International Journal of Agricultural Technology 2018 Vol. 14(7): 2125-2136
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

Effects of tropical forage species on *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and total bacteria population in goat rumen using real-time PCR techniques

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Thongruang, S., Paengkoum, P., Suksombat, W. and Bureenok, S. (2018). Effects of tropical forage species on *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and total bacteria population in goat rumen using real-time PCR techniques. International Journal of Agricultural Technology 14(7): 2125-2136.

Abstract Three ruminally fistulated crossbred Saanen male goats (approximately 33 ± 3.0 kg BW) were used to determine the effect of 6 tropical forage diets on *B. fibrisolvens*, *F. succinogenes* and total bacteria population in goat rumen using real-time PCR techniques. The results showed that in grass experiment, three grass species including Purple Guinea (*Panicum maximum* TD58), Chinese Pennisetum (*Pennisetum purpureum* x *Pennisetum alopecuroides*) and Napier Pak Chong 1 (*Pennisetum purpureum* x *Pennisetum americanum*) were not significantly ($p>0.05$) different in the population of *B. fibrisolvens* (1.51-2.97 x 10^6 copies/ml), *F. succinogenes* (4.21-5.98 x 10^6 copies/ml) and total bacteria (4.08-6.58 x 10^{10} copies/ml) in goat rumen at 0, 2, 4 and 6 h after feeding. Similarly, legumes experiment, the population of ruminal *B. fibrisolvens* (1.31-3.01 x 10^6 copies/ml), *F.succinogenes* (4.01-5.73 x 10^6 copies/ml) and total bacteria (3.12-5.40 x 10^{10} copies/ml) of goats were not significantly ($p>0.05$) different between three legume species including Hamata (*Stylosanthes hamate*), Hedge Lucern (*Desmanthus virgatus*) and Leucaena (*Leucaena leucocephala*) at 0, 2, 4 and 6 h after feeding.

Keywords: forage species, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, total bacteria, population

Introduction

Microorganisms including bacteria, protozoa, and fungi in the anaerobic rumen fibrolytic family perform degradation of fibrous material, thus allowing ruminant animals to access nutrition otherwise locked within the plant fibers. Bacterial species are the most abundant of these microbial life and play a major role in performing such function. Major cellulolytic bacterial that are found within the rumen include: *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* (Forster *et al*., 1997; Shinkai and Kobayashi, 2007) while *Butyrivibrio fibrisolvens* is identified to undertake biohydrogenation of

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fatty acids and to form conjugated linoleic acid (CLA) which is isomer of C18:2 as intermediate in the process. Natural CLA is found in milk and meat of ruminants. The presence of CLA in milk fat from ruminants results from the isomerization and biohydrogenation of unsaturated fatty acid (linoleic acid and α-linolenic acid) by rumen bacteria *B. fibrisolvens* as well as the Δ9-desaturase activity in the mammary gland. CLA has potential to be anti-carcinogenic, anti-oxidant, anti-atherosclerosis, positive effects on cardiovascular diseases, anti-diabetic, anti-obesity, immunomodulating and reduces the plasma cholesterol concentrations (O'Shea *et al*., 2000; Laso *et al*., 2007). O'Shea *et al*. (1998) found total CLA in milk being between 2-30 mg/g fat and found cis-9, trans-11 octadecadienoic acid about 90% of total CLA.

Dewhurst *et al*. (2003) described that linoleic acid and α-linolenic acid are the predominant unsaturated fatty acids in forages, which they are the main precursors of c9t11 CLA and t10c12 CLA in milk. Herbage lipids are the cheapest and safest sources of these fatty acids. The forages and pastures are, therefore, important long-term strategy of CLA. Tropical forages are low in fatty acid composition, Khan *et al.* (2015) reported that in all 12 tropical forages, C18:2n6 content averaged 2.72 g/kg DM, contributing 16% to the total FAs and C18:3n3 was the predominant FA with an average content of 9.21 g/kg, contributing on average 51% to the total measured FAs.

