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Original Article

Effects of levels of ensiled pineapple waste and pangola hay fed as roughage sources on feed intake, nutrient digestibility and ruminal fermentation of Southern Thai native cattle

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Abstract

The objective of this experiment was to determine the effect of ensiled pineapple waste (P/A) as roughage source on rumen fermentation and feed utilization of native cattle. Four rumen-fistulated Southern Thai native cattle with initial weight 286.1±48.5 kg were arranged to receive dietary treatments in a 4x4 Latin square design. Each period was lasted for 21 days. Cattle were fed concentrate to roughage at the ratio of 65:35. The roughage used was as follows; 1) 100% ensiled pineapple waste, 2) 65% P/A and 35% pangola hay (HAY), 3) 35% P/A and 65% HAY and 4) 100% HAY. The results revealed that dry matter intake among dietary treatments was not significantly different (P>0.05) (3.70, 3.81, 3.52 and 3.59 kgDM/d). Apparent digestibility of DM, OM, CP, NDF and ADF in cattle fed only P/A as roughage source was higher (P<0.05) than in cattle fed only HAY. Increased HAY resulted in linearly decreased apparent digestibility of DM, OM, CP, NDF and ADF. Caloric density of diet with only P/A as roughage source (2.96 Mcal/kgDM) was higher (P<0.05) than that of diet with only HAY (2.67 Mcal/kgDM). Ruminal temperature, pH and ammonia-nitrogen concentration were not different (P>0.05). Acetic acid, propionic acid and butyric acid concentrations were not significantly different (P>0.05) among dietary treatments. Blood urea-nitrogen was similar among dietary treatments. In conclusion, P/A is promising to use as a roughage source for Southern Thai native cattle.

Keywords: Southern Thai native cattle, ensiled pineapple waste, pangola hay, nutrient digestibility, ruminal fermentation

1. Introduction

Beef production in Thailand increased from 4.6 to 9.1 million head between 1999 and 2008 at an average 8.32% per year. In 2009, the total population decreased slightly with 8.6 million head, 5.4 million head of native cattle and, particularly, the highest ratio of native cattle to other breeds (84:16) with 0.6 million head in the South of Thailand (DLD, 2010). This population was built up due to the Thai governmental support policy, especially for smallscale farms that raised their cattle by free grazing in communal land or public area without supplementation. This no-cost raising may be unsuitable at this time, when the economic return of land-use is concern. Pineapple, one of the important economic plants, is usually consumed fresh, or as pineapple juice, fruit pulp or canned. Pineapple residue is a by-product of the pineapple

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processing industry and consists basically of the residual pulp, peels, and skin with high moisture content. By-products can account for a large proportion (70-75% w/w) of a crop. Whilst the pineapple crop production in Thailand was 2.5 million tons in 2008 (OAE, 2009), much of this in areas of Petchaburi and Prachuap Khiri Khan provinces ($1.0x10^6$ tons) it resulted in $1.6x10^6$ to $1.7x10^6$ tons of processing wastes. These residues, like many other agricultural wastes, can cause serious environmental problems from both solid wastes and their effluents (Correia *et al.*, 2004). Thus, feeding these by-products to livestock should be considered not only to lessen environmental problems, to diminish dependence of livestock on grains that can support human, and to eliminate the costly waste management programs (Grasser *et al.*, 1995), but also to support sustainable development among the agricultural community. Therefore, the present study aimed to determine the effect of ensiled pineapple waste fed as a roughage source on feed intake, nutrient digestibility and ruminal fermentation of Southern Thai native cattle.

