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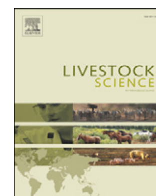

# Comparison of ruminal fermentation characteristics and microbial population in swamp buffalo and cattle

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ABSTRACT

The aim of this study was to determine the ruminal cellulolytic bacterial population in swamp buffalo and beef cattle fed on rice straw by real-time PCR techniques. Four, rumen-fistulated swamp buffalo and four beef cattle (Native × Brahman) were randomly assigned to receive rice straw as a main feed with minimal amount of concentrate. All animals were allowed to adapt to feeds respective before samplings were conducted. It was found that diurnal ruminal pH and $NH_3$–N concentration of buffalo were not different with those found in cattle, population of bacteria and fungal zoospores while the protozoal population were lower ($P < 0.05$). In addition, the nutrient digestibilities of DM, OM, CP, NDF and ADF were found higher in buffalo than in cattle. Employing the real-time PCR techniques for the quantification of cellulolytic bacterial numbers (*Ruminococcus albus*) in the digesta of swamp buffalo, the results revealed significant higher in buffalo than those in cattle. However, at 4 h post-feeding *R. albus* were significantly higher in buffalo than those in cattle measured in rumen fluid, while *F. succinogenes* resulted in higher number in cattle than those in buffalo. Interestingly, the values found in the digesta samples were higher in cellulolytic bacteria than those found in the rumen fluid. This finding indicates higher ability of swamp buffalo in digesting low-quality roughages, especially rice straw.

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## 1. Introduction

Ruminants are capable of utilizing fibrous feeds, however current researches revealed significant differences in feed use for example between Gayal and native cattle (Deng et al., 2007). While, Infascelli et al. (1997) found a significant increase in milk yield by supplementing the diet for buffalo cows with *Aspergillus oryzae*. However, Wanapat (1989) found major differences between swamp buffaloes and cattle in terms of rumen fermentation and nutrient digestibilities. Evidently, buffaloes could utilize feed more efficiently, particularly where the feed supply is of low quantity and/or quality, with the digestibility of feed 2–5% higher than in cattle. Wanapat (2000) also suggested that this may be explained by buffaloes having different rumen ecology than in cattle with higher population of cellulolytic bacteria, fungal zoospores, lower protozoal population and a greater capacity to recycle nitrogen to the rumen. Nevertheless, little is known about ruminal fermentation characteristics and microbial populations, particularly ruminal cellulolytic bacteria in swamp buffalo and cattle. Recent advances in molecular biology techniques allow the analysis of such bacteria without cultivation, thereby identifying many functional, but uncultured, bacteria as new targets for basic and applied research. Up to date, very limited research data have been reported in regards to comparative rumen microorganisms of swamp buffalo and cattle. Therefore, the purpose of this study was to determine the ruminal cellulolytic bacterial population in swamp buffalo and beef cattle fed on rice straw to order to

*Abbreviations:* ADF, acid detergent fiber;BW, body weight;CP, crude protein;CT, condensed tannins;$C_2$, acetic acid;$C_3$, propionic acid;$C_4$, butyric acid; $C_2/C_3$, acetic to propionic acid;DM, dry matter;NDF, neutral detergent fiber; OM, organic matter;RS, rice straw;VFA, volatile fatty acid

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understand the possible differences in both species by real-time PCR techniques.

## 2. Materials and methods

### 2.1. Animals, diets and experimental design

Four, rumen-fistulated, beef cattle and swamp buffalo, about 3-year-old males with 270 ± 10 and 250 ± 20 kg live-weight, respectively, were randomly allotted to receive dietary treatments. Rice straw was fed on ad libitum and concentrate supplement was provided at 0.3% of body weight. The animals were kept in individual pens and received free choice of water and mineral block. The animals were adjusted to feeds for 3 weeks allow the animal and the rumen microorganisms to adapt to the feeds before samplings were done.

