Effects of Direct-fed Exogenous Fibrolytic Enzymes on Rumen Fermentation and Fibrolytic Bacteria Population in Ruminants Fed a Rice Straw-based Diet

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ABSTRACT

The experiment investigated the effects of direct-fed exogenous fibrolytic enzymes (EFE) on feed efficiency and ruminal bacteria population in ruminants fed a rice straw-based diet. Nine rumen-fistulated animals, three crossbred dairy cattle (>75% Holstein Friesian), three crossbred beef cattle (> 75% Brahman) and three swamp buffaloes, were arranged according to a cross-over design trial. The dietary treatments were a diet without EFE (control) and a diet with direct-fed EFE (500 mg/kg DM). The results revealed that the administration of direct-fed EFE did not alter feed intake but slightly increased the digestibility of nutrients (P > 0.05). Ruminal fermentation as illustrated through pH, ammonia-nitrogen (NH_3-N), molar proportion of volatile fatty acids and blood urea nitrogen (BUN), was not altered (P > 0.05) by the administration of direct-fed EFE. Among the fibrolytic bacteria, the population of *Ruminococcus albus* increased significantly as a results of the administration of direct-fed EFE (P < 0.01). Among ruminant species, swamp buffalo showed better feed utilization and had more fibrolytic bacteria, particularly *R. flavefaciens*, than did beef cattle. Based on these results, we conclude that direct-fed EFE does not affect intake and rumen fermentation but does alter the number of fibrolytic bacteria in ruminants.

Keywords: Exogenous fibrolytic enzymes (EFE), rice straw, dairy cattle, beef cattle, swamp buffalo

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INTRODUCTION

Feed resources, particularly low-quality roughages and agricultural crop-residues, are of prime importance for ruminants raised in the tropics. These feeds exhibit close relationships with rumen ecology, microbes, and rumen fermentation patterns (Wora-anu *et al.*, 2007). A number of dietary factors, especially the physical forms and fermentation end products of basal roughage sources, could influence rumen fermentation (Wanapat, 2000). In general, rice straw is a main roughage source for ruminants in Thailand (Wanapat *et al.*, 2009). However, rice straw is characterized by low levels

of crude protein (CP) and high levels of structural carbohydrates such as cellulose, hemicellulose, and lignin, which drastically affect dry matter (DM) intake, digestibility, and animal performance (Safari *et al.*, 2011). Researchers have reported several ways of improving the utilization of rice straw. For example, supplementation with the exogenous fibrolytic enzymes (cellulase and xylanase) has improved the nutritive value of fiber-rich diets and as the performance of cattle (Kung *et al.*, 2000; Elwakeel *et al.*, 2007; Kocyigit*et al.*, 2015), sheep (Cruywagen and Van Zyl, 2008; Ahmed, 2016), and goats (Titi, 2003). However, measurements of fiber digestibility in ruminants have generally shown non or slightly responses to fibrolytic enzymes (Yang *et al.*, 2000; Beauchemin *et al.*, 1999; Dean *et al.*, 2005; Knowlton *et al.*, 2007). Fiber utilization in ruminants not only affect by EFE activity, but also depends on the activity of ruminal fibrolytic bacteria such as *Fibrobacter succinogenes*, *Ruminococcus albus, R. flavefaciens*, *Butyrivibrio fibrisolvens* and *Prevotella ruminicola*, which produce cellulase and xylanase to degrade plant cell walls and release more reducing sugar as an energy source for both rumen microbes and ruminants (Forsberg and Cheng, 1992; Alsersy *et al.*, 2015). Therefore, the objective of this experiment was to investigate the effects of exogenous fibrolytic enzymes on feed utilization and ruminal fibrolytic bacteria population in ruminants fed rice straw-based diets.

