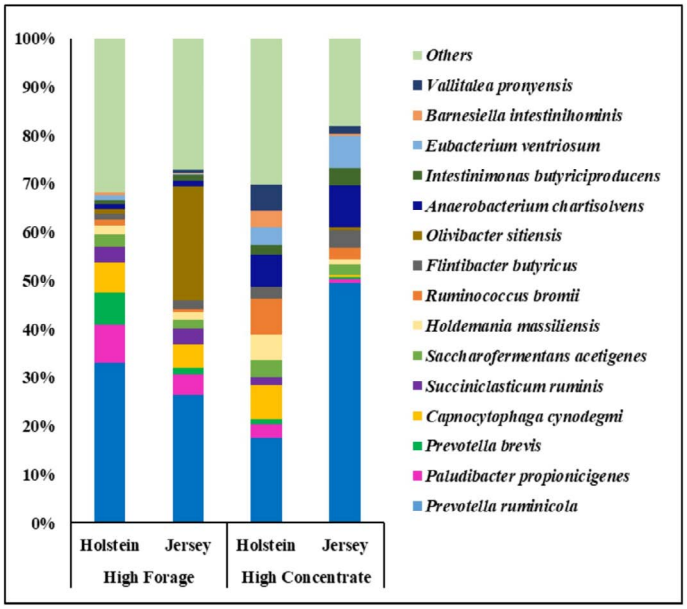

Figure 2: Relative abundance of top 15 rumen bacterial species of Holstein and Jersey steers with high forage and concentrate diets.

DISCUSSION

This study evaluated the influence of HF and HC diets on the rumen fermentation characteristic and rumen bacterial community composition of Holstein and Jersey steers during the summer season but not evaluated the effect of heat stress. The ruminal pH and the VFA correlated negatively, and feeding a HC diet decreased the pH, while increased the VFA concentration in the rumen fluid of ruminants (Sato, 2016). In this study, we observed similar findings of low ruminal pH and high total VFA concentration in the rumen of steers received HC diet. In our study, we observed significantly higher acetate concentration in the steers received HF diet, while significantly higher propionate and numerically higher butyrate concentration in the steers received HC diet. These findings were strongly supported by Grubb and Dehority (1975) and Zhang et al. (2017) who reported that dietary changes from HF to HC reduced molar proportion of acetate and A:P ratio, while increasing propionate and butyrate proportion. Our study also observed that acetate production was significantly higher in the Jersey steers, while propionate production in the Holstein steers. The reason might be due to the more efficient utilization of HF diet by the Jersey steer, while HC diet by the Holstein steers was consistent with the findings of Olijhoek et al. (2018).

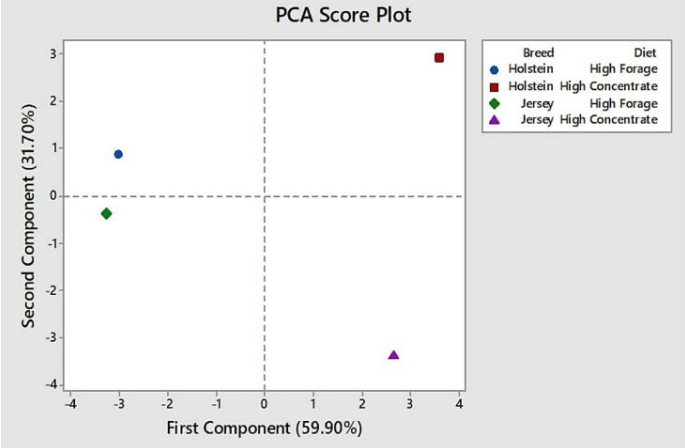

Figure 3: Principal component analysis (PCA) score plot represented the separation of both breeds and diets based on their rumen fermentation characteristics and species level relative abundance of rumen bacteria.

Table 2: Changes in the rumen fermentation characteristics of steers with different diets.