### 2.2. Data collection and sampling procedures

During the last 2 days of the 21-day feeding period, rumen fluid and feces (by rectal sampling) were collected. During the preliminary feeding period, the animals were offered with rice straw ad libitum with 0.3% BW of concentrate for 3 weeks. Measurement of rice straw and concentrate was measured daily. The amount of feed offered was adjusted daily so that feed refusals were weighed and discarded daily. Feeds (rice straw and concentrate mixture) were sampled on alternative days, stored at −18 °C until the end of the experiment and then composite for later chemical analyses. Feed intake and apparent diet digestibility were determined by the method as described by Moloney and O'Kiely (1997).

Feed samples were pooled from all animals and analysed for chemical composition; DM, Ash, CP and $NH_3$–N by the methods of AOAC (1990) and NDF, ADF by Van Soest et al. (1991). Samples of rumen fluid (80 ml) were taken from the fistulated rumen at 0, 2, 4, 6, 8, 12 and 24 h post-feeding of each animal and was immediately measured for pH and temperature using a portable pH temperature meter (HANNA Instruments HI 8424 microcomputer, Singapore) and later analyzed for $NH_3$–N by Kjeltech Auto 1030 Analyzer (AOAC, 1990; ID 973.18). The blood sample (about 10 ml) drawn from the jugular vein into EDTA containing tubes was separated by centrifugation at 500 × g for 10 min at 4 °C to sequent blood which was stored at −20 °C until analysis of blood urea N according to the method of Crocker (1967). Rumen fluid was separated into two parts, with one part was used to determine volatile fatty acids (VFA) were analyzed using High Pressure Liquid Chromatography (HPLC, Instruments by controller water model 600E; water model 484 UV detector; column Novapak C18; column size 3.9 mm×300 mm; mobile phase 10 mM $H_2PO_4$ pH 2.5) according to Samuel et al. (1997). The second part was immediately fixed with 10% formalin solution (1:9 v/v, rumen fluid: 10% formalin) to measure microbial populations by total direct counts of bacteria, protozoa and fungal zoospores (Galyean, 1989). Methane gas production was used standard equations according to $CH_4 = 0.45(\text{acetate}) - 0.275\ (\text{propionate}) + 0.40(\text{butyrate})$ (Moss et al., 2000). Rumen fluid was used for direct counts of protozoa and fungal zoospores using methods of Galyean (1989) by haemacytometer (Boeco, Singapore).

During the last 2 days of the 21 days, rumen fluid (fluid only) and digesta (particle only) were collected at 0, 4 and 0, 4, 8 h post-feeding, respectively and were immediately prepared for DNA extraction. Community DNA was extracted from1.0-ml aliquots of each sample by the RBB + C method (Yu and Morrison, 2004), which was shown to substantially increase DNA yields. The quality and quantity of these DNA samples were also determined by agarose gel electrophoresis and spectrophotometry. In total, 40 samples belonging to two treatments (buffalo and cattle), five incubation times (0, and 4) and (0, 4, and 8) of rumen fluid (fluid only) and digesta (particle only), respectively, and four replicates were extracted for genomic DNA.

The primers used for the real-time PCR are as follows: primers for *Fibrobactor succinogenes*, Fs219f (5′-GGT ATG GGATGA GCT TGC-3′) and Fs654r (5′-GCC TGC CCC TGA ACT ATC-3′), were selected to allow amplification (446-bp product) of all 10 F. *succinogenes* strains deposited in GenBank. For *Ruminococcus albus* primers, Ra1281f (5′-CCC TAA AAG CAG TCTTAG TTC G-3′) and Ra1439r (5′ CCT CCT TGC GGT TAG AAC A-3′) (175-bp product). *Ruminococcus flavefaciens* primers, Rf154f (5′-TCT GGA AAC GGA TGG TA-3′) and Rf425r (5′-CCT TTA AGA CAG GAG TTT ACA A-3′), were also selected to allow species–species amplification (295 bp) of all seven R. *flavefaciens* strains deposited in GenBank. All these primer sets were previously published by Koike and Kobayashi (2001). Regular PCR conditions for F. *succinogenes* were as follows: 30 s at 94 °C for denaturing, 30 s at 60 °C for annealing and 30 s at 72 °C for extension (48 cycles), except for 9-min denaturation in the first cycle and 10-min extension in the last cycle. Amplification of 16S rRNA for the other two species was carried out similarly except an annealing temperature of 55 °C was used. Quantification of anaerobic fungal population, primer and condition, was previously published by Denman and Mcsweeney (2006).