2. Materials and Methods

2.1 Animals, diets and experimental design

The experiment was conducted on the experimental farm at Faculty of Technology and Community Development, Thaksin University, Phatthalung Campus. Four rumen-fistulated Southern Thai native cattle (average ± SD: 286.1± 48.5 kg BW) were arranged to receive dietary treatments in a 4x4 Latin square design in order to investigate the effects of level of different roughage sources on feed intake, nutrient digestibility and ruminal fermentation. The roughage used was as follows: 1) 100% ensiled pineapple waste (P/A), 2) 65% P/A and 35% pangola (*Digitaria eriantha*) hay (HAY), 3) 35% P/A and 65% HAY and 4) 100% HAY. Cattle were offered concentrate and roughage separately *ad libitum* twice daily in the equal amounts in the morning and the afternoon. The concentrate to roughage ratio was adjusted daily to maintain the ratio at 65:35 (DM basis). All animals were kept in individual pens and received free access to water. Each period lasted for 21 days. The first 14 days was for adaptation while the last 7 days was for total collection in metabolism crates.

P/A was packed in plastic bags and kept at least 21 days before opening. Pangola hay, cut at 45 days, was derived from farmers in Prachuap Khiri Khan province.

2.2 Data collection, analysis and sampling procedures

During the adaptation period, feed consumption was recorded daily by weighing feeds offered and orts. All animals were weighed three times (d1, d14 and d21 of each period) to calculate intake and weight change. Regular samples of feed and feces from the total collection of each cattle were collected during the last 7 days of each period. Samples were analyzed for chemical compositions (DM, ash, organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF)) (AOAC, 1985; Goering and Van Soest, 1970).

Rumen fluid samples (about 200 ml) were taken using an aspiration technique on the last day of each period at 0, 2 and 4 h post-feeding. The pH and temperature of rumen fluid was immediately measured using a mobile digital pH meter (Ecoscan pH5, Eutech instruments Pte Ltd., Singapore). Rumen fluid was strained through four layers of cheesecloth to remove particulate matter and 10 ml of 1M H_2SO_4 was added to 90 ml of rumen fluid. The mixture was centrifuged at 16,000g for 15 min and the supernatant was removed and stored at -20°C prior to ammonia-nitrogen (NH_3-N) analysis (Bremner and Keeney, 1965) and volatile fatty acid (VFA) analysis using Gas Chromatography (GC6890, Agilent Technologies).

Blood samples (about 10 ml) were drawn from the jugular vein at the same time as rumen fluid sampling. Samples were centrifuged at 500g for 10 min and stored at -20°C until blood urea nitrogen (BUN) analysis (Crocker, 1967).

2.3 DNA Extraction

Community DNA was extracted from 0.25 ml aliquots of content (rumen fluid and digesta) by the RBB+C method described by Yu and Morrison (2004). In brief, cell lysis was 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 protect the released DNA from degradation by DNases, which were very active in the rumen and gastrointestinal sample. 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 be purified via sequential digestion with RNase A and proteinase K, and the DNA was purified using columns from QIAgen DNA Mini Stool Kit (QIAGEN, 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. Ten-fold serial dilutions were made in Tri-EDTA prior to real-time PCR (Yu *et al.*, 2005). In total, 4 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 amplication. All PCRs were performed in duplicate.

2.4 Statistical analyses

The repeated measurement in a 4x4 Latin square design was conducted for each parameter and the means were compared by Duncan's new multiple range test (DMRT) and trend analysis using orthogonal polynomial. The analysis was carried out using the Proc GLM (SAS, 1998).

3. Results

3.1 Chemical composition of feeds

Values of nutrient composition (DM basis) for P/A, HAY and concentrate are shown in Table 1. P/A contained low DM (13.85%) and had low pH (3.06) in accordance with the results of Nguyen Thi Hong Nhan *et al.* (2009) (2.97). However, crude protein content of P/A was 2-fold higher than that of HAY. Fiber constituents of P/A were also lower than those of HAY. The concentrate used in this experiment contained 18.36 %CP, 24.61%NDF, 15.37%ADF and 2.90 Mcal ME/kgDM.

3.2 Feed intake, nutrient digestibility and nitrogen balance

Dry matter intake and nutrient intake values are presented in Table 2. In all parameters of intake, the results showed that there was no significant difference (P>0.05) among dietary treatments. Ratio of roughage source and feeding roughage to concentrate ratio were found to be similar as expected.