MATERIALS AND METHODS

The protocol of the study was approved by the Animal Care and Use Committee of Rajamangala University of Technology Isan. Nine rumen-fistulated animals, three Holstein-Friesian crossbred dairy cattle (> 75% HF), three beef cattle (Brahman) and three Thai swamp buffaloes, were randomly assigned according to a cross-over design experiment. The animals were fed at 2% body weight (BW) on a standard diet (roughage:concentrate = 50:50) with long form rice straw used as roughage and concentrate (Table 1). The dietary treatments included a diet without EFE supplementation (control) and with direct-fed EFE (80,000 and 15,000 IU/g DM of cellulase (β-1,4-endoglucanase, EC 3.2.1.4) and xylanase (β-1,4-endoxylanase, EC 3.2.1.8) activity, respectively) at 500 mg/kg DM as follow as previous *in vitro* experiment (Wongnen *et al.,* 2016).

Animals were housed in individual pen and offered concentrate and rice straw at ratio 50:50 twice a day at 8 am and 4 pm in an equal amounts. Clean water and mineral blocks were free access at all time. The experiment was conducted over 2 periods of 21 days, the first 14 days for treatment adaptation and the last seven days were for sample collections. Feeds and orts were daily weighed and sampled the last week of the experiment. Feces samples were collected from each animal by rectal sampling at the same time for the last 7 days of each period. Both composited feeds and feces were dried at 60°C, ground pass through 1-mm screen and then analyzed for DM, ash, CP and Ether Extract (EE) according to AOAC (1997). Fiber fractions (neutral detergent fiber; NDF, acid detergent fiber; ADF and acid detergent lignin; ADL) were analyzed according to Van Soest *et al.* (1991). Acid insoluble ash (AIA) was used as an internal marker to evaluate apparent digestibility of nutrients (Van Keulen and Young, 1977).

On the last day of each period, 200-ml rumen fluid samples were collected via the fistula at 0 and 4 h post-feeding and then immediately measured for the pH by portable pH meter (Eutech instruments CyberScan pH 11). The first portion of rumen fluid samples, 5 ml of 1 *M* H_2SO_4 were added to 50 ml of rumen fluid. This mixture was then centrifuged at 16,000 ×g for 15 min and the supernatant stored at −20°C until analyzed for ammonia-nitrogen (NH_3-N) (Bremner and Keeney, 1965) and volatile fatty acids (VFAs) (HPLC; model RF-10AXmugiL; Shimadzu; Japan) according to Mathew *et al.* (1997).

Table 1 The chemical composition of rice straw and concentrate used in this experiment

Item	Concentrate	Rice straw
Dry matter (DM), %	96.3	93.4
Chemical composition, % of DM basis		
Organic matter (OM)	90.3	87.6
Ash	9.7	12.4
Crude protein (CP)	11.0	3.0
Ether extract (EE)	1.1	0.8
Neutral detergent fiber (NDF)	33.2	80.7
Acid detergent fiber (ADF)	11.4	53.0
Acid detergent lignin (ADL)	1.7	8.0
TDN[1], %	71.7	41.9
ME[2], Mcal/kgDM	2.59	1.51

[1] Calculation according to feed composition (Harris *et al.*, 1982)

[2] ME (Mcal/kgDM) = %TDN * 0.0361

Another portion of rumen fluid was immediately placed on ice and stored at -20°C for DNA extraction. Total genomic DNA was extracted from 500 µl of rumen fluid by the repeated bead-beating plus column (RBB+C) method (Yu and Morrison, 2004). In brief, cell lysis is achieved by bead-beating in the presence of 4% (w/v) sodium dodecyl sulfate (SDS), 500 mM NaCl, and 50 mM EDTA. The buffer should also protect the released DNA from degradation by DNases, which were very active in the rumen and gastrointestinal samples. After bead-beating, most of the impurities and the SDS were removed by precipitation with ammonium acetate and then the nucleic acids were removed by precipitation with isopropanol. Genomic DNA could then purified via sequential digestion with RNase A and proteinase K, and the DNA was purified using columns from High Pure PCR Template Preparation Kit (Roche, Germany) according to the manufacturer guidelines. The targeted bacteria were total bacteria, Archaea, cellulolytic bacteria (*F. succinogenes, R. albus, R. flavefaciens, B. fibrisolvens* and *P. ruminicola*) and other bacteria (*Anaerovibrio lipolytica, Megasphaera elsdenii and B. proteoclasticus*) were determined by using qPCR analysis (CFX Connect™ Real-time PCR Detection System, Biorad, USA). The primers used for measurement total bacteria and *Archaea* were chosen from previously published sequences as follows: primers for total bacteria, 1369F (CGG TGA ATA CGT TCY CGG; Suzuki *et al.*, 2000) and 1492R (GYY ACC TTG TTA CGA CTT) were employed as described by Nicol *et al.* (2008). Total archaeal 16S rRNA gene copies were quantified using 787f (ATT AGA TAC CCS BGT AGT CC) and 1059r (GCC ATG CAC CWC CTC T) according to Takai and Horikoshi (2000). Other species were designed by primer3 free software as follows: *F. succinogenes*, Fs1100f (5′ CAA CCC ACG TTT CCA GTT -3′) and Fs1218r (5′-TGT GTA GCC CAG GAT GTA A - 3′) (119-bp product) of all thirty eight *F. succinogenes* referent strains deposited in GenBank, For *R. albus* primers, Ra698f (5′-GCT TAC TGG GCT