Parameters	High forage		High concentrate		SEM	P- value		
	Holstein	Jersey	Holstein	Jersey		Diet	Breed	Diet × Breed
pH	6.66	6.64	6.25	6.31	0.104	0.012	0.866	0.717
NH_3-N (mg/dl)	7.36	7.18	7.57	8.82	0.813	0.304	0.546	0.420
Acetate (mM)	58.62	60.62	54.84	56.99	0.425	<.001	0.002	0.865
Propionate (mM)	18.52	17.14	24.54	20.84	0.328	<.001	<.001	0.010
Butyrate (mM)	10.81	10.62	12.06	12.13	0.235	0.001	0.806	0.594
TVFA (mM)	87.95	88.37	91.44	89.97	0.555	0.004	0.426	0.170
A: P	3.16	3.54	2.24	2.74	0.042	<.001	<.001	0.273

SEM: standard error of the means; NH_3-N: ammonia nitrogen; TVFA: total volatile fatty acid; A:P: acetate: propionate ratio.

Table 3: Changes in the richness and diversity indices of the rumen bacterial community of steers with different diets.

Parameters	High forage		High concentrate	
	Holstein	Jersey	Holstein	Jersey
Observed OTUs	585	542	598	502
Chao1	628	579	645	526
Shannon	6.68	6.23	6.73	5.17

OUT: operational taxonomic unit.

The diversified rumen microbiome regulates the fermentation of feed particles with in the rumen. However, the low number of observed OTUs (ranges from 502 to 598), and Chao 1 (ranges from 526 to 645) might be due to the adverse effect of heat stress during the study period. Usually, the rumen microbial diversity is increasing with the increasing of forage portion in the diet (Wang et al., 2020). Likewise, the Jersey steers received HF diet had higher OTUs, Chao 1 and Shannon index in this study; however, Holstein steer had similar richness and diversity with both diets. In contrast, numerically higher OTUs, Chao 1 and Shannon index were observed in the Holstein steer compared to the Jersey steer. Similar findings also observed by Paz et al. (2016), who reported significantly higher Chao1, and observed OTU estimates in Holstein cows than that in Jersey lactating cows. In our study, we observed Bacteroidetes, and Firmicutes, together representing >97% of all bacteria, were the highest bacterial phyla in both Holstein and Jersey steers with HF and HC diets which was consistent with the findings of several other ruminant studies (Bharanidharan et al., 2018; Xue et al., 2018). However, Bacteroidetes was more abundant in the steers received HF diet, while the relative abundance of Firmicutes was increased with HC diet. This finding is in agreement with the findings of Wang et al. (2020). Though *Prevotella ruminicola* was recorded as the most abundant bacterial species in both breeds and diets; however, highest relative abundance in Jersey steer with HC diet might be associated with their highest NH_3-N concentration. This is because *Prevotella* spp. are the major species triggers proteolytic activity to breakdown of protein rich diets in the rumen (Bharanidharan et al., 2018; Xue et al., 2018). In this study, the highest relative abundance of *Ruminococcus bromii, Holdemania massiliensis, Barnesiella intestinihominis* and *Vallitalea pronyensis* in Holstein steer received HC diet and *Olivibacter sitiensis* in the Jersey steer received HF diet suggested the influence of both breeds and diets on rumen bacterial community. The *I. butyriciproducens and F. butyricus* are the two important butyrate producing bacteria in ruminants (Bui et al., 2016; Petri et al., 2018). The higher relative abundance of those bacteria in the steers received HC diet might be associated with their significantly higher butyrate production. In addition to this, both breeds with different diets had distinct group of bacterial species having higher relative abundances compared to other groups. The PCA score plot further supported that overall rumen bacterial community along with their fermentation products were distinct among the four different groups.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the rumen fermentation characteristics differs according to the variation in the diets and breeds which was regulated by a distinct bacterial community with higher relative abundances. These results strengthen our current understanding of rumen fermentation and bacterial community composition of ruminant steers at different dietary condition. Future study included detail metagenomics and metabolomics analysis with large number of animals at different environmental conditions to explore the complex metabolic pathways of ruminants.

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NOVELTY STATEMENT

This is the first study focused the effects of dietary changes on the rumen fermentation characteristics and bacterial community composition of steers of the dairy breed particularly in the summer.

AUTHOR'S CONTRIBUTION

S-HK, MI, and S-SL designed and conceptualized the experiments. S-HK, MI, and A-RS performed the animal experiment and laboratory tests. S-HK, MI, and S-SL performed data checking. S-HK, and MI performed statistical analysis. MI wrote the first draft of the manuscript which was revised by S-HK, MI, and S-SL. All authors contributed to the final manuscript revision and approval.

Conflict of interest

The authors have declared no conflict of interest.

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