Apparent digestibility, digestible nutrient intake, energy intake and microbial crude protein synthesis of Southern Thai native cattle are reported in Table 3. The apparent digestibilities of DM, OM, CP, NDF and ADF were significantly influenced (P<0.05) by different proportions of roughage sources. The cattle fed only P/A as roughage source had higher (P<0.05) nutrient digestibilities than cattle fed only HAY as roughage source. Increased proportion of HAY in the diet resulted in a linear decrease in apparent digestibilities of DM, OM, CP, NDF and ADF, but not of EE (P>0.05). None of the digestible nutrient intakes were significantly different (P>0.05); consequently estimated metabolizable energy (ME) intake and microbial crude protein (MCP) synthesis were not also different (P>0.05) among dietary treatments. Nevertheless, caloric density of diet with only P/A as roughage source (2.96 Mcal/kgDM) was significantly higher (P<0.05) than that of diet with only HAY as roughage source (2.67 Mcal/kgDM). Nitrogen balance was positive and did not differ (P>0.05) among dietary treatments (Table 4). In addition, energy intake, caloric density, microbial crude protein synthesis, nitrogen absorption and retention increased linearly (P<0.05) as roughage ratios of HAY decreased.

3.3 Ruminal environment and blood metabolites

Means of ruminal temperature, pH and NH_3-N concentration were unaffected (P>0.05) by dietary treatments (range 38.18-38.30°C; 6.88-7.02, and 20.53-37.85 mg/L, respectively). Moreover, no differences of BUN were found (P>0.05) among treatment diets. The values of BUN in cattle fed dietary treatments ranged from 9.07 to 11.46 mg/dl (Table 5). Table 6 shows the concentration of VFA produced in the rumen. Acetic acid (C2), and propionic acid (C3) concentrations were not significantly different (P>0.05) among dietary treatments. Differences of butyric acid (C4) concentration were detected (P<0.05), the highest value was in cattle fed only HAY as roughage source while the lowest value was in

Table 1. Chemical composition of feeds used in the experiment

Item[1]	P/A	HAY	Concentrate[2]
DM	13.85	85.39	88.50
		% of DM	
Ash	7.01	8.03	8.38
OM	92.99	91.97	91.62
CP	6.04	3.08	18.36
EE	3.98	3.83	7.18
CF	18.94	30.80	7.32
NFE	64.03	54.26	58.76
NDF	61.10	71.66	24.61
ADF	40.44	41.69	15.37
ADL	2.18	4.06	1.07
pH	3.06	-	-
Metabolizable energy[3], Mcal/kgDM	-	-	2.90

[1] DM = dry matter, OM = organic matter, CP = crude protein, EE = ether extract, CF = crude fiber, NFE = nitrogen free extract, NDF = neutral detergent fiber, ADF = acid detergent fiber, ADL = acid detergent lignin.

[2] Concentrate comprised of cassava chip 41.0%, fined rice bran 11.0%, palm kernel cake 30.0%, soybean meal 9.2%, molasses 4.0%, rock phosphate 0.9%, urea 2.0%, salt 1.0%, mineral premixed 0.7% and sulfur 0.2%.

[3] Calculated based on energy concentration of ingredients.

Table 2. Effects of P/A and HAY on dry matter intake and nutrient intake of Southern Thai native cattle