TTA ACT GA-3′) and Ra808r (5′-CCC ACA CCT AGT AAT CAT CG- 3′) were selected to allow amplification (94 bp) of all fourteen *R. albus* referent strains deposited in GenBank. *R. flavefaciens* primers, Rf279f (5′-GTA GCC GGA CTG AGA GGT TG-3′) and Rf394r (5′- ATC GCT GCA TCA GGG TTT C-3′), were also selected to allow species-species amplification (113 bp) of all nineteen *R. flavefaciens* strains deposited in GenBank. *B. fibrisolvens* primers, Bf476f (5′- AAA GCT CTA TCA GCA GGG AA-3′) and Bf602r (5′- GTA AAT CCG GAT AAC ACT TG-3′), were also selected to allow species-species amplification (126 bp) of all 19 *B. fibrisolvens* strains deposited in GenBank. *P. ruminicola* primers, Pr1117f (5′- TCT TTA GTT GCC ATC AGG TAA-3′) and Pr1239r (5′- TGT AAC ACG TGT GTA GCC C-3′), were also selected to allow species-species amplification (111 bp) of all 5 *P. ruminicola* strains deposited in GenBank. *A. lipolytica* primers, Al627f (5′- CAG GTG GGA TTG TAA GTC AG -3′) and Al731r (5′- TAC ACT AGG AAT TCC GCT TT -3′), were also selected to allow species-species amplification (105 bp) of two *A. lipolytica* strains deposited in GenBank and *M. elsdenii* primers, Me1063f (5′- AGG ACA AGA AAA CAG GTG G -3′) and Me1166r (5′- CGC TGG TAA CAG AAG ATA GG -3′), were also selected to allow species-species amplification (104 bp) of all 16 M. elsdenii strains deposited in GenBank and B. proteoclasticus primers, Bp72f (5′- GGG ATA TTG CAC AAT GGA GG -3′) and Bp182r (5′- TCT CTT GCG AGC CTT TCT TC -3′), were also selected to allow species-species amplification (106 bp) of all three *M. elsdenii* strains deposited in GenBank. All primers are shown in Table 2.

Twenty ng of template DNA was used in a total reaction volume of 10 μL contained 5 μL Master mix (Biorad, USA), 0.2 μL of forward and reverse primer (10 μM/μL), 0.125 μL visiblueand 20 ng/μL of template DNA. Reactions used the following cycling parameters: 10 min of an initial denaturation at 95°C, followed by 20 s at 95 °C (denaturing), annealing for 30 s and 30 s at 72°C (elongation) for 35 cycles for total bacteria, *F. succinogenes*, *R. flavefaciens, R. albus, B. fibrisolvens, P. ruminicola* and *B. proteoclasticus* and 40 cycles for *Archaea*, *A. lipolytica* and *M. elsdenii*, with a final extension at 72°C for 1 min. Annealing temperature were differ depended on each pair of species (60.0, 60.0, 55.0, 56.9, 55.0, 55.2, 53.7, 59.4, 51.0 and 56.0 for total bacteria, *Archaea*, *F. succinogenes, R. flavefaciens, R. albus, B. fibrisolvens, P. ruminicola, A. lipolytica, M. elsdenii* and *B. proteoclasticus*, respectively). Positive and negative controls without a template were included for each primer pair.

