Comparative study of rumen fermentation and microbial community differences between water buffalo and Jersey cows under similar feeding conditions

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ABSTRACT

Differences in rumen volatile fatty acids (VFAs), microbial population and community composition between water buffalo and Jersey cows were compared to explore the effects of animal species on ruminal microbiota. Ruminally fistulated buffalo (n = 4) and Jersey cows (n = 4) with similar body weights were selected and provided with similar diets. After feeding for 30 days, rumen fluid was collected to quantify the ruminal VFA, and determine microbial population and community composition. Results revealed that concentrations of total VFA, acetate and propionate in buffalo were higher (P < .05), while butyrate concentration and acetate/propionate ratio were lower than those in Jersey cows (P < .05). Populations of ruminal bacteria, protozoa, fungi and F. succinogenes were higher in buffalo (P < .05). The abundance of Firmicutes was higher (P < .05), while the abundance of Bacteroidetes was lower in buffalo (P < .05). The abundances of dominant bacterial genus Prevotella and dominant methanogen genus Methanobrevibacter were higher in Jersey cows (P < .05). Prozoal genus Metadinium was dominant in buffalo, while Entodinium was dominant in Jersey cows. Concluded that not only rumen microbial population, but also microbial community composition were different between two species. The higher bacterial, protozoal and fungal populations, and different microbial community can explain why buffalo are more efficient to digest fibre.

1. Introduction

The water buffalo and the Jersey cows are very important livestock species which are kept primarily for supplies of meat and milk in subtropical areas, such as southern China. China has the third largest population of buffalo in the world, and Jersey cows as the second most popular dairy breed, while their milk fat and protein contents are much higher than Holstein cows (Coffey et al. 2016). Both buffalo and Jersey cows are well acclimatized to hot and humid environments and roughage feeding (Smith et al. 2013). However, a key point of difference is that buffalo have a different digestion physiology from cattle.

It has been reported that, when cattle and buffalo are kept under similar conditions, buffalo utilize feed more efficiently with higher digestibility (Chanthakhoun et al. 2012). Another study revealed that buffalo ingest the same quantity of feed dry matter as cattle, but buffalo spend 50% more time on rumination; which results in a 30% lower residence time for particulate matter in the rumen (McSweeney et al. 1989). It has also been reported that the excretion of purine derivatives is lower in buffalo, because they have the ability to recycle nitrogen which makes the buffalo more effective to use feed that contains less nitrogen (Thanh 2012). These distinct characteristics are evidence about probable differences in rumen microbial community of both species, which affects the formation of fermentation products (Khejornsart et al. 2011). Wanapat et al. (2000) reported that buffalo have higher forage digestibility due to higher population of cellulolytic bacteria, fungal zoospores and lower protozoa in the buffalo rumen than those in the cattle rumen (Gurbuz 2009). Therefore, it is important to reveal ruminal microbiota differences existing between buffalo and Jersey cows, which is the basis to formulate proper feed for them.

Recent microbial molecular techniques, especially, real-time PCR (Wanapat and Cherdthong 2009), and high-throughput sequencing technology (Jiao et al. 2014; Henderson et al. 2015), are being successfully used to monitor the population and community composition of ruminal microbes. Jersey cows and water buffalo have similarity in heat tolerance and milk composition, whereas they have differences in physiology, genetics and behaviour. Thus, comparing their ruminal fermentation and microbial community under the same feeding condition is helpful to understand the differences and effects of animal species on gut microbial community (Gurbuz and Davies 2010). To the best of our knowledge, such type of study with latest techniques has not been reported. The purpose of this study was to explore the effects of animal species on the rumen microbial population and community composition by comparing rumen fermentation, microbial population and community composition differences between water buffalo and Jersey cows, with modern microbial molecular techniques.
2. Materials and methods

**Ethical statement**

The animals included in this study were housed at Buffalo Research Institute, Chinese Academy of Agricultural Sciences, Nanning, Guangxi province, China. All experimental protocols regarding animal handling and treatment were approved by the Animal Care Committee, Guangxi University, under the guidance of the International Cooperation Committee of Animal Welfare, China.

