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EFFECT OF *CENTELLA ASIATICA* POWDER (CAP) AND MANGOSTEEN PEEL POWDER (MPP) ON RUMEN FERMENTATION AND MICROBIAL POPULATION IN SWAMP BUFFALOES

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ABSTRCT

Four, rumen-fistulated swamp buffalo bulls were randomly assigned to receive dietary treatments according to a 4x4 Latin square design. Four treatments were as follows; un-supplementation (control); supplementation with *Centella asiatica* powder (CAP) at 25 g/kg; supplementation with Mangosteen peel power (MPP) at 25 g/kg; CAP at 25 g/kg and MPP at 25 g/kg (CAMP) of total dry matter intake (DMI). Animals were fed with concentrate at 30 g/kg BW. Rice straw, water and mineral salt block were offered ad libitum. The experiment was conducted for 4 periods, and each period lasted for 21 days, while the last 7 days was for sample collection. The results revealed that the proportion of ruminal acetic acid was decreased whereas propionic acid increased ($P<0.05$) by supplementation as compared to control group. Similarly, methane emission was lesser ($P<0.05$) in the CAP and CAMP as compared to control group. While, ruminal protozoal population was dramatically decreased ($P<0.05$) with the CAP and MPP supplementation; whilst, the CAMP treatment had a higher ($P<0.05$) fungal zoospore population when compared to the control group. Moreover, community of DNA was extracted from 0.5 g of rumen fluid and digesta by the repeated bead beating plus column (RBB+C) method, Real-time PCR amplification and detection were performed in a Chromo 4™ system (Bio-Rad, USA), the use of real-time PCR technique provided the data that the population of protozoa was reduced ($p<0.05$) by CAMP supplementation; whereas instead, the population of *F. succinogenes* were increased ($p<0.05$) by the CAP and CAMP supplementation. Dietary supplementation had no effect on total bacterial population, and *R. flavefaciens*, *R. albus*. In conclusion, CAP or MPP supplementation improved rumen fermentation by positively affecting the ruminal microbial population in swamp buffaloes fed on rice straw.

Keywords: *Centella asiatica* powder, mangosteen peel powder, rumen fermentation, methane production, real-time PCR, swamp buffaloes**,** rice straw.

INTRODUCTION

Enteric methanogenesis represents an energy loss to the ruminants by as much as 12% of the gross energy (GE) intake (Johnson and Johnson, 1995) due to, fermentation process, and is a major contributor to the global greenhouse gas (GHG) emissions. Cattle continue to be highlighted as the main contributors to the global enteric methane budget (Thorpe, 2009). Reducing methane production can be highly beneficial indicating efficient use of energy and feed by the animal (Beauchemin and McGinn, 2006). However, most successful mitigation options will be those that are persistent, resulting in profitable milk or meat production (Grainger *et al*., 2008). Various approaches have been reported in mitigating rumen methane emission but their practical use have not yet been clearly shown (Hook *et al*., 2009).

Asiatic Pennywort (*Centella asiatica (L.) urban*) is a stoloniferous perennial herb, commonly growing in humid areas in several tropical countries. It is used as a remedy for many diseases in the folk medicine of several countries (Mamedov, 2005). The chemical composition of asiatic pennywort consists of several groups; Triterpenoid saponins and Aglycones. It also contains other groups, including amino acids, flavonoids, alkaloids and volatile oils. However, Devkota *et al.* (2010) reported that the asiatic pennywort containing triterpene reduced the bacterial growth and inflammation. This herb may result in the degradation of carbohydrates (starch and sugar) in the rumen. Moreover, Dhar *et al*. (1968) found that alcoholic extract of entire plant showed antiprotozoal activity against Entamoeba histolytica. The essential oil from *C.asiatica* grown in South Africa contains 11 monoterpenoid hydrocarbons (20.2%), 9 oxygenated monoterpenoid (5.5%), 14 sesquiterpenoid hydrocarbons (68.8%), 5 oxygenated sesquiterpenoid (3.9%) and 1 sulphide sesquiterpenoid (0.8%). The predominant constitutes were b-caryophyllene (19.1%), bicyclogermacrene (11.2%), germacrene B (6.3%) and myrcene (6.6%) (Oyedeji and Afolayan, 2005).

Although a number of studies have reported on using plant secondary compounds on reducing rumen methanogenesis, but the results have been variable (Pilajun and Wanapat, 2011). Puchala *et al*., (2005) reported that feeding plants containing condensed tannin to ruminants reduces methane emission. Supplementation of pellets containing condensed tannins and saponins (mangosteen peel powder and soapberry fruit) influenced rumen ecology by reducing methanogen population and consequent methane emission (Poungchompu *et al*., 2009). In another investigation, Guo *et al*. (2008) reported that tannins reduced the methane emission by inhibiting protozoa as well as presumably lowering the methanogenic activity of protozoal-associated methanogens. Other investigation reported that supplementation of plant containing condensed tannin and saponin (mangosteen peel powder), although reduced protozoal population but had no effect on methane production (Ngamsaeng and Wanapat, 2006). However, information on the use of *Centella asiatica* and/or tropical plant (mangosteen peel powder or MPP) in ruminants has been limited. Therefore, objective of this study was to evaluate the effect of *Centella asiatica* powder (CAP) and Mangosteen peel powder (MPP) supplementation on microbial population, rumen VFA concentration and their diversity in the rumen of swamp buffalo.