A blood sample was taken from the jugular vein immediately after rumen fluid sampling. The blood was drawn into 6-ml vacutainer tubes without anticoagulant, refrigerated at 5 °C for 1 h then centrifuged at 3,000×g for 15 min. The supernatant was stored at –20 °C until analysis for blood urea nitrogen (BUN) according to procedure described by Crocker (1967).

All data were analyzed according to a cross-over design using PROC GLM (SAS, 1996). Comparisons among individual treatment means were performed by the procedure of possible difference (PDIFF). Least square means are reported throughout, and significance was indicated at $P < 0.05$

Table 2 Details of qPCR primers for reaction of molecular techniques in this study

Rumen microbes	Primer	Sequence	Tm (°C)	Source
Total bacteria	1369F 1492R	CGGTGAATACGTTCYCGG GYYACCTTGTTACGACTT	60.0	Suzuki *et al.* (2000) Nicol *et al.* (2008)
Archaea	787F 1059R	ATTAGATACCCSBGTAGTCC GCCATGCACCWCCTCT	60.0	Takai and Horikoshi (2000)
Fibrobactor succinogenes	Fs1100f Fs1218r	CAACCCACGTTTCCAGTT TGTGTAGCCCAGGATGTAA	55.0	Present study
Ruminococcus flavefaciens	Rf279f Rf394r	GTAGCCGGACTGAGAGGTTG ATCGCTGCATCAGGGTTTC	56.9	Present study
Ruminococcus albus	Ra698f Ra808r	GCTTACTGGGCTTTAACTGA CCCACACCTAGTAATCATCG	55.0	Present study
Butyrivibrio fibrisolvens	Bf476f Bf602r	AAAGCTCTATCAGCAGGGAA GTAAATCCGGATAACACTTG	55.2	Present study
Prevotella ruminicola	Pr1117f Pr1239r	TCTTTAGTTGCCATCAGGTAA TGTAACACGTGTGTAGCCC	53.7	Present study
Anaerovibrio lipolytica	Al627f Al731r	CAGGTGGGATTGTAAGTCAG TACACTAGGAATTCCGCTTT	59.4	Present study
Megasphaera elsdenii	Me1063f Me1166r	AGGACAAGAAAACAGGTGG CGCTGGTAACAGAAGATAGG	51.0	Present study
Butyrivibrio proteoclasticus	Bp72f Bp182r	GGGATATTGCACAATGGAGG TCTCTTGCGAGCCTTTCTTC	56.0	Present study

RESULTS AND DISCUSSION

Researchers have observed no interaction between dietary treatments and ruminant species according to any parameters. Total feed intake was the same between dietary treatments, control and direct-fed EFE, but intake differed significantly among ruminant species (P < 0.05). Intake of rice straw among dairy cattle, measured in %BW, was significantly lower than that among beef cattle and swamp buffalo (P < 0.05) owing to the fact that the proportion of concentrate intake was significantly higher among dairy cattle than among other species (P < 0.05) (Table 3). This result was related to the slightly lower nutrient digestibility of dairy cattle compared to beef cattle and swamp buffalo, particularly with respect to NDF and ADF digestion (Allen, 2000).