Item	P/A:HAY ratio[2]				SEM	P- value
	100:0	65:35	35:65	0:100		
Dry matter intake						
Roughage intake						
kg/d	1.23	1.29	1.13	1.20	0.070	0.528
% BW	0.42	0.44	0.39	0.42	0.028	0.604
g/kg $W^{0.75}$	17.28	18.24	15.95	17.19	1.115	0.582
Concentrate intake						
kg/d	2.47	2.53	2.38	2.39	0.114	0.775
% BW	0.85	0.88	0.82	0.82	0.036	0.675
g/kg $W^{0.75}$	34.92	36.01	33.76	33.77	1.504	0.689
Total intake						
kg/d	3.70	3.81	3.52	3.59	0.168	0.645
% BW	1.27	1.32	1.21	1.24	0.060	0.623
g/kg $W^{0.75}$	52.20	54.24	49.71	50.97	2.413	0.616
P/A:HAY ratio	100:0	69:31	36:64	0:100	-	-
R:C ratio	33:67	33:67	32:68	33:67	-	-
Nutrient intake[1], kg/d						
OM	3.40	3.51	3.23	3.29	0.155	0.626
CP	0.528	0.529	0.484	0.475	0.023	0.311
EE	0.226	0.232	0.215	0.217	0.010	0.642
NDF	1.36	1.45	1.36	1.45	0.070	0.626
ADF	0.876	0.914	0.833	0.869	0.042	0.638

[1] For explanations of the presentation of chemical composition of feed, see footnotes of Table 1.
[2] Values within a row with no superscript are not significantly different (P>0.05)

cattle fed only P/A as roughage source. The ratio of C2:C3 ranged between 2.92 to 4.02; the difference was not significant (P>0.05).

3.4 Bacteria population in the rumen

Three dominant cellulolytic bacteria were elucidated by using molecular technique. For each standard, linear regressions derived from the threshold cycle (Ct) of each DNA dilution versus the log quantity were calculated. The coefficient of determination (r^2) of linear regressions obtained was 0.997, 0.986, 0.962 and 0.975 for total bacteria (TB), *F. succinogenes*, *R. flavefaciens* and *R. albus*, respectively. The results showed that *F. succinogenes*, a gram-negative organism, was the predominant cellulolytic bacteria in the rumen as compared to *R. flavefaciens* and *R. albus*, gram- positive organisms. The populations of the three cellulolytic species at 4 h after feeding were in the ranges of 6.82-7.10, 8.47-8.95 and 9.44-9.77 log copies/g content, equivalent to $1.23x10^7$-$1.63x10^7$, $3.00x10^8$-$9.62x10^8$ and $2.88x10^9$-$5.92x10^9$ copies/g content for *F. succinogenes*, *R. flavefaciens* and *R. albus*, respectively (Table 7). Source of roughage seems to have effect on bacterial population. In cattle fed solely P/A as roughage source had significantly higher *F. succinogenes* and *R. flavefaciens* populations than that fed solely HAY as roughage source (9.77 and 8.95 vs 9.44 and 8.47 log copies/g content). Populations of total bacteria and *R. albus* did not change (P>0.05) in cattle fed different proportions of roughage source.

4. Discussion

HAY used in this experiment was brought from farmers around Petchaburi province. Crude protein content (3.08%) of HAY was relative low probably owing to farmers not properly applying fertilizer. However, HAY had the same quality as observed in other experiments such as these of Nitipot *et al.* (2008) and Chaorkuar (2009). They reported that pangola grass hay contained 3.37 and 3.31 %CP of DM, respectively. Moreover, the crude protein content varied from 3.9 to 11.6%CP of DM (Bryan and Sharpe, 1965). However, some studies reported that pangola hay cut at 45 days con-

Table 3. Effects of P/A and HAY on apparent digestibility and digestible nutrient intake of Southern Thai native cattle

Item[1]	P/A:HAY ratio				SEM	P- value
	100:0	65:35	35:65	0:100		
Apparent digestibility, %						
DM[2]	82.66[a]	79.14[ab]	76.30[ab]	74.54[b]	1.256	0.016
OM[2]	84.52[a]	80.94[ab]	78.42[ab]	76.73[b]	1.083	0.010
CP[2]	84.10	81.48	81.27	78.89	1.105	0.081
EE	94.68	92.00	92.91	92.09	0.837	0.188
NDF[2]	76.64	70.51	66.94	66.18	2.227	0.054
ADF[2]	68.84[a]	63.18[ab]	57.56[ab]	54.61[b]	2.414	0.023
Digestible nutrient intake, kg/d						
OM[2]	2.86	2.83	2.52	2.51	0.114	0.140
CP[2]	0.441	0.429	0.393	0.374	0.017	0.104
EE	0.213	0.213	0.199	0.200	0.009	0.565
NDF[2]	1.024	1.01	0.91	0.95	0.045	0.340
ADF[2]	0.588	0.575	0.478	0.468	0.030	0.054
Energy intake						
Mcal/d[2,3]	10.86	10.75	9.59	9.55	0.434	0.140
Mcal/kgDM[2]	2.96[a]	2.83[ab]	2.74[ab]	2.67[b]	0.038	0.009
Microbial crude protein synthesis[2,4], kg/d	0.371	0.368	0.328	0.327	0.015	0.140