MATERIALS AND METHODS

Animals, diets, experimental design, and animal management: Four, rumen-fistulated swamp buffalo (*B. bubalis*) bulls, age 4-year-old, weighing 350± 30 kg BW were used in this experiment. All animals were housed in individual pens. Concentrate was fed at 3 g/kg BW twice a day (at 07:00 h and at 16:30 h). Rice straw, water and mineral salt block were offered *ad libitum*. The experiment was a 4x4 Latin square design. Bulls were randomly assigned to four dietary treatments: without supplementation (Control), supplementation with *Centella asiatica* powder (CAP) at 25 g/kg of total DMI, with Mangosteen peel powder (MPP) at 25 g/kg of total DMI, and with *Centella asiatica* powder (CAP) at 25 g/kg and Mangosteen peel powder (MPP) at 25 g/kg of total DMI. Feed ingredients and chemical composition are presented in Table 1. The experiment was conducted for four periods; each experimental period lasted for 21 days. First 14 days were used as adaptation period in which, all animals were fed with their respective diets followed by a 7-day collection period. During the collection period, bulls were kept in metabolism crates for the daily feces and urine collection.

Data collection, sampling procedures, and statistical analysis: Roughage and concentrate were sampled for feeds analysis daily during the collection period. Feed and fecal samples were collected each day during a 7-day collection period. Composited samples were dried at 60°C in a forced-air oven for 48 h and ground to 1-mm screen using Cyclotech Mill (Tecator, Sweden) to determine DM, OM, CP according to AOAC (1990), NDF and ADF according to Van Soest *et al*. (1991). During the final day of collection period, rumen fluid and blood samples were collected at 0, 2, 4, and 6-h post-feeding. Rumen fluid pH was immediately measured for pH (HANNA Instrument HI 8424 microcomputer, Singapore), and then divided into four aliquots. A subsample was mixed with 5 ml of 1 M H_2SO_4 to stop fermentation process of microbial activity and centrifuged at 16,000 x g for 15 minute. About 20-30 ml of supernatant were collected and frozen at –20 °C until analyzed in the laboratory and to determine NH_3-N analysis using Kjeldahl methods (AOAC, 1990); whereas, rumen volatile fatty acid (VFA) were determined using high-pressure liquid chromatography (HPLC, Instruments by Water and Novapak model 600E; water mode l484 UV detector; column novapak C18; column size 3.9 mm × 300 mm; mobile phase10 mM H2PO4 [pH 2.5]) according to the method of Samuel *et al*. (1997). Remaining aliquots were determined for direct count of protozoa, and fungal zoospores according to the methods described by Galyean (1989) using a haemocytometer (Boeco, Singapore), and three groups of bacteria (cellulolytic, proteolytic, and amylolytic bacteria) were determined using roll-tube technique (Hungate, 1969).

A 10 ml blood sample was drawn from the jugular vein, plasma was harvested by centrifugation at 5,000×g for 10 min and store at −20°C until further analysis. Plasma urea nitrogen (BUN) was determined according to the method of Crocker (1967).

Rumen microbial population analysis: Community DNA was extracted from 0.5 g of rumen fluid and digesta by the repeated bead beating plus column (RBB+C) method (Yu and Morrison, 2004), which was shown to significantly increased in DNA yields. The quality and quantity of these DNA samples were also determined by agarose gel electrophoresis and spectrophotometry.

To establish a quantitative assay, the target 16s rDNA of each species was amplified using specific primers; the purified DNA was quantified by spectrophotometer with multiple dilutions. The target DNA was quantified by using serial ten-fold dilutions from 10^3 to 10^9 DNA copies of the previously quantified DNA standards. Real-time PCR amplification and detection were performed in a Chromo 4™ system (Bio-Rad, USA). All primer set sequences (forward and reverse primers) are shown in Table 6.

Statistical analysis: Data were analyzed according to a 4x4 Latin square design using Proc GLM/Proc Mix (SAS, 1996). Data were analyzed using the model

Yijk=μ+Mi+Aj+Pk+εijk where Yijk is the observation from animal j, receiving diet i, in period k; μ, the overall mean; Mi, effect of treatment (i=1 to 4); Aj, the effect of animal (j=1 to 4); P_k, the effect of period (k=1 to 4); and ε_{ijk}, the residual effect. Treatment means were statistically compared by Duncan's New Multiple Range Test (Steel *et al.*, 1997). Differences among means with P<0.05 were accepted as representing statistically significant differences.

RESULTS

Feed intake and nutrient digestibility: Feed intake and nutrient digestibility were affected by the CAP and/or MPP supplementation (Table 2). Total feed intake, intake of rice straw and digestibility of DM, OM, and CP were not affected (P>0.05) by feed supplementation. However, fiber digestibility (NDF, ADF) was increased (P<0.05) after supplementation. CAMP supplementation resulted in higher NDF and ADF digestibility as compared to the control treatment (P<0.05).