Although the nutrient digestibility and nutrient digestible intake of the direct-fed EFE group trended higher than those of the control group, this difference was not statistically significant (P > 0.05) (Table 3). In according with previous research that EFE addition was seem to promising in ruminants but mostly non significantly or slightly improved (Beauchemin *et al.*, 1999; Kung *et al.*, 2000; Yang *et al.*, 2000; Titi, 2003; Dean *et al.*, 2005; Elwakeel *et al.*, 2007; Knowlton *et al.*, 2007; Cruywagen and Van Zyl, 2008; Kocyigit*et al.*, 2015; Ahmed, 2016). Previous experiments using the *in vitro* gas production technique have revealed that EFE supplementation improves the digestibility of DM and OM (Wongnen *et al.*, 2016); by contrast, the administration of direct-fed EFE in this experiment *in vivo* did not improve the digestibility of DM and OM. Hristov *et al.* (2000), Wang *et al.* (2001), and Reddish and Kung (2007) have reported that in *in situ* experiments, EFE does not improve the extent of dry matter digestion. These results could signify that supplementary direct-fed EFE is rapidly destroyed by rumen proteases (Beauchemin *et al.*, 2004) before cellulase was active, since the lag time of cellulose degradation by rumen microbes was estimated between 30-45 min after inducer addition (Johri *et al.*, 1999). Moreover, Lee *et al.* (2011) reported that fiber fraction was slowly degraded and the highest fibrolytic enzymes activity obtained after 4 days of fermentation. Thus, attempts to improve feed efficiency among ruminant species using direct-fed fibrolytic enzymes applied to the feed during or only hours before feeding would have variable digestion responses.

Indeed, responses to feed intake have generally been small and inconsistent (Rode *et al.*, 1999; Schingoethe *et al.*, 1999; Phipps *et al.*, 2000; Vicini *et al.*, 2003), with only occasional significant increases (Lewis *et al.*, 1999). Furthermore, the research suggests that exogenous enzymes digest only those substrates normally digested by enzymes produced by the autochthonous microbiota in the rumen (Hristov *et al.*, 2000; Wang *et al.*, 2001; Reddish and Kung, 2007). Long form rice straw is difficult to degrade via EFE and cellulolytic bacteria activity until broken down into small particles. Bowman and Firkins (1993) have found that small-particle (2 mm) forages result in greater carboxymethyl cellulase (CMCase) activity than do large-particle (5 mm) forages. The greater surface area of the small particles enhances both cellulolytic enzyme activity (Grethlein, 1985; Grous *et al.*, 1986) and the binding of cellulase to cellulose (Oshima *et al.*, 1983; Kyriacou *et al.*, 1989).

Table 3 Least square means of dry matter intake and nutrients digestibility in animals offered diet without EFE and with direct-fed exogenous fibrolytic enzymes (DFE)

Items	Treatment		SEM	Ruminant species			SEM
	Control	DFE		Dairy	Beef	Buffalo	
Body weight, kg	505	512		603	509	415	
Total DM intake							
Kg/day	10.1	10.2	0.97	11.5	10.4	8.6	1.15
%BW	2.00	1.99	0.02	1.89[a]	2.04[b]	2.06[b]	0.02
C:R ratio	0.52	0.53	0.01	0.56[b]	0.51[a]	0.51[a]	0.01
Apparent digestibility,%							
DM	57.1	58.9	1.56	57.5	56.6	60.0	1.86
OM	62.3	63.9	1.62	62.4	61.6	65.4	1.92
CP	48.5	51.2	1.68	51.1[ab]	45.4[a]	53.0[b]	2.00
EE	60.1	58.8	2.16	59.9	57.6	60.9	2.57
NDF	52.7	55.5	2.10	53.0	51.7	57.5	2.50
ADF	37.5	40.7	2.32	36.4	38.5	42.5	2.76
Digestible nutrient intake, kg/d							
OM	5.60	5.75	0.54	6.35	5.69	4.98	0.64
CP	0.35	0.37	0.035	0.44	0.33	0.32	0.04
EE	0.06	0.06	0.001	0.07	0.06	0.05	0.001
NDF	2.99	3.11	0.32	3.31	3.04	2.79	0.38
ADF	1.18	1.27	0.14	1.26	1.27	1.16	0.17
Metabolizable Energy							
Mcal/d	21.4	21.7	2.05	24.1	21.6	18.9	2.43
Mcal/kg	2.11	2.16	0.05	2.11	2.08	2.21	0.06

[a, b] Means in the same row with different superscript differ ($P < 0.05$)
EFE = exogenous fibrolytic enzymes.

