

Comparative Analysis of Rumen Bacteria between Water Buffalo and Cattle Fed the Same Diet during their Fattening Period in the Philippines

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Abstract

The objective of this study was to compare the differences in ruminal fermentation and the proportion of rumen bacteria between swamp buffalo and cattle in the Philippines. Six crossbred swamp buffalo and six crossbred cattle used in the study were fed the same diet for 16 weeks. Rumen fluid was collected at weeks 0, 8, and 16 of the fattening period. Diversity of the rumen bacteria was analyzed using 454 pyrosequencing. The population density of major cellulolytic bacteria was determined by real-time PCR. Animal performance was not significantly different between buffalo and cattle. The concentration of total short chain fatty acids was found to be higher in the buffalo than in the cattle; however, the proportions of each short chain fatty acid were not significantly different. The proportion of Bacteroidetes increased in buffalo during the sampling period, whereas it was constant in cattle. In contrast, the proportion of Firmicutes and Fibrobacteres decreased in both groups of animals throughout the experiment. Although the density of cellulolytic bacteria between the two groups of animals was not found to be significantly different, the proportion of cellulolytic bacteria decreased during the fattening period. Both animal species exhibited similar traits for composition and population change of bacteria during the fattening period, suggesting that the microbial community structure might be dependent on feed rather than animal species.

Discipline: Animal Science

Additional key words: cattle, pyrosequencing, rumen bacteria, short chain fatty acid, swamp buffalo

Introduction

The rumen of ruminants is populated by highly diverse and dense obligately anaerobic microorganisms, including bacteria, protozoa, fungi, and methanogens. These microorganisms digest and ferment feed components, and produce short-chain fatty acids and microbial proteins, which are utilized by the hosts as energy and protein sources, respectively (Stewart et al. 1997). Swamp buffalo and cattle are both known for having a high capability for forage utilization and both

represent a significant proportion of all domesticated animals in the world. Cattle are raised in a variety of regions, whereas swamp buffalo, which have adapted to tropical and subtropical climates, are concentrated in low-latitude regions. Approximately 2.85 million buffaloes are raised each year in the Philippines (FAOSTAT 2015) as draft animals or for meat and milk production, and are thus important.

A study reported that swamp buffalo, receiving high levels of roughage rations, are better at utilizing the roughage (Lapitan et al. 2008). Swamp buffalo digest

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fibrous fractions, crude protein, and crude fiber better than cattle (Katiyar & Bisth 1988, Robles et al. 1971). Nutrient digestibility of dry matter, organic matter, crude protein, neutral detergent fiber, and acid detergent fiber were found to be greater in buffalo than in cattle (Chanthakhoun et al. 2012). These results may indicate differences in rumen microbial ecology or activities between buffalo and cattle. However, comparative studies on rumen microbial ecology between swamp buffalo and cattle are limited.

Wanapat et al. (2009) first investigated rumen microbial ecology of swamp buffalo using molecular ecological techniques such as PCR-DGGE and quantitative real-time PCR. They showed changes in the diversity and populations of rumen microbes of animals fed different diets. We previously compared short-term changes in the microbial community structure after feeding in the rumen of swamp buffalo and cattle that were fed the same diet, in the Philippines, using molecular ecological techniques (Lwin et al. 2012a). The results showed that the profiles of the fermentation process, the bacterial and fungal populations, and diversities were similar in crossbred swamp buffaloes and crossbred cattle until 6 h post feeding. Although the diversity of methanogen was similar in both animals (Lwin et al. 2012b), the population density of methanogen was lower in the buffalo (Lwin et al. 2012a). Although short-term changes in the microbial community structure have been examined, long-term changes remain unknown. New molecular techniques such as next-generation sequencing (NGS) are now available and provide a higher resolution of microbial community structures. However, no study has examined rumen microbial ecology in the swamp buffalo using NGS.

The objective of the present study was to compare ruminal fermentation profiles, transitions, and the diversity of rumen bacteria and major rumen microbes, by meta 16S rRNA gene amplicon analysis using NGS, between swamp buffalo and cattle in the Philippines that were fed the same diet over a 16-week period. A part of this study was presented at the 16th Asian-Australasian Association of Animal Production Societies Congress (Asai et al. 2014).