Eight sample-derived standards were prepared from treatment (4 buffalo and 4 cattle) set of community DNA. The regular PCR was used to generate sample-derived DNA standards for each real-time PCR assay. Then the PCR product was purified using a QIA quick PCR purification kit (QIAGEN, Inc., Valencia, CA) and quantified using a spectrophotometer. For each sample-derived standard, copy number concentration was calculated based on the length of the PCR product and the mass concentration. Tenfold serial dilution was made in Tri–EDTA prior to real-time PCR (Yu et al., 2005). In total, 8 real-time PCR standards were prepared. The conditions of the real-time PCR assays of target genes were the same as those of the regular PCR described above. Biotools QuantiMix EASY SYG KIT (B&M Labs, S. A., Spain) was used for real-time PCR amplification. All PCRs were performed in duplicate.

### 2.3. Statistical analysis

The means of all parameters measured were statistically analyzed by Analysis of Variance Procedure and means were statistically compare between digesta and rumen fluid at 0, 4, 8 and 0, 4 h post-feeding respectively, with both animals (species) by *T*-Test (SAS, 1996).

## 3. Results and discussion

### 3.1. Chemical composition of feeds

Cassava chip (cassava root), rice bran and molasses were energy sources while rice bran, palm kernel meal and urea were CP sources in the concentrate mixture (Table 1). Rice straw was fed as basal roughage, while concentrate mixture was in a powder form and was readily consumed by the buffaloes and cattle throughout the entire experimental period. Rice straw used in this experiment contained similar values of crude protein (2.7%) and NDF (78.4%), respectively as compared to other values reported by Wanapat et al. (1986) (Table 1). However, the crude protein content of these rice straws was lower than that reported in China by Liu et al. (2002). The differences could be due to different rice straw varieties, locality and management practices.

All rumen parameters between species are presented in Table 2. Results revealed that ruminal pH and $NH_3$–N concentration of buffalo were not different with those found in cattle. Furthermore, the most pronounced effects were remarkably significantly higher in nutrient digestibilities of buffalo than those in cattle particularly those of DM, OM, CP, NDF and ADF of digestibility. However, the feed efficiency utilization by cattle and buffalo depends on the type of diet and of the animal ability to digest and utilize the end products of the digestion keeping losses to a minimum. The utilization of poor quality feeds rich in structural carbohydrates of cell wall only is possible due the presence of microorganisms exiting in the rumen-reticulo and large intestine. Wide differences have been detected between cattle and buffalo in relation to the total process of digestion and utilization of various feeds. These differences include: anatomy and physiology of digestive system, feed intake and digestibility, rumen microbial metabolism, rumen ciliate protozoa population, etc. (Franzolin, 1994) Calabro et al. (2008) have carried out in vitro studies with rumen fluid incubated with common feedstuffs for ruminants. It was found that gas production was lower for inoculum derived from buffalo than for samples from the rumen of cattle. On the other hand, Wanapat (1984) who reported that DM degradability in buffalo was higher than that in cattle, regardless of type of rice straw fed. In addition, Chanthakhoun et al. (2009) reported that cattle rumen fluid was higher in rumen total VFAs, acetate ($C_2$), $NH_3$–N than in buffalo fluid, while total gas production, propionate ($C_3$) and butyrate ($C_4$) were higher in buffalo. Therefore, the higher digestibility of nutrients could be attributed by the rumen microorganisms and their possible fermentation in the buffalo more than those found in cattle.