2.1. Experimental animals and their feeding

Four Water buffalo (*Bubalus bubalis*), a hybrid of the Murrah and the local Chinese buffalo, and four Jersey cows (*Bos taurus*) with fistulated rumen were selected to be part of this study. All animals were dry pregnant females with the same body weights (~500 kg). Both animal groups were fed with three kilograms (kg) of concentrate per head per day, and corn silage was offered *ad libitum*. Water supply was freely accessed by the animals. All animals were housed at the farm of buffalo research institute, CAAS, Nanning, Guangxi, China. The measured feed intake and its nutritional composition are shown in Table 1. The composition of the feed concentrate was as follows (based on dry matter): maize 52%, wheat bran 18.5%, soybean meal 8%, cotton seed meal 15%, stone dust 2%, maize 52%, wheat bran 18.5%, soybean meal 8%, cotton seed meal 15%, stone dust 2%, and premix 1%. Per kg of the premix contained 19 g MgSO₄·H₂O; calcium hydrogen phosphate 1.5%, sodium chloride 2% and soybean meal 8%, cotton seed meal 15%, stone dust 2%, (based on dry matter): maize 52%, wheat bran 18.5%, soybean meal 8%, cotton seed meal 15%, stone dust 2%.

The feed consumption by experimental animals/day and its nutritional intake and its nutritional composition are shown in Table 1.

2.2. Rumen fluid collection, analysis and DNA extraction

On the last day of the experiment, rumen fluid samples were collected from all of the animals through their fistulas before the morning feeding, filtered with four layered cheese-cloth and separated into two aliquots. One aliquot was shifted for ruminal volatile fatty acids (VFAs) analysis using a gas chromatograph (GC-2010, Shimadzu, Tokyo, Japan), equipped with a flame ionization detector and a capillary column (HP-INNOWAX, 1909N-133, Agilent Technologies, Santa Clara, CA, USA) as described by Zhang et al. (2008), and the other aliquot was preserved at −20°C, for metagenomic DNA extraction. DNA was extracted from 1.5 millitres (mL) of the preserved rumen fluid sample following the procedure as reported by Rius et al. (2012), and was further used to perform real-time PCR and high-throughput sequencing.

2.3. Quantitative real-time PCR analysis

Quantitative real-time PCR (qRT-PCR) was performed to quantify the populations of bacteria, methanogen, fungi, protozoa, *Ruminococcus albus*, *Fibrobacter succinogenes* and *Prevotella ruminicola* using the method as described by Jiao et al. (2014). Primers used were the same as described by Jiao et al. (Table 2). Briefly, standard curves were generated by tenfold serial dilutions of plasmid DNAs containing the extracts of 16S and 18S rRNA gene inserts from each of the microbial groups and bacterial species. The quantitative PCR assay was performed using SYBR Green Master Mix (Takara, Japan) on a Roche light cycle 480 real-time PCR system (Riche, Basel, Switzerland) with a 10-μL reaction mixture volume. Each reaction mixture contained 5 μL of Fast SYBR Green Master Mix, 0.5 μL of each primer (20 pmol μL⁻¹), 3.5 μL of nuclease-free water and 0.5 μL of DNA template (10 ng μL⁻¹). Every standard dilution and sample were assayed in triplicate, with the amplification carried out according to the following program: 95°C for 10 min for initial denaturation, 30 cycles at 95°C for 20 s, annealing for 1 min. at 62°C, followed by terminal elongation at 72°C for 5 min. The corresponding qRT-PCR efficiency for every microbial group and bacterial species ranged from 90% to 100%. Total 16S rRNA or 18S rRNA gene copy numbers in the samples were determined by relating the threshold cycle (Ct) values to standard curves. Copy numbers for the 16S rRNA gene in mL of rumen fluid were calculated as proposed by Li et al. (2009), and values were converted to log₁₀ for further statistical analysis.