Materials and methods

1. Animals and sample collection

Animal handling was performed according to the Philippine Carabao Center (PCC) guidelines for animal experiments. Six crossbred female swamp buffalo (*Bubalus bubalis*, Philippine native buffalo × Murrah) and six crossbred female cattle (*Bos indicus*, native cattle

× Brahman) were used at the research farm of the PCC, University of the Philippines at Los Baños. At the beginning of the experiment (week 0), the cattle showed an average body weight (BW) of 247.1 ± 16.6 kg and the swamp buffalo showed an average BW of 274.2 ± 15.0 kg (Table 1). The animals were kept in individual pens for 16 weeks during the experiment. Their diets consisted of 50% Napier grass and 50% commercial concentrate on a dry matter basis. The animals were fed separately twice a day (at 07:00 and 13:00) at 3% BW for the first 12 weeks and at 2.5% BW for the last 4 weeks. The concentrate consisted of copra meal, rice bran, pollard, molasses, yellow corn, limestone, salt, urea, mineral premix, and vitamin premix. The nutrition information for the concentrate was as follows: dry matter 89.27, crude protein 16.00, crude fat 9.03, crude fiber 7.51, calcium 1.3, phosphorus 0.14, and total digestible nutrients 74.63. The animals were allowed free access to water and mineral blocks. Rumen fluid was orally collected just before the morning feeding at weeks 0, 8, and 16 using a stomach tube. The rumen fluid was filtered through four layers of surgical gauze, transported on ice, and stored at -80°C until analysis.

2. Short chain fatty acids (SCFAs) analysis

The SCFA content was analyzed using high-performance liquid chromatography (HPLC) as described by Uddin et al. (2010).

3. DNA extraction

DNA was extracted from rumen fluid using the QIAamp[®] DNA Stool Mini Kit (Qiagen) according to the manufacturer's protocol, and stored at -30°C until analysis. The DNA concentration of all samples was adjusted to 10 ng/ μL .

4. Microbial composition by 16S rRNA gene amplicon pyrosequencing

A pooled DNA solution was used as the template. Amplification of the partial 16S ribosomal RNA gene (16S rDNA) was performed using the primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3'; Lane, 1991) and 519R (5'-GWATTACCGCGGCKGCTG-3'; Lane, 1991). Each primer contained a sequencing adaptor sequence and the forward primer contained a unique barcode sequence on the 5' end. The PCR mixture (30 μL) contained 1.0 μL of template, 0.5 μM of each primer, 200 μM of dNTP mixture, 1 \times Ex Taq buffer, and 0.75 units of Ex Taq polymerase. PCR was carried out under the following conditions: initial denaturation at 95°C for 3 min., followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and elongation at 72°C for

1 min., with a final extension at 72°C for 10 min. The PCR products were purified using the QIAquick PCR purification kit (Qiagen). Equal amounts of amplicons from each sample were mixed and sent to a commercial pyrosequencing service using Roche 454 GS FLX pyrosequencer (Hokkaido System Science, Sapporo, Japan). All sequence reads were processed by using the next-generation sequencing analysis pipeline of the QIIME program (Caporaso et al. 2010). Reads shorter than 150 aligned nucleotides, with more than 2% ambiguity, or with more than 2% homopolymers were excluded from further processing. Reads with low alignment quality (50 alignment identity, 40 alignment score reported by SINA) were considered as putative contaminations or artifacts and excluded from downstream analysis. A dendrogram for similarity analysis was visualized with the MEGA6 program.

5. Real-Time PCR assay for major cellulolytic bacteria

Quantitative analyses of *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, and total bacteria were performed using SYBR[®] Green Master Mix (Applied Biosystems) and Real-Time PCR system (Step One Plus[™]; Applied Biosystems) as described in a previous study (Lwin et al. 2012a).

6. Statistical analysis

All data were subjected to paired t-test analysis with SPSS software (2008). A value of $P < 0.05$ was considered statistically significant.

Results and discussion

1. Animal performance

Table 1 shows the animal performance in this study. Initial and final average body weights were not different between cattle and buffalo ($P > 0.05$). Feed intake, daily gain, and feed efficiency were also not different between cattle and buffalo ($P > 0.05$).