Recent advances in molecular biology techniques allow the analysis of bacteria without cultivation. The recent development of real-time polymerase chain reaction (PCR) has been successfully used for quantifying protozoa, cellulolytic fungi, and cellulolytic bacterial species (McSweeney and Denman, 2007; Tajima *et al*., 2001). Real-time PCR (RT-PCR) is a molecular biological technique that can sensitively detect and quantify target bacterial species even when the organisms in question is present at low levels (Zimmermann and Mannhalter, 1996). RT-PCR has been utilized to perform analysis of numerous environmental samples, such as water (Leser *et al*., 1995) and rumen digesta (Reilly and Attwood, 1998). This technique is relatively simple to perform and yields reliable results. The objective of this experiment was to investigate the effects of forage species on *B. fibrisolvens* and also *F. succinogenes* and total bacteria population in goat's rumen using real-time PCR techniques.

Materials and methods

Animals and treatments

Three male crossbred Saanen goats (body weight of approximately 33 ± 3 kg), which have been ruminally fistulated, were used as replicates to determine *B. fibrisolvens*, *F. succinogenes* and total bacteria population in goat's rumen.

The study was devided into 2 experiments by type of forages (grasses and legumes), Experiment 1: 3 treatments from 3 grasses: Purple Guinea (*Panicum maximum* TD58), Chinese Pennisetum (*Pennisetum purpureum* x *Pennisetum alopecuroides*) and Napier Pak Chong 1 (*Pennisetum purpureum* x *Pennisetum americanum*), and Experiment 2: 3 treatments from 3 legumes: Hamata (*Stylosanthes hamata*), Hedge Lucern (*Desmanthus virgatus*) and Leucaena (*Leucaena leucocephala*). Goats were housed in individual pen and feed *ad libitum* roughage and 16% CP concentrate at 1.5% BW.

Each experiment used 3 ruminally fistulated crossbred Saanen male goats which were randomly assigned in 3 × 3 Latin Square Design to receive 3 treatments. Each experiment was conducted in 3 periods; each period lasted 28 days of which the first 7 days were used as adjustment period to the experimental diets. Overall experimental period was 84 days. At the end of each period, rumen content of each animal was collected at 0, 2, 4 and 6 h post-feeding in the morning and was used for DNA extraction of *B. fibrisolvens*, *F. succinogenes* and total bacteria using real-time PCR technique, (LightCycler® Nano System version 1.0.1, Roche).

Seven-day metabolism trial was also conducted to determine goat's nutrient utilization. We designed the metabolic cage facility specially in such a way that allows spatial separation of feces and urine sample collection. Before sample collection, animals were held in the metabolic cages for 3 days to acclimatize the animals to the new environment. After that, we collected the samples for 7 consecutive days. During this time period, we preserved aliquots of feed offered, residue left, and feces for downstream chemical analyses, We also recorded the body weight of the animals before and after the metabolism trials.

For further analysis, amount of the feed offered and remaining residues were quantified and recorded, and approximately 10% of feces (fresh weight) from each goat was taken daily and stored in a deep freezer at -20^{o}C until the end of the experiment. Collected feces from all seven days were pool and thoroughly mixed, and then samples were taken and then dried at 60^{o}C for 72 hours. After that the dried samples were ground with a mortar and pestle. The determination of dry matter (DM) was achieved by drying the samples at 105^{o}C for 24h. Ash content was assayed by samples incineration at 550^{o}C, and quantity of organic matter (OM) could therefore be calculated. Nitrogen (N) content was determined by the Macro Kjeldahl technique (AOAC, 1985) and crude protein content was calculated as N x 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) determination were analyzed according to following the procedure described by Van Soest *et al*. (1991).

Blood samples were taken from the jugular vein at 0 (prior to morning feeding), 2, 4 and 6 h post-feeding. After collection, the blood samples were centrifuged at 3,000 xg for 15 min to separate the plasma from the rest of the blood content. Plasma samples were then stored at -20^{o}C for futher analysis of blood urea nitrogen (BUN) concentration.

Laboratory analyses

For forage's nutritive value analysis, the forages were freeze-dried, ground, and pass through a 1 mm^2 mesh screen before analyzing for chemical composition. Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25. Ether extract (EE) and ash contents were quantified according to the method of AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated by the methods described by Van Soest *et al*. (1991).