2.4. High-throughput sequencing for microbial community analysis

Metagenomic DNA samples were sent to the BGI genomic research centre in Wuhan, China, for the rumen microbial community composition analysis. High-throughput sequencing technique was conducted using Illumina Miseq PE 250 platform (Illumina, Santiago, CA, USA). Bacterial and methanogen communities were analysed by using 16S rRNA gene sequencing.

### Table 1. The feed consumption by experimental animals/day and its nutritional profile (dry matter basis).

| Index                  | Buffalo | Jersey cow |
|------------------------|---------|------------|
| Concentrate intake     | 3.00    | 3.00       |
| Forage intake          | 4.19    | 3.32       |
| Total feed intake      | 7.19    | 6.32       |
| Crude protein          | 13.79   | 14.21      |
| Acid detergent fibre   | 39.12   | 37.29      |
| Neutral detergent fibre| 39.12   | 37.29      |

### Table 2. Primers used for real-time PCR.

| Primers name                | Sequence (5’–3’) | Size (bp) |
|-----------------------------|------------------|-----------|
| Bacteria-F                  | CGGCAACGAGCCCAACC | 146       |
| Bacteria-R                  | CCATTGTACAGCTGTAAGC | 223       |
| Fungi-R                     | GAGGAATCGAAAAAAGTCGAAACAGGTTC | 120       |
| Fungi-R                     | CAAGTTACAAAGGTTAGGATT | 120       |
| Protozoa-F                  | GCCTCCGATGATGATGATT | 120       |
| Protozoa-R                  | CTGCCCTCYATTAGT | 140       |
| Methanogen-F                | TTCGGTGGATATCRRGC | 140       |
| Methanogen-R                | GBARGTCGAWCGTAGAATCC | 140       |
| Fibrobacter succinogenes-F  | GTTCGGAATTACCCGCGCAATTAA | 121       |
| Fibrobacter succinogenes-R  | GCCGGCCGCTGCACTATC | 121       |
| Ruminococcus albus-F        | CCTCATAAGACTTTAGTTCG | 176       |
| Ruminococcus albus-R        | CCCTCCTGGCGATAGAACA | 176       |
| Prevotella ruminicola-F     | GAAGCTCGGATATGCTTGTTG | 74        |
| Prevotella ruminicola-R     | CATCCCTTACCGGGAACCTTGG | 74        |
while protozoal community was analysed by using 18S rRNA gene sequencing (Kittelmann and Janssen 2011). The primers used for PCR amplification are shown in Table 3. The sequence data reported in this study have been deposited in the NCBI database (accession no. 5306539–5306562). All high-throughput sequence data processing including sequence quality control, operational taxonomic unit (OTU)-based analysis, taxonomy analysis and diversity indices calculation were performed using the MOTHUR V 1.31.2. Sequences were grouped into OTUs sharing 97% similarity of bacterial, methanogen and protozoal sequences, and were put into phylogenetic groups by using the Basic Local Alignment Search Tool (BLAST). Bacterial 16S rRNA genes were blasted against the Green genes database (V201305), methanogen 16S rRNA genes were blasted against databases provided by Seedorf et al. (2014); and protozoal 18S rRNA genes were blasted against databases provided by Kittelmann and Janssen (2011). Bacterial data were summarized at phylum and genus levels, protozoal data were summarized at the genus level, while methanogen data were summarized using a mixed taxonomic rank scheme. Pie charts for bacteria, methanogen and protozoa were drawn using the Microsoft Excel program 2013. Venn diagrams were also drawn using the software R (v3.2.3) to display the number of individual and common OTUs. Bray–Curtis distance heat maps were drawn using ‘ahetmap’ in the package ‘NMF’ of the software R (v3.2.3), to reflect the differences between microbial communities of both animal groups. Rarefaction curves were drawn using software R (v3.2.3) to reflect species richness. Principal component analysis (PCA) diagrams were drawn using package ‘ade4’ of software R (v3.2.3) to display the differences in OTU compositions in different samples. Spearman’s rank correlations were calculated and plotted using the ‘corrplot’ packages within the R software (v3.2.3). Microbial groups those represented >1% of the total community within each of the three microbial groups (bacteria, methanogen and protozoa) were included in the analysis.