2. Fermentation profile in the rumen

Total SCFAs concentration in the rumen of buffalo tended to be higher than that in cattle ($P > 0.05$) (Table 2). At week 8, the lowest total SCFAs concentration was observed in cattle ($P < 0.05$). No significant difference was observed in the proportion of acetate, propionate, and butyrate between the animals and sampling periods. Khejornsart et al. (2011) reported that total SCFAs, acetate, and propionate were significantly different between swamp buffalo and beef cattle that were fed the same diet. In contrast, in our previous study, when swamp buffalo and cattle were fed the same diet, total SCFAs

Table 1. Performance of animals

Parameters	Cattle	Buffalo
Initial body weight (kg)	247.1 ^a ± 16.6	274.2 ^a ± 15.0
Final body weight (kg)	358.3 ^a ± 23.4	390.1 ^a ± 10.2
Feed intake (kg/d)	7.14 ^a ± 0.51	7.59 ^a ± 0.33
Daily gain (kg/d)	0.99 ^a ± 0.14	1.03 ^a ± 0.08
Feed efficiency (kg/kg)	0.139 ^a ± 0.016	0.137 ^a ± 0.014

Values are mean ± standard deviation (n = 6).

^a Means with different superscripts in the same row differ significantly ($P < 0.05$).

Table 2. Short chain fatty acids (SCFAs) profiles in the rumen of buffalo and cattle

Parameters	Animal Species	Week			Significance Cattle vs. Buffalo
		0	8	16	
Total SCFA (mM)	Cattle	43.1 ^{ab} ± 8.2	31.1 ^a ± 6.3	52.7 ^b ± 14.4	NS
	Buffalo	51.7 ± 13.4	42.3 ± 11.4	56.6 ± 23.5	
Acetate (%)	Cattle	78.5 ± 1.7	84.4 ± 2.6	76.9 ± 4.8	NS
	Buffalo	80.8 ± 3.9	82.0 ± 4.6	74.9 ± 2.2	
Propionate (%)	Cattle	16.4 ± 1.9	11.9 ± 1.7	16.9 ± 3.7	NS
	Buffalo	14.7 ± 2.3	13.2 ± 2.8	19.4 ± 1.1	
Butyrate (%)	Cattle	5.1 ± 1.3	3.8 ± 1.6	6.2 ± 2.0	NS
	Buffalo	4.5 ± 2.2	4.8 ± 2.9	5.8 ± 2.2	
A:P ratio	Cattle	4.9 ^a ± 0.6	7.2 ^b ± 1.2	4.8 ^a ± 1.5	NS
	Buffalo	5.6 ^a ± 1.1	6.5 ^a ± 1.6	3.9 ^b ± 0.3	

Values are mean ± standard deviation (n = 6).

^{a, b} Means with different superscripts in the same row differ significantly ($P < 0.05$).

NS: not significant

Table 3. Diversity indices of microbiome in the rumen of cattle and buffalo

	Cattle (week)			Buffalo (week)		
	0	8	16	0	8	16
Shannon index	8.061	7.453	7.345	6.999	7.694	6.837
Chao-1	587	582	675	450	661	454