Nutrient digestibility among swamp buffalo seems higher than among other species, albeit not at a level of statistical significance ($P > 0.05$) except regarding crude protein, the digestibility of which was significantly higher among swamp buffalo than among beef cattle ($P < 0.05$), but was similar to that in dairy cattle (Table 3). Similar CP digestibility in buffalo in relation to dairy cattle was reported by Gandra *et al.* (2011). These findings agree with a previous report by Wanapat *et al.* (2003), who indicated that the digestibility of nutrients, particularly of OM and crude protein, was higher among swamp buffalo than among cattle. These results confirm those of Skunmun *et al.* (2002), who demonstrated that swamp buffalo can utilize poor-quality feeds such as crop residues more efficiently than can cattle. Swamp buffalo are also more efficient than cattle in aspects of N-recycling and fiber digestion, according to Devendra (1985) and Wanapat *et al.* (1994), perhaps because swamp buffalo have a greater amount of ruminal microorganisms than do dairy or beef cattle. To confirm this theory, Wanapat *et al.* (2000) and Khejornsart *et al.* (2011) demonstrated that the number of total bacteria in swamp buffalo was higher than in beef cattle. Wora-anu *et al.* (2006)

confirmed that the ruminal cellulolytic, proteolytic, and amylolytic bacteria of swamp buffalo are significantly more numerous than those found in beef cattle, resulting in higher fiber utilization and degradation in swamp buffalo than in beef cattle, as found by Kennedy *et al.* (1992) and Wanapat *et al.* (1994).

Administration of direct-fed EFE did not affect ruminal pH, NH_3-N, VFA proportion, or BUN (Table 4) but did alter the ruminal microorganism population (Table 5), as the number of *R. albus* in the direct-fed EFE group was higher than in the control group ($P < 0.05$). This finding was in accordance with the work of Montanez-Valdez *et al.* (2010). Although the treatment of wheat straw with an EFE preparation containing xylanase and β-gluconase activities increased fiber digestibility and VFA production in experiments by Giraldo *et al.* (2007) and Arriola *et al.* (2011), the response to direct-fed EFE was lesser in this experiment, most likely because EFE was demolished in the rumen (Beauchemin *et al.*, 2004) as mention earlier.

Table 4 Least square means of rumen fermentation and blood urea nitrogen in animals offered diet without EFE and with direct-fed exogenous fibrolytic enzymes (DFE)

Items	Treatment		SEM	Ruminant species			SEM
	Control	DFE		Dairy	Beef	Buffalo	
Ruminal pH	7.02	6.95	0.07	7.08[b]	7.12[b]	6.75[a]	0.08
NH_3-N, mg%	12.3	12.9	0.67	13.2	11.4	13.0	0.79
Volatile fatty acids (VFAs)							
Total VFAs, mM	100.2	98.4	5.45	93.6[a]	88.4[a]	115.9[b]	6.50
Acetic acid (C_2), %	58.1	58.8	0.96	57.1[a]	54.9[a]	63.3[b]	1.14
Propionic acid (C_3), %	29.3	28.8	0.91	29.9[b]	31.0[b]	26.3[a]	1.08
Butyric acid (C_4), %	12.5	12.1	0.72	13.0[b]	13.3[ab]	10.5[a]	0.85
C_2:C_3 ratio	2.03	2.11	0.10	1.96[a]	1.80[a]	2.44[b]	0.11
BUN, mg %	10.7	10.1	0.87	7.8[a]	8.9[a]	14.4[b]	1.03

[a, b] Means in the same row with different superscript differ ($P < 0.05$)
EFE = exogenous fibrolytic enzymes, NH3-N = ammonia nitrogen, BUN = blood urea nitrogen

Nevertheless, Mendoza *et al.* (2014) have reported that EFE supplementation results in more reducing sugars being released from roughage, thereby stimulating a greater concentration of VFAs and microbial protein synthesis. Likewise, EFE supplementation results in greater attachment and colonization of rumen microorganisms to the plant cell wall, altered ruminal fermentation, increased particle outflow rate from the rumen associated with a reduction in rumen liquid viscosity (Alvarez *et al.*, 2009), and improved fiber digestion (Nsereko *et al.*, 2002). The current research demonstrates that BUN concentration were not affected by direct-fed EFE supplementation, as in the work of Shekhar *et al.* (2010).