2.5. Statistical analysis of data

All of the preliminary data including rumen fermentation parameters, microbial population and microbial relative abundances were sorted by Microsoft Excel 2013, and were statistically analysed with SAS 8.02 software using ANOVA (Duncan’s multiple comparison method). Significant differences were achieved when $P < 0.05$.

### Table 3. Primers used for microbial community composition analysis.

| Microbes     | Primer sequence (5′–3′)                  | Size (bp) | Literature cited |
|--------------|-----------------------------------------|-----------|------------------|
| Bacteria-F   | GGGCGVAGCGTGGTAGATAA                    | 427       | Hristov et al. (2012) |
| Bacteria-R   | CCGCNCGGCTGGCAC                        | 427       | Jeyananthan et al. (2011) |
| Methanogen-F | AGGAATTGCGGGAGGAC                      | 427       |                  |
| Methanogen-R | GCGGTGTGCGAAGAGGAC                     | 511       | Kittelmann et al. (2013) |
| Protozoa-F   | AATTGCAAGATCTATCCC                     |           |                  |
| Protozoa-R   | GACTAGGGATGGATGGG                      |           |                  |

### 3. Results

#### 3.1. Rumen fermentation parameters and microbial populations

The gas chromatography analysis of rumen fluid samples revealed that concentrations of acetate, propionate and total VFA in buffalo were higher, although not significant ($P > 0.05$); while butyrate concentration and acetate to propionate (A/P) ratio were significantly lower than those in Jersey cows ($P < 0.05$) (Table 4). After qRT-PCR analysis, overall populations of bacteria, fungi, protozoa and *F. succinogenes* in buffalo were found significantly higher than those in Jersey cows ($P < 0.05$) (Table 4). No significant differences were observed in the population of methanogens, *R. albus*, and *P. ruminicola* between the two animal groups ($P > 0.05$).

#### 3.2. Rumen microbial community composition

##### 3.2.1. Bacteria

High-throughput sequencing analysis revealed that Bacteroidetes and Firmicutes were the dominant bacterial phyla in both buffalo and Jersey cows (Figure 1) (Table s1). The abundance of Firmicutes in buffalo was significantly higher ($P < 0.05$), while the abundance of Bacteroidetes was significantly lower than those in the Jersey cows ($P < 0.05$). Bacterial genus *Prevotella* was the dominant genus in both animal groups, while its abundance in Jersey cows was significantly higher ($P < 0.05$) (Figure 2) (Table s1). The abundance of genus *Ruminococcus* in buffalo was higher than in the Jersey cows ($P < 0.05$). The rarefaction analysis showed that sequencing results had reasonable depth (Figure s1). No differences were observed for bacterial OTUs, Chao1 and Simpson diversity indices, while Shannon index of buffalo was significantly higher than that of Jersey cows ($P < 0.05$) (Table 5). The PCA and Bray–Curtis distance analysis showed that there were differences in bacterial OTUs, abundance and community compositions between buffalo and Jersey cows (Figure 4) (Figure s7). The Venn diagram expressed that buffalo and Jersey cows shared 62.9% of bacterial OTUs, operational taxonomic unit (OTU)-based analysis, taxonomic analysis and diversity indices calculation were performed using the MOTHUR V 1.31.2. Sequences were grouped into OTUs sharing 97% similarity of bacterial, methanogen and protozoal sequences, and were put into phylogenetic groups by using the Basic Local Alignment Search Tool (BLAST). Bacterial 16S rRNA genes were blasted against the Green genes database (V201305), methanogen 16S rRNA genes were blasted against databases provided by Seedorf et al. (2014); and protozoal 18S rRNA genes were blasted against databases provided by Kittelmann and Janssen (2011). Bacterial data were summarized at phylum and genus levels, protozoal data were summarized at the genus level, while methanogen data were summarized using a mixed taxonomic rank scheme. Pie charts for bacteria, methanogen and protozoa were drawn using the Microsoft Excel program 2013. Venn diagrams were also drawn using the software R (v3.2.3) to display the number of individual and common OTUs. Bray–Curtis distance heat maps were drawn using ‘ahetmap’ in the package ‘NMF’ of the software R (v3.2.3), to reflect the differences between microbial communities of both animal groups. Rarefaction curves were drawn using software R (v3.2.3) to reflect species richness. Principal component analysis (PCA) diagrams were drawn using package ‘ade4’ of software R (v3.2.3) to display the differences in OTU compositions in different samples. Spearman’s rank correlations were calculated and plotted using the ‘corrplot’ packages within the R software (v3.2.3). Microbial groups those represented >1% of the total community within each of the three microbial groups (bacteria, methanogen and protozoa) were included in the analysis.