Fatty acids in grasses and legumes were extracted using a method previously descrived by Folch *et al*. (1957) and Metcalfe *et al*. (1966) with slight modifications. Fresh forage samples were cut, immediately frozen at -20°C, subsequently freeze-dried, then ground and pass through a 1 mm^2 mesh screen before the extraction. Each sample, consisting of 15 grams, was homogenized for 2 min with 90 ml of chloroform-methanol (2:1) (Nissel AM-8 Homogenizer, Nihonseikikaisha, LTD., Japan). Each sample was then further homogenized for 2 min with 30 ml of deionized water and 5 ml of 0.58% NaCl was added. The under layer of fatty acid methyl esters (FAME) was removed and placed in screw-cap test tube and stored at -20°C until methylation. Fatty acid methel ester (FAME) were prepared by the procedure described by Ostrowska *et al*. (2000). Community DNA was extracted from 1.5 ml aliquots of rumen fluid and digesta by the RBB+C method described by Yu and Morrison (2004).

The real-time PCR, species specific PCR primers (*B. fibrisolvens*, *F. succinogenes* and total bacteria) used to amplify 16S rDNA regions (target DNA) were chosen from Kobayashi *et al*. (2000) for *B. fibrisolvens* (FR-27: 5'-AGAGTTTGATCCTGGCTCA GGA-3', Prb-156: 5'-CACGTTGTCATGCAACATCGT-3', 213 bp), and Denman and McSweeney (2006) for total bacteria (Forward: 5'-CGGCAACGAGCGCAACCC-3' and Reverse: 5'-CCATTGTAGCACGTGTGTAGCC-3') and *F. succinogenes* (Forward: 5'-GTTCGGAATTACTGGGCGTAAA-3' and Reverse: 5'-CGCCTGCCCCTGAACT ATC-3'). Real-time PCR amplification and detection were performed using a LigthCycler Nano (LightCycler® Nano System version 1.0.1, Roche). 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 of denaturation in the first cycle and 10 min of extension in the last cycle. Amplification of 16S rDNA for the other two species was carried out similarly, except at an annealing temperature of 55°C.

Statistical analysis

All data were statistically analyzed as repeated measurements for a 3 × 3 Latin squares design by the Analuysis of Variants (ANOVA) procedure using specializesd statistical program, SAS (SAS, 2001). Significant differences among treatment were assessed by Duncan's New Multiple Range Test. A significant level of $P < 0.05$ was used (Steel and Torries, 1980).

Results

Chemical composition, linoleic and linolenic acid contents of grasses were demonstrated in the Table 1 and of legumes were showed in Table 2. The average DM content of grass was 20.58% and of legume was 27.82%. The CP contents ranged from 9.16% to 14.12% in grasses and 15.33% to 22.96% in legumes. The NDF contents ranged from 64.34% to 69.29% in grasses and 59.44% to 64.14% in legumes, whereas that of C18:2n6 ranged from 11.50% to 13.92% of total fatty acid in grasses and 10.95% to 14.20% of total fatty acid in legumes.

Table 1. Chemical composition of concentrate and experimental grasses

Items	Concentrate (16% CP)	Purple Guinea	Chinese pennisetum	Napier Pak Chong 1
DM	94.16	23.28	19.46	19.01
	---------- % on a dry matter basis ----------			
CP	16.57	9.16	12.05	14.12
CF	14.90	32.17	31.23	30.11
NDF	44.24	69.29	65.20	64.34
ADF	24.56	42.19	41.14	41.10
Ash	7.33	10.03	12.46	12.17
Fat	3.44	2.53	3.27	3.34
C18:2n6 (% of total fatty acid)	3.29	11.50	11.85	13.92
C18:3n3 (% of total fatty acid)	0.41	16.72	30.69	38.74

DM = dry matter, CP = crude protein, CF = crude fat, NDF = neutral detergent fiber, ADF = acid detergent fiber, C18:2n6 = linoleic acid, C18:3n3 = α-linolenic acid.

The average DMI, BW change, DM, OM, CP, NDF, ADF and fat digestibilities were not significantly different among the treatments in both grasses and legumes (Table 3 and Table 4 respectively).

Ruminal pH decreased gradually and reached the minimum at 4 h after feeding and then increased, both grass and legume experiments showed the same manner (Table 5 and Table 6). The pH values ranged between 6.49 and 6.97, and were not significantly influenced by time of sampling and forage species. In both experiments, the maximum NH_3-N concentration was achieved at 4 h after feeding, but overall ruminal NH_3-N concentrations were not significantly ($p > 0.05$) different among the treatments. Similarly, BUN concentrations were highest at 4 h after feeding and were also not significantly different among goats fed different forage species.