**Table 1**
Chemical composition of rice straw and concentrate mixture.

| Item | Concentrate | Rice straw |
|---|---|---|
| Ingredient, g/kg | | |
| Cassava chip | 630 | |
| Rice bran | 150 | |
| Palm kernel meal | 150 | |
| Molasses | 30 | |
| Urea | 10 | |
| Salt | 10 | |
| Sulphur | 10 | |
| Mineral mixture | 10 | |
| Chemical composition, g/kg dry matter | | |
| Dry matter | 886 | 930 |
| Organic matter | 943 | 854 |
| Crude protein | 126 | 27 |
| Neutral detergent fiber | 208 | 784 |
| Acid detergent fiber | 87 | 462 |

**Table 2**
Ruminal pH, rumen temperature and concentrations of rumen $NH_3$–N and VFA, and feed intakes and apparent digestibility of beef cattle and buffalo.

| Item | Buffaloes | Cattle | P value |
|---|---|---|---|
| Voluntary feed intake | | | |
| Dry matter intake, kg/day | 2.8 | 2.3 | 0.95 |
| g/kg $W^{.75}$ | $67.5^a$ | $53.8^b$ | <0.001 |
| Apparent digestibility, % | | | |
| DM | $54.8 \pm 1.4^a$ | $49.9 \pm 0.8^b$ | <0.001 |
| OM | $60.8 \pm 1.2^a$ | $56.0 \pm 0.8^b$ | <0.001 |
| CP | $68.8 \pm 1.7^a$ | $53.1 \pm 5.0^b$ | 0.001 |
| NDF | $78.4 \pm 0.7$ | $71.6 \pm 6.3$ | 0.12 |
| ADF | $42.9 \pm 1.9^a$ | $34.8 \pm 5.9^b$ | 0.04 |
| Rumen characteristics | | | |
| Rumen pH | $6.78 \pm 0.55$ | $6.51 \pm 0.81$ | 0.66 |
| Rumen $NH_3$–N, mg/100 ml | $14.7 \pm 2.5$ | $12.7 \pm 0.5$ | 0.17 |
| Total VFAs, (mol/100 mol) | $110.1 \pm 8.8$ | $112.1 \pm 8.2$ | 0.91 |
| Acetate, % | $65.8 \pm 1.1^a$ | $63.9 \pm 5.0^b$ | 0.03 |
| Propionate, % | $22.4 \pm 1.6$ | $22.6 \pm 3.4$ | 0.25 |
| Butyrate, % | $11.7 \pm 0.7$ | $13.3 \pm 1.6$ | 0.21 |
| C2:C3 | $2.9 \pm 0.2$ | $2.8 \pm 0.6$ | 0.17 |
| (C2 + C4):C3 | $3.6 \pm 0.3$ | $3.9 \pm 0.4$ | 0.62 |
| Methane, (mol/100 mol) | $28.1 \pm 1.1$ | $28.9 \pm 1.6$ | 0.58 |

[a,b]Means in the same row with different superscripts differ (P<0.05, P<0.01).

### 3.2. Rumen microorganism population

Table 3 shows the rumen microorganism population between beef cattle and swamp buffaloes. The results revealed relatively higher bacterial and fungal zoospores but lower protozoal population in swamp buffalo than those in cattle. However, under earlier experiment, cattle and swamp buffaloes also showed differences in rumen bacterial, protozoal

**Table 3**
Rumen population of bacteria, protozoa and fungal zoospores in the rumen of swamp buffaloes and cattle.

| Items | Buffaloes | Cattle | P value |
|---|---|---|---|
| Microbial population | | | |
| Bacteria, $\times 10^8$ cells/ml | | | |
| 0 h post-feeding | $2.01 \pm 0.46$ | $1.61 \pm 0.32$ | 0.29 |
| 4 | $3.60 \pm 0.39^a$ | $2.84 \pm 0.10^b$ | 0.02 |
| 8 | $3.35 \pm 0.40^a$ | $2.29 \pm 0.73^b$ | 0.04 |
| Mean | $3.3 \pm 1.3$ | $2.2 \pm 0.6$ | 0.25 |
| Protozoa, $\times 10^6$ cells/ml | | | |
| 0 h post-feeding | $2.01 \pm 0.27$ | $3.75 \pm 0.51$ | 0.23 |
| 4 | $4.63 \pm 0.23^a$ | $6.63 \pm 0.62^b$ | 0.03 |
| 8 | $4.25 \pm 0.40$ | $5.22 \pm 0.43$ | 0.06 |
| Mean | $3.6 \pm 1.4$ | $5.0 \pm 1.7$ | 0.34 |
| Fungal zoospore, $\times 10^5$ cells/ml | | | |
| 0 h post-feeding | $5.55 \pm 0.11^a$ | $2.22 \pm 0.29^b$ | 0.04 |
| 4 | $7.54 \pm 0.62^a$ | $3.54 \pm 0.12^b$ | 0.01 |
| 8 | $8.35 \pm 0.95^a$ | $5.58 \pm 0.36^b$ | 0.03 |
| Mean | $7.1 \pm 1.4$ | $3.8 \pm 1.7$ | 0.06 |