#### Table 4. RT-PCR analysis showing the concentrations of ruminal microbes and VFAs of buffalo and Jersey cow.

| Item          | Buffalo     | Jersey cow | $P$  |
|---------------|-------------|------------|------|
| Total VFAs (mmol/L) | 78.92 ± 7.12 | 74.65 ± 4.35 | .4262 |
| Acetate (mmol/L)      | 59.14 ± 5.13 | 54.74 ± 2.61 | .2567 |
| Propionate (mmol/L)    | 12.37 ± 2.82 | 10.09 ± 1.36 | .0693 |
| Butyrate (mmol/L)      | 7.41 ± 1.28  | 9.8 ± 0.50   | .0395 |
| Acetate/propionate (A/P) | 4.77 ± 0.11b | 5.60 ± 0.48a | .0151 |
| Bacteria log$_{10}$ (copy/mL) | 10.22 ± 1.070 | 9.872 ± 0.17P | .0181 |
| Fungi log$_{10}$ (copy/mL) | 8.237 ± 0.0550 | 7.349 ± 0.10P | .0001 |
| Protozoa log$_{10}$ (copy/mL) | 8.237 ± 0.0550 | 7.350 ± 0.10P | .0001 |
| Methanogen log$_{10}$ (copy/mL) | 8.332 ± 0.28 | 8.122 ± 0.16 | .3197 |
| Ruminococcus albus log$_{10}$ (copy/mL) | 6.789 ± 0.19 | 6.684 ± 0.23 | .3159 |
| Fibrobacter succinogenes log$_{10}$ (copy/mL) | 7.643 ± 0.27a | 6.970 ± 0.06b | .0133 |
| Prevotella ruminicola log$_{10}$ (copy/mL) | 8.993 ± 0.23 | 8.941 ± 0.27 | .8022 |

Note: Means within a row with different superscripts differ ($P < 0.05$).
3.2.2. Methanogens
High-throughput sequencing analysis revealed that Methanobrevibacter was the most dominant genus in both buffalo and Jersey cows (Figure 3) (Table s2). The most abundant species was M. gottschalkii. However, its abundance was comparatively higher in Jersey cows ($P > 0.05$) (Figure 3). The abundances of M. boviskoreani and M. ruminantium were comparatively higher in buffalo but only M. boviskoreani achieved significance ($P < 0.05$) (Table s2). The overall abundance of Methanomassiliicoccales was higher in buffalo ($P > 0.05$), but the abundance of Methanomassiliicoccales Group 12 was significantly higher ($P < 0.05$) (Table 2). The abundance of Methanobacterium alkaliphilum in buffalo was significantly higher than that of Jersey cows ($P < 0.05$) (Table s2). The rarefaction analysis showed that sequencing results had reasonable depth (Figure s3). No differences were observed in the OTUs, Chao1, Shannon and Simpson diversity indices of methanogens between both animal groups ($P > 0.05$) (Table 5). The PCA and Bray–Curtis distance analysis showed that there were differences in methanogen OTUs, abundance and community compositions between buffalo and Jersey cows (Figure 4) (Figure s8). The Venn diagram expressed that buffalo and Jersey cows shared 92% of methanogen OTUs (Figure s4). The Spearman rank correlations analysis showed that M. gottschalkii had strong positive correlations with Prevotella, M. boviskoreani, Entodinium and A/P ratio, and strong negative correlations with Oscillospira and M. ruminantium (Figure 6).