Table 2. Chemical composition of concentrate and experimental legumes

Items	Concentrate (16% CP)	Hamata	Hedge Lucern	Leucaena
DM	94.16	27.11	28.21	28.14
	---------- % on a dry matter basis ----------			
CP	16.57	15.33	19.17	22.96
CF	14.90	28.28	27.55	24.74
NDF	44.24	64.14	61.23	59.44
ADF	24.56	39.89	36.51	35.33
Ash	7.33	8.02	6.23	6.07
Fat	3.44	2.96	3.58	3.97
C18:2n6 (% of total fatty acid)	3.29	10.95	11.34	14.20
C18:3n3 (% of total fatty acid)	0.41	16.23	17.71	20.49

DM = dry matter, CP = crude protein, CF = crude fat, NDF = neutral detergent fiber, ADF = acid detergent fiber, C18:2n6 = linoleic acid, C18:3n3 = α-linolenic acid.

Table 3. Dry matter intake (DMI), body weight change and digestibility of goats fed different grass species

Items	Purple Guinea	Chinese penisetum	Napier Pak Chong 1	SEM
DMI (g/d)				
Concentrate	440	437	446	9.026
Roughage	620	640	647	17.033
Total	1,060	1,077	1,092	25.632
BW Change (kg)	1.75	1.79	1.89	0.235
Apparent digestibility (%)				
DM	60.36	61.55	63.84	1.342
OM	62.37	63.58	65.92	1.494
CP	70.12	72.54	74.86	1.641
NDF	71.64	73.49	75.16	1.313
ADF	67.32	68.45	69.57	1.184
Fat	85.16	87.64	88.96	1.343

Means within a row followed by the different letter are different ($P < 0.05$) and SEM : standard error of means.

Table 4. Dry matter intake (DMI), body weight change and digestibility of goats fed different legume species

Items	Hamata	Hedge Lucern	Luecaena	SEM
DMI (g/d)				
Concentrate	441	445	451	9.854
Roughage	540	562	580	15.346
Total	981	1,007	1,032	24.855
BW Change (kg)	1.69	1.77	1.82	0.313
Apparent digestibility (%)				
DM	66.21	67.75	68.38	1.052
OM	67.36	69.54	70.23	1.161
CP	74.57	77.92	79.51	1.665
NDF	75.65	78.13	79.73	1.455
ADF	70.36	70.92	74.88	1.543
Fat	87.54	88.46	89.16	1.051

Means within a row followed by the different letter are different ($P < 0.05$) and
SEM : standard error of means.

Table 5. Ruminal pH, ruminal ammonia nitrogen (NH_3-N) and blood urea nitrogen (BUN) in goats fed three grass species

	Purple Guinea	Chinese pennisetum	Napier Pak Chong 1	SEM
Ruminal pH				
0 h	6.94	6.97	6.96	0.071
2 h	6.49	6.51	6.59	0.063
4 h	6.61	6.79	6.74	0.082
6 h	6.74	6.84	6.80	0.071
Ruminal NH_3-N (mg/dl)				
0 h	11.75	12.10	12.70	0.515
2 h	15.80	14.00	13.60	0.873
4 h	17.50	18.40	19.20	0.772
6 h	15.10	16.40	15.90	0.864
BUN (mg/dl)				
0 h	16.21	16.81	17.40	0.485
2 h	16.77	18.38	17.67	0.644
4 h	18.40	18.40	18.92	0.516
6 h	16.80	16.98	16.46	0.397

Means within a row followed by the different letter are different ($P < 0.05$) and
SEM : standard error of means.

The pattern of total VFA concentration (Table 7: grass experiment, Table 8: legume experiment) was similar to the pattern of ruminal NH_3-N and BUN concentrations. Concentrations and proportions (% molar) of individual VFAs were not significantly ($p > 0.05$) affected by goats fed different forage species.

Table 6. Ruminal pH, ruminal ammonia nitrogen (NH_3-N) and blood urea nitrogen (BUN) in goats fed three legume species

	Hamata	Hedge Lucern	Leucaena	SEM
Ruminal pH				
0 h	6.80	6.94	6.94	0.061
2 h	6.64	6.79	6.79	0.063
4 h	6.76	6.71	6.61	0.075
6 h	6.79	6.87	6.84	0.081
Ruminal NH_3-N (mg/dl)				
0 h	12.40	12.90	13.10	0.472
2 h	16.70	18.50	17.80	0.715
4 h	19.10	17.40	18.60	0.844
6 h	15.40	16.30	15.70	0.512
BUN (mg/dl)				
0 h	16.92	17.17	17.70	0.436
2 h	17.78	18.43	18.58	0.513
4 h	19.41	19.88	19.60	0.476
6 h	17.37	17.40	16.80	0.454

Means within a row followed by the different letter are different ($P < 0.05$) and
SEM : standard error of means.