[1] For explanations of the presentation of chemical composition of feed, see footnotes of Table 1.
[2] Linear effect, $P<0.05$
[3] ME intake (Mcal/d) = 3.8 x kg DOMI (Kearl, 1982)
[4] MCP (kg/d) = 0.130 x kg DOMI (ARC, 1980)
[a,b] Values within a row with a common superscript or no superscript are not significantly different ($P>0.05$)

Table 4. Effects of P/A and HAY on nitrogen balance of Southern Thai native cattle

Item	P/A:HAY ratio[2]				SEM	P- value
	100:0	65:35	35:65	0:100		
Nitrogen balance						
Intake, g/d	84.42	84.66	77.51	76.04	3.713	0.311
Excretion via						
Feces, g/d	13.92	15.98	14.70	16.27	1.349	0.604
Urine, g/d	0.65	0.74	1.96	0.51	0.425	0.158
Absorption[1], g/d	70.50	68.69	62.82	59.78	2.792	0.104
Retention[1], g/d	69.85	67.95	60.86	59.27	2.778	0.089

[1] Linear effect, $P<0.05$
[2] Values within a row with no superscript are not significantly different ($P>0.05$)

tained about 9-10% protein (Pitaksinsuk *et al.*, 2007 and Angthong *et al.*, 2006).

P/A used in this experiment contained crude protein close to the value (7.42%CP) reported by Sruamsiri (2007). In addition, Abdullah and Mat (2008) reported that solid pineapple waste silage had 5.18%CP of DM. Chandapillai and Selvarajah (1978) reported that solid pineapple waste silage had 6.10%CP of DM.

Dry matter intake of cattle weighing 286 kg fed dietary treatments in this study was low compared to the predicted DMI value of Southern Thai native cattle reported by WTSR (2008) or by Kearl (1982) (7.29 and 7.68 kg DM/d, respect-

Table 5. Effects of P/A and HAY on ruminal environment and blood metabolites of Southern Thai native cattle

Item	P/A:HAY ratio[2]				SEM	P- value
	100:0	65:35	35:65	0:100		
Ruminal environment						
Temperature, °C						
0 h post-feeding	37.63	38.03	37.85	38.05	0.181	0.396
2 h post-feeding	38.55	38.28	38.28	37.93	0.175	0.198
4 h post-feeding	38.35	38.60	38.48	38.55	0.270	0.918
Mean	38.18	38.30	38.20	38.18	0.147	0.917
pH						
0 h post-feeding	7.03	7.13	7.23	7.06	0.091	0.468
2 h post-feeding	6.69	6.87	6.89	6.80	0.108	0.585
4 h post-feeding	6.93	6.91	6.95	6.88	0.092	0.945
Mean	6.88	6.97	7.02	6.91	0.091	0.706
Ammonia-N, mg/dl						
0 h post-feeding	31.15	37.10	49.08	25.73	5.757	0.377
2 h post-feeding[1]	57.58	45.85	52.68	23.98	8.437	0.111
4 h post-feeding	17.33	15.23	20.80	11.90	5.113	0.676
Mean	35.35	32.73	37.85	20.53	4.501	0.123
Blood metabolites						
BUN[3], mg/dl						
0 h post-feeding	10.24	9.00	11.13	8.01	1.227	0.371
2 h post-feeding	10.07	11.25	11.58	9.16	0.839	0.254
4 h post-feeding	11.22	10.76	11.68	10.03	1.343	0.842
Mean	10.51	10.33	11.46	9.07	1.033	0.490