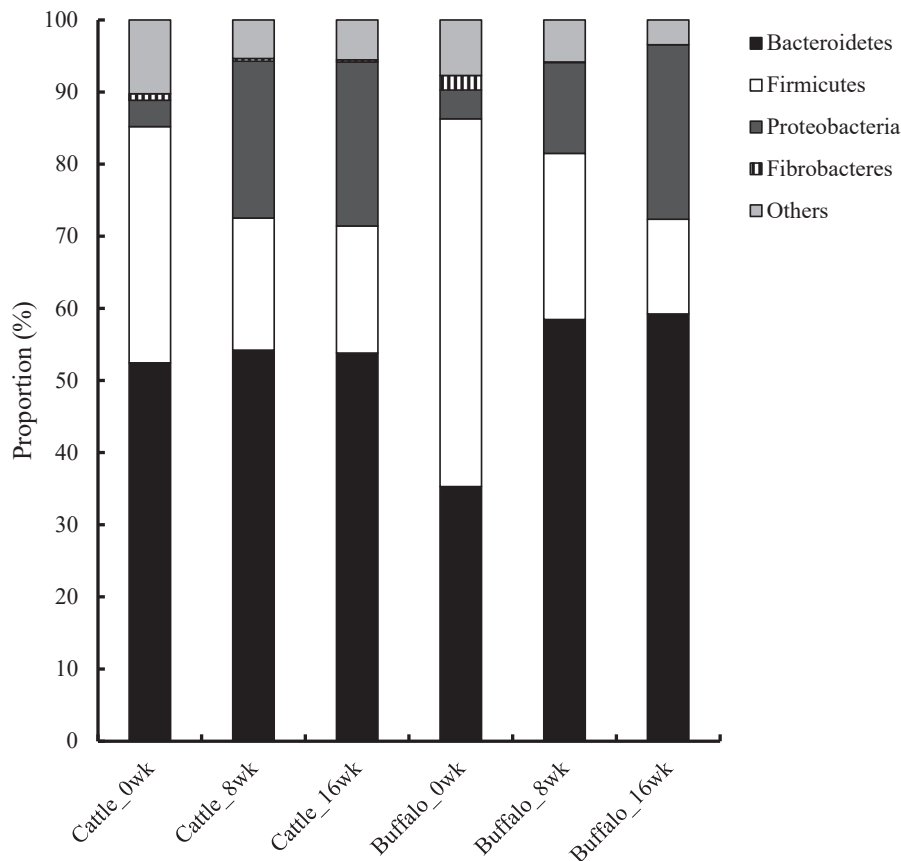


Fig. 1. Composition of the bacterial community structure at phylum level in the rumen of cattle and buffalo

and the molar proportion of acetate, propionate, and butyrate were not significantly different (Lwin et al. 2012a). The present study supports this observation. The acetate: propionate (A:P) ratio in the rumen of buffalo was significantly higher at weeks 0 and 8 than at week 16 ($P < 0.05$), while the highest ratio was observed at week 8 in the rumen of cattle. However, there was no significant difference between buffalo and cattle.

3. NGS analysis

A total of 46,515 reads (average length 359) were obtained from pyrosequencing analysis. After quality control was conducted, the number of reads was reduced to 28,580. There were no large differences in the Shannon index within or between the animals (Table 3). The Chao-1 estimate was highest at week 16 and week 8 in cattle

and buffalo, respectively.

Three major phyla (Bacteroidetes, Firmicutes, and Proteobacteria) constituted more than 88.9% of the total bacteria in all samples (Fig. 1). Both animal species exhibited similar traits of composition change during the fattening period. The proportion of Bacteroidetes was 35.3% in the rumen of buffalo at week 0 and increased to 58 and 59% at weeks 8 and 16, respectively. Conversely, the proportion was constant (52%-54%) in cattle throughout the fattening period. The proportion of Firmicutes decreased during the fattening period in both species; 33%-18% for the cattle and 51%-13% for the buffalo. The proportion of Proteobacteria in both species increased during the fattening period and the proportion of Fibrobacteres was highest at week 0 in both species.