The results in grass experiment showed that the population of ruminal *B. fibrisolvens*, *F. succinogenes* and total bacteria of goats at 0, 2, 4 and 6 h (Table 9) were not significantly ($p > 0.05$) different between three grass species. Similarly, legume experiment showed that the population of ruminal *B. fibrisolvens*, *F. succinogenes* and total bacteria of goats at 0, 2, 4 and 6 h (Table 10) were not significantly ($p > 0.05$) different among three legume species.

Table 7. Total volatile fatty acid (VFA) and proportion of VFAs in goats fed three grass species

	Purple Guinea	Chinese pennisetum	Napier Pak Chong 1	SEM
Total VFA (mM/L) (h)				
0 h	59.45	60.42	61.64	2.471
2 h	63.54	62.44	65.84	2.463
4 h	66.45	67.92	67.38	2.742
6 h	63.12	63.84	62.82	1.676
VFA proportions (% Molar)				
Acetic acid	68.80	69.46	69.85	1.012
Propionic acid	21.02	20.16	20.05	0.441
Butyric acid	10.18	10.38	10.10	0.314

Means within a row followed by the different letter are different ($P < 0.05$) and
SEM : standard error of means.

Table 8. Total volatile fatty acid (VFA) and proportion of VFAs in goats fed three legume species

	Hamata	Hedge Lucern	Leucaena	SEM
Total VFA (mM/L) (h)				
0 h	60.57	61.44	61.85	2.561
2 h	64.34	63.51	66.64	2.243
4 h	67.84	68.45	68.57	1.682
6 h	64.00	64.54	63.87	1.946
VFA proportions (% Molar)				
Acetic acid	65.64	66.54	67.25	1.052
Propionic acid	22.64	21.89	21.11	0.683
Butyric acid	11.72	11.57	11.64	0.254

Means within a row followed by the different letter are different ($P < 0.05$) and
SEM : standard error of means.

Table 9. Rumen microorganisms of goats fed with three grasses including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1

	h	Purple Guinea	Chinese Pennisetum	Napier Pak Chong 1	SEM	Pr > F
B. fibrisolvens	0	1.51	1.72	1.85	0.079	0.414
(10^6 copies/ml)	2	1.73	1.94	1.99	0.027	0.118
	4	2.46	2.53	2.81	0.040	0.138
	6	2.51	2.91	2.97	0.059	0.177
F. succinogenes	0	4.21	4.46	4.51	0.030	0.056
(10^6 copies/ml)	2	4.53	4.56	4.94	0.095	0.292
	4	5.24	5.60	5.76	0.066	0.191
	6	5.22	5.59	5.98	0.124	0.289
Total bacteria	0	4.08	4.32	5.06	0.099	0.076
(10^{10} copies/ml)	2	4.84	4.95	5.52	0.111	0.200
	4	5.14	5.67	6.30	0.159	0.098
	6	5.62	5.79	6.58	0.172	0.154

Means within a row followed by the different letter are different ($P < 0.05$) and
SEM : standard error of means.

Table 10. Rumen microorganisms of goats fed with three legumes including Hamata, Hedge Lucern and Luecaena

	h	Hamata	Hedge Lucern	Leucaena	SEM	Pr > F
B. fibrisolvens	0	1.31	1.32	1.56	0.063	0.188
(10^6 copies/ml)	2	1.76	1.81	1.87	0.062	0.267
	4	2.29	2.30	2.66	0.069	0.190
	6	2.73	2.81	3.01	0.057	0.103
F. succinogenes	0	4.01	4.05	4.31	0.058	0.123
(10^6 copies/ml)	2	4.30	4.54	4.57	0.035	0.071
	4	5.06	5.26	5.41	0.147	0.279
	6	5.56	5.66	5.73	0.143	0.133
Total bacteria	0	3.12	3.40	4.16	0.171	0.287
(10^{10} copies/ml)	2	3.44	3.78	4.64	0.142	0.155
	4	4.47	4.75	5.30	0.119	0.130
	6	4.34	4.97	5.40	0.415	0.569

Means within a row followed by the different letter are different ($P < 0.05$) and
SEM : standard error of means.