3.2.3. Protozoa
High-throughput sequencing analysis revealed that Metadinium, which was the most dominant protozoal genus, accounted for 83.16% of the abundance among all protozoal genera in buffalo ($P < 0.05$) (Figure 5) (Table s3). Entodinium was the most abundant genus in Jersey cows (46.70%) (Figure 5) (Table s3). The abundance of genus Entodinium (46.70%) (Figure 5) (Table s3). The abundance of genus Entodinium was comparatively higher in Jersey cows ($P > 0.05$), and the abundances of

![Figure 1](image1.png)

**Figure 1.** The taxonomic composition of ruminal bacteria and their average relative abundance at the phylum level in buffalo ($n = 4$) and Jersey cow ($n = 4$).

![Figure 2](image2.png)

**Figure 2.** The taxonomic composition distribution of ruminal bacteria and their average relative abundance at the genus level in buffalo ($n = 4$) and Jersey cow ($n = 4$). The genera of which abundance was less than 0.5% in all samples were classified into ‘others’.
genera *Anoplod-Diplodinium* and *Ophryoscolex* were also higher in Jersey cows ($P < .05$). The rarefaction analysis showed that sequencing results had reasonable depth (Figure s5). No differences were observed for protozoal OTUs and Chao1 diversity index between buffalo and Jersey cows ($P > .05$), while Shannon index of buffalo was significantly lower and the Simpson index was significantly higher than that of Jersey cows ($P < .05$) (Table 5). The PCA and Bray–Curtis distance analysis showed that there were differences in protozoal OTUs, abundance and community compositions between buffalo and Jersey cows (Figure s5). The Venn diagram expressed that both buffalo and Jersey cows shared 45% of protozoal OTUs (Figure s6). The Spearman rank correlations analysis showed that *Entodinium* had a strong positive correlation with *M. gottschalkii*, and a strong negative correlation with *M. ruminantium*.

**Table 5.** The OTU numbers and alpha diversity statistics of buffalo and Jersey cow rumen fluid.

| Microbes | Index | Buffalo | Jersey cow | $P$  |
|----------|-------|---------|------------|------|
| **Bacteria** | OTU | 675 ± 29.9 | 610 ± 76.3 | .1641 |
| | Chao | 764 ± 28.1 | 706 ± 59.2 | .1684 |
| | Shannon | 5.44 ± 0.29a | 4.94 ± 0.15b | .0215 |
| | Simpson | 0.01 ± 0.01 | 0.02 ± 0.01 | .2954 |
| **Methanogen** | OTU | 12.5 ± 0.57 | 11.5 ± 1.00 | .1340 |
| | Chao | 12.5 ± 0.57 | 11.5 ± 1.00 | .1340 |
| | Shannon | 0.79 ± 0.21 | 0.53 ± 0.09 | .0740 |
| | Simpson | 0.66 ± 0.11 | 0.78 ± 0.03 | .0966 |
| **Fungi** | OTU | 57.2 ± 37.9 | 61.6 ± 19.8 | .8634 |
| | Chao | 70.1 ± 48.0 | 80.1 ± 21.8 | .7545 |
| | Shannon | 1.84 ± 1.14 | 1.81 ± 0.05 | .9662 |
| | Simpson | 0.35 ± 0.39 | 0.31 ± 0.04 | .8796 |
| **Protozoa** | OTU | 116 ± 37.4 | 124 ± 65.5 | .6614 |
| | Chao | 145 ± 47.0 | 161 ± 18.6 | .5318 |
| | Shannon | 2.15 ± 0.55b | 3.28 ± 0.28a | .0111 |
| | Simpson | 0.30 ± 0.15a | 0.07 ± 0.04b | .0273 |

Note: Means within a row with different superscripts differ ($P < .05$).
Additionally, *Metadinium* had strong positive correlations with *Oscillospira* and Methanomassiliicoccales Group 12, and strong negative correlations with *Blautia* and *Anoplod-Diplodinium* (Figure 6).