[1] Linear effect, P<0.05
[2] Values within a row with no superscript are not significantly different (P>0.05)
[3] BUN = blood urea nitrogen

ively). The high caloric density of diet fed to cattle was responsible for lowering DMI. Caloric density is one way for short-term control of intake in ruminants (Van Soest, 1994). Energy content of diet less than 2.0 Mcal ME/kgDM increases DMI while energy content more than 2.0 Mcal ME/kgDM decreases DMI. Intake by beef cattle fed high-concentrate ratio diets is likely controlled primarily by metabolic factors and not limited by bulk fill (Galyean and Defoor, 2003). By calculation based on energy content of concentrate (2.90 Mcal ME/kgDM), it was found that P/A contained 3.08 Mcal ME/kgDM, whereas HAY contained 2.24 Mcal ME/kgDM. This value of HAY was close to the value (2.12 Mcal ME/kgDM) previously reported by Pitaksinsuk *et al.* (2009).

Diet containing P/A as the sole roughage source showed higher digestibility of DM, OM, CP, NDF and ADF compared to that containing HAY as the sole roughage source (Table 3). P/A used as roughage source resulted in the diet having higher digestibility of DM and OM by 8.11% and 7.79%, respectively, compared with HAY. This means that using P/A as roughage source has a positive associative effect on digestibility. Reasons for increased digestibility with changes in roughage ratio and source are not understood fully. It is possible that fiber degradation in the rumen could have been impaired by a decrease in rumen retention time of feed particles (McCollum and Galyean, 1985). P/A fiber has shorter fibrous particles and is probably more digestible compared to HAY and may increase in the rate of passage of feed particles. Increased the rate of passage is concomitant with an increased ADF digestibility (Guthrie and Wagner, 1988).

In this study, NH_3-N concentration in rumen fluid was 35.35 mg/dl for P/A diet and 20.53 mg/dl for HAY diet, a value that was closer to the optimal ruminal NH_3-N concentration (15 to 30 mg/dl) for increasing microbial protein synthesis and feed digestibility (Perdok and Leng, 1990). Satter and Slyter (1974) have reported that ruminal NH_3-N concentration with ammonia is the main nitrogen source for growth and protein synthesis by ruminal bacteria to achieve maximum fermentation. For optimum rumen fermentation and microbial yield in cow, the ruminal NH_3-N should be between 10-25 mg/dl (Orskov, 1992). The values of nitrogen absorption and retention were also observed to be positive in cattle fed the

Table 6. Effects of P/A and HAY on volatile fatty acids in the rumen of Southern Thai native cattle

Item[1]	P/A:HAY ratio				SEM	P- value
	100:0	65:35	35:65	0:100		
Volatile fatty acids						
C2, mol/100mol						
0 h post-feeding	68.97	64.33	67.33	61.93	2.693	0.343
2 h post-feeding[2]	71.75[a]	68.04[a]	70.37[a]	61.76[b]	1.838	0.037
4 h post-feeding	65.86	63.16	68.80	62.43	4.880	0.772
Mean	68.86	66.11	69.47	62.04	2.606	0.268
C3, mol/100mol						
0 h post-feeding	23.28	22.14	15.76	22.00	2.57	0.260
2 h post-feeding	19.95	21.98	17.57	24.11	1.679	0.171
4 h post-feeding	22.35	24.43	16.80	23.13	3.533	0.488
Mean	21.83	22.16	16.29	23.08	2.288	0.246
C4, mol/100mol						
0 h post-feeding[2]	7.84	13.53	16.91	16.07	2.255	0.100
2 h post-feeding[2]	8.30	9.98	12.06	14.12	0.783	0.011
4 h post-feeding	11.79	12.41	14.39	14.45	1.569	0.537
Mean[2]	9.31[a]	11.73[b]	14.24[b]	14.88[b]	1.029	0.030
C2/C3						
0 h post-feeding	3.11	3.14	4.30	3.11	0.487	0.315
2 h post-feeding[2]	3.77	3.42	4.05	2.71	0.165	0.010
4 h post-feeding	3.18	2.78	4.14	2.93	0.667	0.513
Mean	3.35	3.26	4.31	2.92	0.391	0.174