[a,b]Means in the same row with different superscripts differ (P<0.05, P<0.01).

population and fungal zoospore counts, which could explain why swamp buffaloes exhibit conditional body weight better than cattle especially during long dry season without green grass (Wanapat et al., 2000). Under a similar condition, Malakar and Walli (1995) compared fiber degradation (*in vitro*) by bacteria and fungi using inocula from cow and buffalo rumen, and reported that the average counts of anaerobic bacteria were significantly higher for buffalo than for cow. In addition, the rumen microbes of beef cattle and swamp buffalo fed similar typical local feeds and found to be different, in that buffalo exhibited higher cellulolytic bacteria and fungal zoospores, but had a lower protozoa population than those found in cattle (Wanapat et al., 2000). However, although the mean rumen pH was similar between both animal species but the buffaloes had a higher liquid outflow rate that could result in a lower number of total rumen ciliate protozoa (Dehority, 2003). Currently, Franzolin et al. (2010) reported that the zebu cattle had higher rumen protozoa population than the buffaloes, except for *Dasytricha* and *Charonina*, while lower *Entodinium* population was found in buffalo.

Results revealed interesting results showing, when molecular technique (Real-Time PCR) was employed, the two predominant cellulolytic bacteria were significantly higher in buffalo than those in cattle in rumen digesta, respectively. Table 4 shows that the Real-Time Polymerase Chain Reaction approach could be used to determine the population of cellulolytic bacteria (*Fibrobacter succinogenes, R. albus*, and *R. flavefaciens*) both in digesta and rumen fluid of swamp buffalo and beef cattle. *R. albus*, and *R. flavefaciens* in the digesta of swamp were higher than those in cattle as measured by the

**Table 4**
Quantitative measurement, *R. albus* and *R. flavefaciens* and *F. succinogenes* population in rumen digesta and fluid between swamp buffalo and beef cattle using Real-Time PCR technique.

| Items | Buffalo | Cattle | P value |
|---|---|---|---|
| Rumen digesta | | | |
| *R. albus* ($\times 10^8$ copies/g rumen content) | | | |
| 0 hpost-feeding | $3.27 \pm 0.35^a$ | $0.16 \pm 0.14^b$ | <0.001 |
| 4 h | $5.34 \pm 0.49^a$ | $1.70 \pm 0.98^b$ | 0.04 |
| 8 h | $8.38 \pm 2.31^a$ | $4.32 \pm 1.54^b$ | 0.03 |
| Mean | $6.04 \pm 0.84^a$ | $2.92 \pm 1.96^b$ | 0.03 |
| *R. flavefaciens* ($\times 10^8$ copies/g rumen content) | | | |
| 0 hpost-feeding | $5.65 \pm 6.10$ | $5.06 \pm 6.44$ | 0.91 |
| 4 h | $16.58 \pm 17.98$ | $5.76 \pm 6.12$ | 0.30 |
| 8 h | $1.90 \pm 1.46$ | $9.20 \pm 7.70$ | 0.18 |
| Mean | $8.31 \pm 5.43$ | $5.57 \pm 4.99$ | 0.48 |
| Rumen fluid | | | |
| *R. albus* ($\times 10^7$ copies/g rumen content) | | | |
| 0 hpost-feeding | $3.70 \pm 2.63$ | $1.80 \pm 1.73$ | 0.30 |
| 4 h | $2.34 \pm 0.10^a$ | $1.37 \pm 0.24^b$ | 0.003 |
| Mean | $3.02 \pm 1.67$ | $1.58 \pm 1.13$ | 0.11 |
| *R. flavefaciens* ($\times 10^7$ copies/g rumen content) | | | |
| 0 hpost-feeding | $0.11 \pm 0.11^a$ | $17.97 \pm 10.20^b$ | 0.05 |
| 4 h | $5.07 \pm 4.29$ | $4.11 \pm 1.63$ | 0.74 |
| Mean | $2.09 \pm 2.64$ | $2.95 \pm 1.29$ | 0.64 |
| *F. succinogenes* ($\times 10^8$ copies/g rumen content) | | | |
| 0 hpost-feeding | $1.90 \pm 1.06 \times 10^{4a}$ | $1.53 \pm 0.09^b$ | 0.0021 |
| 4 h | $3.30 \pm 0.49^a$ | $24.7 \pm 7.02^b$ | 0.0062 |
| Mean | $1.17 \pm 0.32^a$ | $14.45 \pm 3.88^b$ | 0.0438 |