### 4. Discussion

Ruminants primarily depend on microbes for effective feed digestion; therefore, understanding the role of the rumen microbiome has comprehensive implications. The present study showed that concentrations of total VFA, acetate and propionate in buffalo were higher than those in Jersey cows; indicating that the ruminal fermentation pattern was different between both animal species. In addition, bacterial population and Shannon diversity index in buffalo were higher than those in Jersey cows. These findings are consistent with a study conducted by Chanthakhoun et al. (2012), who also concluded that buffalo rumen had higher population of the ruminal cellulytic bacteria and fungi than cattle rumen. Firmicutes and Bacteroidetes were the dominant bacterial phyla in rumen fluid of both buffalo and Jersey cows; further affirmed that a core microbiome exists in the ruminant’s rumen (Henderson et al. 2015). The abundance of Firmicutes in buffalo was significantly higher, while the abundance of Bacteroidetes was significantly lower than that in Jersey cows; indicating that bacterial community was different between the two animal species. It has been reported that the abundance of Firmicutes is always higher in gut of efficient feed utilizing animals (Myer et al. 2015). Fernando et al. (2010) also reported that Firmicutes is the largest bacterial phylum and an increasing population of Firmicutes may increase carbohydrates fermentation in the rumen. Thus, higher abundance of Firmicutes in buffalo rumen fluid in the present study indicates that buffalo are more efficient in the breakdown of feed nutrients than Jersey cows. This predication is further strengthened with the higher concentrations of ruminal VFAs in buffalo. Our results were similar to findings of An et al. (2005), who reported that the abundance of Bacteroidetes in cattle rumen was higher than that of yaks. Yaks are typically considered to be a high forage consuming animal; thus, higher abundance of Bacteroidetes is a possible explanation for cattle having lower tolerance for high roughage feeding. The abundance of bacterial genus *Prevotella* was significantly lower in buffalo than in the Jersey cows, while the abundance of *Ruminococcus* was comparatively higher in buffalo. *Prevotella* is involved in protein degradation and polysaccharide digestion (Flint et al. 2012), while *Ruminococcus* is efficient in the breakdown of cellulose and hemicellulose (Biddle et al. 2013). Feed offered to the animals in this study had higher contents of cellulose and hemicellulose, and the forage intake was higher among the buffalo. This resulted in higher abundance of *Ruminococcus* and ultimately led to a higher production of VFAs within the buffalo group.