Figure 2 shows the predominant top ten bacterial

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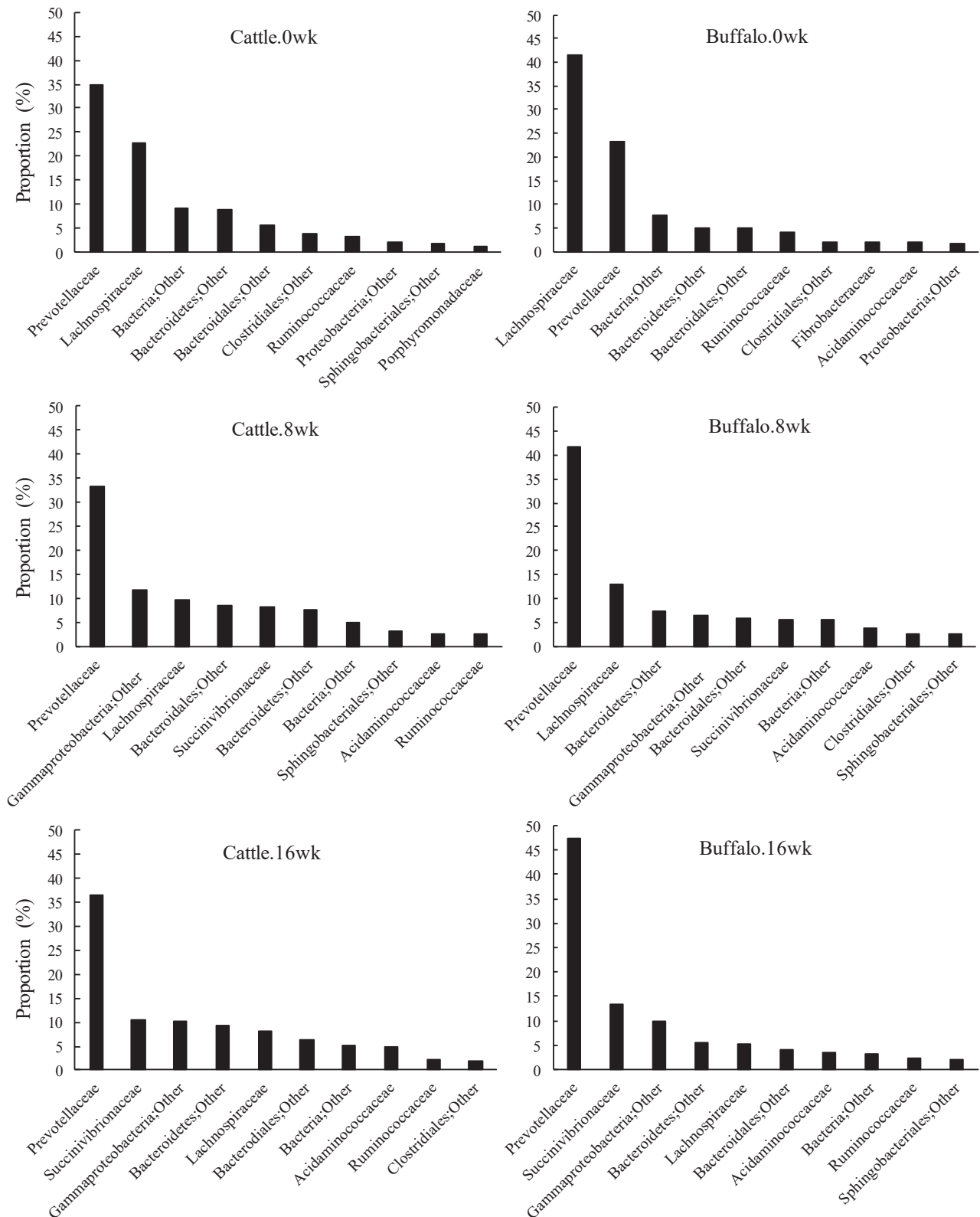


Fig. 2. Predominant bacterial families detected in the rumen of cattle and buffalo (Bacterial families ranked in the top ten are listed.)

families detected in the rumen. These ten most abundant families accounted for at least 93.1% of the sequences analyzed. The family Prevotellaceae was the most dominant except for week 0 in buffalo. Prevotellaceae was the most dominant from weeks 0 to 16 in cattle. Similar bacterial composition was observed at week 16, where Prevotellaceae (36.4%-47.3%), Succinivibrionaceae (10.5%-13.3%), γ -proteobacteria (9.8%-10.2%), Bacteroidetes (5.6%-9.4%), Lachnospiraceae (5.4%-8.0%) and Bacteroidales (4.1%-6.5%) were observed in both animal species. These results suggest that the compositions of the different microbiomes gradually become similar when the animals are fed the same diet. The compositions of bacteria of both animal species was basically similar to those reported by Henderson et al. (2015). Although the proportion of Prevotellaceae was constant in the cattle rumen throughout the fattening period (approximately 35%), it increased in the buffalo rumen (23%-47%). In the present study, animals were fed a relatively high concentrate diet. Prevotellaceae and Flavobacteriaceae were most abundant in the rumen of

cows fed on diet with a high starch content (Thoetkiattikul et al. 2013). Therefore, Prevotellaceae is a key family of bacteria in the rumen of animals fed a starchy diet.

In the clustering analysis of the microbiomes of each sample, cattle and buffalo clustered together during the fattening period (Fig. 3). The microbiomes in cattle and buffalo were distantly related at week 0. However, they became more closely related at weeks 8 and 16. The results also suggest that the microbiomes gradually become more similar when the animals are fed the same diet.

The proportion of bacterial taxon in the rumens and clustering analysis indicate that the microbial community structure is more dependent on feed than animal species. The denaturing gradient gel electrophoresis (DGGE) analysis demonstrated that bacterial diversity tended to be slightly different between cattle and buffalo (Khjornsart et al. 2011, Lwin et al. 2012a). The present results support these observations.