[1] C2 = acetic acid, C3 = propionic acid, C4 = butyric acid

[2] Linear effect, P<0.05

[a,b] Values within a row with a common or no superscript are not significantly different (P>0.05)

Table 7. Effects of P/A and HAY on bacteria population in the rumen of Southern Thai native cattle by using real-time PCR

Item	P/A:HAY ratio				SEM	P- value
	100:0	65:35	35:65	0:100		
	Log copies/g content					
Total bacteria	10.00	10.19	9.99	10.09	0.093	0.463
F. succinogenes[1]	9.77[a]	9.75[a]	9.71[a]	9.44[b]	0.049	0.010
R. flavefaciens[1]	8.95[a]	8.71[a]	8.82[a]	8.47[b]	0.097	0.059
R. albus	7.06	7.00	7.10	6.82	0.168	0.667

[1] Linear effect, P<0.05

[a,b] Values within a row with a common or no superscript are not significantly different (P>0.05)

diets in this study.

With respect to ruminal pH, no significant difference due to roughage source was observed. The overall mean values for pH in rumen fluid was 6.88 for P/A diet, and 6.91 for HAY diet. Previous studies (Stewart, 1977; Russell and Dombrowski, 1980) demonstrated that when ruminal pH was above 6.0, cellulolytic enzymes and cellulolytic bacteria were not inhibited. The range of pH across dietary treatments and sampling times in the current study was 6.69 to 7.23, indicating that pH should not have affected microbial activities in the rumen.

In this study, P/A diet had lower butyric acid concentration ($P<0.05$) than other treatments. The result showed that the starch-fermenting bacteria, i.e., *F. succinogenes* and *R. flavefaciens,* including the fiber fermenting bacteria, *R. albus,* were high in P/A diet as compared to HAY diet which produced large amounts of acetic acid. The concentration of acetic acid was higher especially at 2 h post-feeding, whilst the concentration of butyric acid was lower. Consequently, the concentration of acetic acid decreased associated with increasing of butyric acid concentration at 4 h post-feeding because acetic acid is usually an intermediate in the synthesis of butyric acid (Hungate, 1966).

In present study, the result demonstrated that P/A was a more degradable roughage compared to HAY. Increasing the proportion of P/A in the diet resulted in improved nutrient digestibility except for ether extract. Moreover, the population of cellulolytic bacteria was increased when P/A was offered. It appears that feeding roughages in the form of silage did not exert a detrimental effect on rumen microbial activity as has been suggested to occur (Sniffen and Robinson 1987).

Dominant cellulolytic species found in this study were in accordance with Wanapat and Cherdthong (2009), who made similar observation in the swamp buffalo. Moreover, high populations of cellulolytic bacteria in this study were probably due to high ruminal pH in cattle fed either P/A or HAY diet. Fibrolytic activity and growth of these organisms are actively affected by high ruminal pH (Stewart, 1977). Chow and Russell (1992) found that *F. succinogens* had a high growth rate at high pH (6.5-7) with glucose available. This result is likely to support our observation. It is surprising that both *F. succinogenes* and *R. flavefaciens* are high in P/A diet as compared to HAY diet. Smaller and more degradable particles found in P/A diet may contribute to a higher population of both bacterial species. This observation was supported by higher NDF and ADF digestibility observed in this study.

In conclusion, P/A has been shown to be a feasible alternative to use as roughage source for Southern Thai native cattle. There are several benefits in terms of increasing caloric density, digestibility and feed utilization as compared to pangola hay. Moreover, it also enhances populations of dominant cellulolytic bacteria in the rumen.

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