Methanogen population is approximately $10^7$–$10^8$ cells/g of the rumen contents and the only source of methane production in the rumen. The major phylogenetic methanogen groups in the rumen are: *Methanobrevibacter* ($>62\%$ of methanogens); *Methanosphaera* ($>15\%$ of methanogens); and the rumen cluster C’ recently named as *Methanoplasmatales* ($>16\%$ of methanogens). The rest belong to minority genera such as *Methanimicrococcus*, *Methanosarcina* and *Methanobacterium* (St-Pierre and Wright 2013). In the present study, a resembling methanogen community between buffalo and Jersey cows was found, which was dominated by the genus *Methanobrevibacter* with more than 90% abundance in both animal groups. This is consistent with the findings of Franzolin et al. (2012) and Xue et al. (2016) which have also verified that *Methanobrevibacter* is the most dominant genus in Holstein cattle, water
buffalo and other ruminants. However, some studies conducted in India reported that methanogen community in buffalo was dominated by *Methanomicrobium* (Chaudhary and Sirohi 2009; Singh et al. 2011). The present study further verified that ruminal methanogen community of buffalo in China is quite similar to other ruminants. The abundances of Methanomassiliicoccales Group 10 and *Methanobacterium* *alkaliphilum* were higher, while the abundance of *Methanobrevibacter* was lower in buffalo than that of Jersey cows, indicating the host species influenced methanogen community as well. Reports are available which expressed the substantial host specificity in community structure of the rumen microbes of different ruminants (Paul et al. 2015). The higher abundance of *Methanobrevibacter*, which is a strongly hydrogenotrophic methanogen in Jersey cow rumen, indicated that ruminal hydrogen production was higher in Jersey cows. This was also evidenced by higher acetate/propionate (A/P) ratio in Jersey cows. These results are similar to the findings of McCabe et al. (2015), who reported a strongly positive Spearman correlation between A/P ratio and *Methanobrevibacter gottschalkii* OTUs in cattle rumen fluid. Furthermore, higher ratio of A/P in Jersey cows not only lead to higher hydrogen concentration and *Methanobrevibacter*, but also higher Bacteroidetes and lower Firmicutes abundances in Jersey cows. This is because Bacteroidetes are considered net H (hydrogen) utilizer, and Firmicutes phylum contains a higher number of known H producers (Stewart et al. 1997). In addition, the abundance of genus *Prevotella* was much higher in Jersey cows than in buffalo, which might be due to higher hydrogen production in Jersey cows. This was further evidenced by strong positive Spearman correlation between *Prevotella* and A/P ratio. Denman et al. (2015) reported that population of *Prevotella* was promoted when methanogenesis (which is a hydrogen-consuming process) was inhibited. In the present study, the higher abundance of *Methanobacterium* and Methanomassiliicoccales in buffalo might be due to the decreased abundance of *Methanobrevibacter* in buffalo rumen, which promoted the growth and abundance of *Methanobacterium* and Methanomassiliicoccales (Wright et al. 2009).

Protozoa constitute half of the microbial biomass in the rumen, and contribute to methanogenesis through the
formation of hydrogen. The results of present study revealed that protozoal population in buffalo was significantly higher, which was consistent with the results of Franzolin and Dehority (1999). *Metadinium* was the most dominant genus in buffalo, and *Entodinium* was the most dominant genus in Jersey cows. An obvious variation in the protozoal community within individual animals of the same group was also found, which is coherent with other studies on the ruminal protozoal community (Kittelmann and Janssen et al. 2011; Lin et al. 2015). *Entodinium* was reported to be the dominant protozoa in the rumen of many kinds of ruminants including cattle, sheep, deer and Water buffalo (Tymensen et al. 2012; Kittelmann et al. 2013; Lin et al. 2015). However, buffalo rumen fluid was dominated by the protozoal genus *Metadinium* (73.2%) in the present study, which was unexpected and different from many other studies on the buffalo ruminal protozoal community (Tymensen et al. 2012; Lin et al. 2015). *Metadinium* is a fermenter of cellulosic materials and was found to have the highest abundance in the rumen of hay fed cattle (Tymensen et al. 2012). The methanogen and protozoal populations had specific differences between buffalo and Jersey cows in the present study, which indicated no strong correlations between the two kinds of microbes (Henderson et al. 2015). However, an inter-community correlation between protozoa and methanogenesis was observed. The strong positive correlations between *Entodinium* and *M. Gottschalkii, Metadinium* and Methanomas-siliicoccales were observed, indicating that they were symbiotically living together in the rumen in the form of pairs. This study predicts that there is some relationship between high fermenting capabilities of buffalo rumen and its special protozoal community; however, this finding is suggested for further exploration.

**5. Conclusion**

The present study concluded that the rumen of water buffalo had higher bacterial, fungal and protozoal populations than those in Jersey cows. Ruminal bacterial, methanogen and protozoal communities were different between the two species; even though, they were fed with similar diet and kept with the same condition. Animal species influenced not only ruminal microbial population, but also community composition. The higher total VFA in buffalo rumen might be due to its higher bacterial and fungal populations, and lower A/P ratio might be due to its higher Bacteroidetes and *Methano-brevibacter* abundances. Whereas, reason how the animal species influenced ruminal microbiota in this study needs to be further explored.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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