Table 5 Least square means of ruminal bacteria population in animals offered diet without EFE and with direct-fed exogenous fibrolytic enzymes (DFE)

Items	Treatment		SEM	Ruminant species			SEM
	Control	DFE		Dairy	Beef	Buffalo	
Ruminal bacteria population, copies/ml							
Total bacteria (× 10^{11})	2.68	3.12	0.44	3.28	2.99	2.43	0.53
Archaea (× 10^{8})	1.96	2.43	0.32	1.94	2.38	2.27	0.38
F. succinogenes (× 10^{8})	7.22	4.72	2.45	6.42	8.45	3.04	2.91
R. flavefaciens (× 10^{10})	2.20	2.41	0.25	2.27^{ab}	1.77^{b}	2.89^{a}	0.30
R. albus (× 10^{10})	1.90^{a}	4.18^{b}	0.48	2.15	3.16	3.82	0.57
B. fibrisolvens (× 10^{7})	7.35	7.68	1.87	6.09	7.20	9.24	2.09
P. ruminicola (× 10^{8})	1.75	3.82	1.03	3.48	2.25	2.62	1.23
A. lipolytica (× 10^{5})	5.19	4.47	1.47	3.70	4.76	6.03	1.76
M. elsdenii (× 10^{5})	1.52	1.47	0.47	1.50	2.11	0.87	0.56
B. proteoclasticus (×10^{9})	1.56	1.96	0.31	1.89	1.53	1.86	0.36

$^{a, b}$ Means in the same row with different superscript differ ($P < 0.05$)
EFE = exogenous fibrolytic enzymes

Among ruminant species, swamp buffalo showed a lower molar proportion of propionic acid (C_3) and butyric acid (C_4) but a higher concentration of total VFAs, acetic acid (C_2), C_2:C_3 ratio, and BUN compared to beef and dairy cattle ($P < 0.05$). Higher BUN concentration may related to higher CP digestibility in swamp buffalo in relation to dairy and beef cattle, as also observed by Gandra *et al*. (2011). Additionally, we have observed a greater population of predominant rumen fibrolytic bacteria (*R. flavefaciens*) in swamp buffalo than in beef cattle ($P < 0.05$), but was equal to that in dairy cattle (Table 5). While the population of *R. albus* in swamp buffalo was to some extent higher than in dairy cattle ($P < 0.05$) but was no different than in beef cattle. (Table 5). Moreover, Chanthakoun *et al.* (2012) have found that *R. flavefaciens* and *R. albus* are the predominant cellulolytic bacteria in the rumen and are higher in swamp buffalo than in beef cattle offered rice straw as a main feed source with minimal amounts of concentrate. Wora-anu *et al.* (2006) and Khejornsart *et al.* (2011) have additionally reported that swamp buffalo have a higher number of ruminal fibrolytic bacteria such as *B. fibrisolvens*, *F. succinogenes* and *R. flavefaciens* than do cattle, probably leading to higher fiber utilization and degradation in swamp buffalo than in beef cattle (Kennedy *et al.*, 1992). Furthermore, Gandra *et al.* (2011) suggested that there is a great metabolic diversity among ruminant species when offered to the diet.

Thus, differences in swamp buffalo and cattle rumen fermentation and blood metabolite could be explained by different microbial activity levels among ruminant species as a result of different microbial population numbers or of microbial populations constituted by different species, as observed in this experiment. In addition, any variations between swamp buffalo and cattle in the nature of rumen microbial populations and numbers of ruminal bacteria might explain differences in the types of fermentation occurring, the resulting end-products, and digestive capability (Wanapat *et al.*, 2000).

CONCLUSION

Supplementation with direct-fed EFE resulted in an increased number of fibrolytic bacteria (*R. albus*) among ruminants, with a lesser response in rumen fermentation. Further study would shed light on the effects of pretreatment of low-quality roughage with EFE to enhance fiber utilization in ruminants. Swamp buffalo showed a greater response to fiber utilization, rumen fermentation and ruminal cellulolytic bacteria population as compared to beef and dairy cattle. The results of this experiment may provide valuable information allowing for the manipulation of feeding for different ruminant species.

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