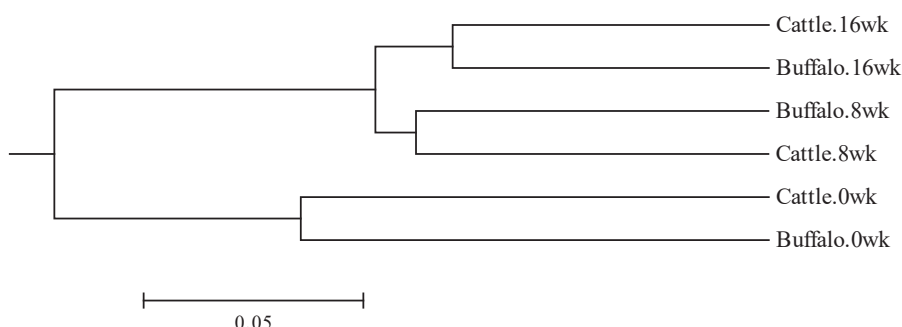


Fig. 3. A dendrogram generated using the UPGMA (unweighted pair group method with arithmetic mean algorithm) of rumen microbial communities of cattle and buffalo

Table 4. Proportion (%)¹ of three major cellulolytic bacteria against the total bacteria in the rumen of buffalo and cattle measured by species-specific real-time PCR assays

Parameters	Animal Species	Week			Significance Cattle vs. Buffalo
		0	8	16	
Rumen bacteria					
<i>Fibrobacter succinogenes</i>	Cattle	6.39 ± 5.49	2.21 ± 3.04	1.09 ± 2.14	NS
	Buffalo	6.00 ± 5.15 ^a	0.58 ± 0.74 ^b	0.11 ± 0.13 ^b	
<i>Ruminococcus albus</i>	Cattle	0.28 ± 0.56	0.05 ± 0.03	0.03 ± 0.02	NS
	Buffalo	0.30 ± 0.39	0.04 ± 0.01	0.03 ± 0.01	
<i>Ruminococcus flavefaciens</i>	Cattle	12.12 ± 22.57	2.98 ± 1.10	3.07 ± 2.18	NS
	Buffalo	12.38 ± 14.43	4.64 ± 1.94	1.86 ± 0.47	

Values are means ± standard deviation (n = 6).

¹ Proportion to total rumen bacteria

^{a, b} Means with different superscripts in the same row differ significantly ($P < 0.05$).

NS: not significant

4. Real-time PCR assay of cellulolytic bacteria

Table 4 shows the proportions of three major cellulolytic bacteria against total bacteria in the rumen of cattle and buffalo. In buffalo, the proportion of *F. succinogenes* was significantly ($P < 0.05$) higher at week 0 than at weeks 8 and 16. During the fattening period, the proportions of *F. succinogenes*, *R. albus*, and *R. flavefaciens* decreased in both buffalo and cattle. The changes and population in the major cellulolytic bacteria examined changed in similar traits. These results also suggest that the changes were influenced by diet rather than animal species. Wanapat suggested that higher nutrient digestibility in buffalo may be explained by buffalo having different rumen ecology than cattle with higher populations of cellulolytic bacteria, fungal zoospores, and lower protozoal populations (Wanapat 2000). Since the populations of cellulolytic bacteria did not differ between buffalo and cattle, our results conflict with this suggestion. The proportion of *R. flavefaciens* was the highest of the three cellulolytic species examined in this study. In previous reports, inconsistent results were observed. *F. succinogenes* or *R. albus* was the predominant fibrolytic bacteria in the rumen of buffalo (Lwin et al. 2012a, Wanapat et al. 2009). These differences may be due to the feed or origin of the animals used in each experiment.

Conclusions

Performance and the concentration and composition of fermentation products were similar between both animals. The microbiomes were slightly different between cattle and buffalo at the beginning of the experiment, but became more similar over the duration of the experiment. The results suggest that the microbiome in the rumen were affected largely by the diet rather than the animal species. Further studies on microbial molecular ecology of ruminal fungi, methanogens, and protozoa are required for a better understanding of the rumen microbiology of swamp buffalo.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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