[a,b]Means in the same row with different superscripts differ ($P<0.05$, $P<0.01$).

Real-Time PCR technique. However, at 4 h *R. albus* were significantly higher in buffalo than those in cattle in the rumen fluid, but *R. flavefaciens* and *f. succinogenes* tended to be higher in cattle than those in buffalo, in rumen fluid. Importantly, the digesta sample had higher cellulolytic bacteria than those found in the rumen fluid. This finding indicates higher ability of buffalo in digesting low-quality roughages especially rice straw.

However, *F. succinogenes*, *R. albus*, *and R. flavefaciens* are presently recognized as the major cellulolytic bacterial species found in the rumen (Forster et al., 1997; Shinkai and Kobayashi, 2007). Current research was concluded and reported that when swamp buffaloes received various ratio of roughage to concentrate (R:C), it was found that *F. succinogenes* were the highest in population followed by *R. flavefaciens* and *R. albus* measured in the rumen digesta and fluid using Real-time PCR, respectively (Wanapat and Cherdthong, 2009). Under this study, *F. succinogenes* was found highest in the digesta, and the three cellulolytic bacterial numbers were $3.21 \times 10^9$, $4.55 \times 10^7$, and $4.44 \times 10^6$ copies/ml for *F. succinogenes*, *R. flavefaciens*, and *R. albus*, respectively. Similarly, Hungate (1966) reported that cellulolytic bacteria were more abundant in the whole digesta, including solid, than in the liquid left when the solid floats to the top of freshly drawn contents, presumably because many attached to the solids particles. However, the predominant cellulolytic bacteria; *R. flavefaciens* tended to be higher in cattle than those found in buffalo in the rumen fluid, respectively. In addition, Khejornsart et al. (2011) found that swamp buffaloes utilize feeds more efficiently with higher rumen fermentation efficiency and with higher rumen microorganism population when fed with urea–lime treated rice straw as a roughage source. Therefore, the results found under this study offered additional data in explaining why swamp buffaloes could perform better when fed on low-quality roughages and/or during the scarce season of feeds.

## 4. Conclusion and recommendations

Based on this study, it could be concluded that ruminal pH and $NH_3$–N concentration of buffalo were not different with those found in cattle. However, DM, OM, CP, NDF and ADF of digestibility in buffaloes were higher than in cattle. Furthermore, applicability of real-time PCR techniques for quantification of cellulolytic bacterial numbers (*R. albus*) in the digesta and rumen fluid of swamp buffalo was higher than in the digesta and rumen fluid of cattle. Total cellulolytic bacteria count in both rumen digesta ($\times 10^8$) and in rumen fluid ($\times 10^7$) was higher in buffalo than in cattle. However, *F. succinogenes* was found higher in cattle than buffalo. Results obtained herein could be used in manipulating the feeding regimes for swamp buffalo.

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