Discussion

The nutritive value of Napier Pak Chong 1 was closed to the report of Keawthong (2002) and the nutritive value of Leucaena was slightly higher than that studied by Paengkoum and Traiyakun (2011). No significant differences were found in ruminal pH, ruminal NH_3-N, BUN and total VFA. The ruminal NH_3-N values of both grass and legume experiments ranged between 11.75 and 19.20 mg/dl and were within the rang of 10-20 mg/dl required for optimum digestion (Krebs and Leng, 1984; Leng, 1990).

The lack of difference in rumimal *B. fibrisolvens*, *F. succinogenes* and total bacteria population among the treatments of this experiment were similar to the study of Khaing *et al*. (2016) who reported that fifteen male Boer crossbred goats around six months old of approximately 18.54 ± 1.83 kg of BW were fed Napier grass in comparison to whole corn plant silage. The mean concentrations of rumen NH_3-N (mg/dl) and the total VFA production in the rumen fluid of the goats were not significantly different among the treatments. The total bacteria population of rumen content was not significantly different among the treatments, total bacteria of goats fed Napier grass were 10.0 $\log_{10}$ or 1.00×10^{10} copies/ml of rumen fluid, while those of goats fed whole corn plant silage were 10.2 $\log_{10}$ or 1.58×10^{10} copies/ml of rumen fluid. While the *F. succinogenes* of goats fed Napier grass were 6.3 $\log_{10}$ or 2.00×10^6 copies/ml of rumen fluid which were significantly ($P < 0.05$) higher than that of goats fed whole corn plant silage (5.6 $\log_{10}$ or 3.98×10^5 copies/ml of rumen fluid), comparable to this experiment that used only fresh grasses and legumes which had greater population of *F. succinogenes* ranging from $4.21\text{-}5.98 \times 10^6$ copies/ml of rumen fluid in grass experiment and $4.01\text{-}5.73 \times 10^6$ copies/ml of rumen fluid in legume experiment. Additionally, Metzler-Zebeli *et al*. (2013) showed the greater number of *F. succinogenes* and total bacteria population in Boer, White German Noble and Toggenburg goats fed a sole Meadow grass hay (5.2% CP) being 5.87×10^9 copies *F. succinogenes* /ml of rumen fluid and 11.1 $\log_{10}$ or 1.26×10^{11} copies total bacteria/ml of rumen fluid.

For the *B. fibrisolvens* population in Saanen goat's rumen fluid in this study, they were lower when compared to those found in steers descrived in Guo *et al*. (2010) who showed that four ruminally cannulated Chinese Luxi steers (BW 559.4 ± 30.1 kg) were used in a crossover design experiment with an experimental period of 28 days. The forage to concentrate ratio of the basal diet was 35:65 on when measured on the basis of dry matter. The daily feeding quantity was fixed at 7.5 kg/head, which includes the Chinese wildrye at 10.2% CP of total feed. Rumen fluid was collected at 07:30 pre-feeding, at 11:30 and 17:30 postfeeding on day 27 and 28. A fraction of the pooled sample from rumen fluid was analyzed for species-specific real-time PCR quantification.

The numbers of *B. fibrisolvens* were 4.74 $\log_{10}$ copies/µl or 5.49×10^7 copies/ml and total bacteria were 10.91 $\log_{10}$ copies/µl or 8.13×10^{10} copies/ml.

It is conbcluded that the grass experiment, *B. fibrisolvens*, *F. succinogenes* and total bacteria population of goat's rumen fluid using real-time PCR were not significantly ($p > 0.05$) different between three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 at 0, 2, 4 and 6 h after feeding. Similarly, in legume experiment, population of ruminal *B. fibrisolvens*, *F.succinogenes* and total bacteria of goats were not significantly ($p > 0.05$) different between three legume species including Hamata, Hedge Lucern and Leucaena at 0, 2, 4 and 6 h after feeding.

Acknowledgement

The authors acknowledge Suranaree University of Technology for their facilities and for financially supporting this research.

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(Received: 8 September 2018, accepted: 7 November 2018)