Rumen fermentation and blood metabolites: Table 3 shows the effect of CAP, MPP and CAMP supplementation on blood urea nitrogen (**BUN**), pH and temperature in swamp buffalo which were not statistically different among treatments (P>0.05). Whereas, NH_3-N was increased (P<0.05) by CAMP supplemented when compared to control group.

Total rumen VFA concentration and butyric acid were not influenced by dietary treatments (P>0.05; Table 3). The concentration of acetic acid was decreased whereas propionic acid was increased (P<0.05) by the supplementation compared to control group. Furthermore, CH4 was decreased (P<0.05) as result of supplemented group compared to the control group.

Table 1. Feed ingredient and chemical compositions of concentrate, *Centella asiatica* powder, mangosteen peel powder and rice straw

Item	Experimental diets			
	Con-Centrate	CAP	MPP	RS
Ingradients				
Cassava chip	64.9			
Rice bran	13.6			
Brewer's grain	5.9			
Palm kernel meal	8.3			
Urea	3			
Molasses	1.5			
Salt	0.5			
Sulfur	0.3			
Tallow	1			
Mineral mix	1			
Chemical composition				
Dry matter, %	91.2	88.5	93.1	90.5
		dry matter		
Organic matter	92.5	80.7	96.3	88.9
Crude protein	14.1	12.4	15.3	2.7
Neutral detergent fiber	28.9	33.0	60.6	84.1
Acid detergent fiber	11.5	25.3	55.9	44.9
Condensed tannin	-	12.1	17.7	-
Saponins	-	11.2	12.3	-

MPP, mangosteen peel powder; CAP, *Centella asiatica* powder; RS, rice straw

Table 2. Effect of *Centella asiatica* powder and mangosteen peel powder supplementation on voluntary feed intake and nutrient digestibility

Items	Control	CAP	MPP	CAMP	SEM	P-Values
Total DM intake,						
Kg	5.8	5.9	5.8	5.8	0.11	0.84
%BW	1.7	1.7	1.7	1.7	0.23	0.50
g $kg^{-1}BW^{0.75}$	71.8	73.2	73.9	72.4	1.21	0.65
Rice straw intake,						
Kg	4.8	4.9	4.8	4.8	0.10	0.83
%BW	1.4	1.4	1.4	1.4	0.03	0.50
g $kg^{-1}BW^{0.75}$	58.8	60.2	61.0	59.4	1.21	0.63
Apparent digestibility, %						
Dry matter	60.3	63.8	63.6	65.3	1.76	0.33
Organic matter	64.5	67.7	67.0	68.7	1.61	0.37
Crude protein	67.1	68.9	65.7	67.6	3.53	0.93
Neutral detergent fiber	42.3^{a}	49.2^{b}	50.2^{bc}	53.2^{c}	1.12	0.001
Acid detergent fiber	40.3^{a}	46.6^{b}	47.8^{b}	51.1^{c}	0.48	<0.0001

[a, b, c,] Means in the same row with different superscripts differed (P<0.05)

CAP, *Centella asiatica* powder; MPP, mangosteen peel powder; CAMP, combination of CAP and MPP; SEM, standard error of the means

Rumen microorganism population: As shown in Table 4, the protozoal population was decreased (P<0.05) in the supplemented group compared to the control group. Supplementation with CAMP resulted in a higher

zoospore population when compared to the control treatment. Populations of total bacteria and amylolytic bacteria were not affected by treatment supplementation (P>0.05). Supplementation with MPP resulted in a higher population of cellulolytic bacteria compared to the control, CAP and CAMP treatments (P<0.05). In contrast, the CAMP fed buffaloes resulted in a higher population of proteolytic bacteria compared with the control treatments (P<0.05). Results of microbial population estimated by real-time PCR are presented in Table 4. It was found that *F. succinogenes* was increased (P<0.05) in the CAP and CAMP supplementation compared with the control group. In contrast, CAMP supplementations reduced populations of protozoa (P<0.05) compared to control group. Dietary supplementation did not affect on total bacteria, *R. flavefaciens*, or *R. albus* abundances (P>0.05).

Nitrogen utilization and efficiency of microbial protein synthesis: Nitrogen intake was higher in buffalo supplemented with CAMP when compared with control (P<0.05). Nitrogen absorption and retention were not different among treatments (P>0.05). Microbial protein synthesis in terms of both quantity and efficiency were not affected by either CAP or MPP supplementation (P>0.05; Table 5).

Table 3. Effect of *Centella asiatica* powder and mangosteen peel powder supplementation on blood urea nitrogen, ruminal pH, temperature, NH_3-N concentration and volatile fatty acids (VFA)

Items	Control	CAP	MPP	CAMP	SEM	P-Values
Ruminal pH	6.5	6.5	6.6	6.6	0.03	0.87
Temperature	38.5	38.6	38.6	38.6	0.09	0.98
NH_3-N, mg/dl	8.8^{a}	9.5^{b}	9.8^{b}	9.9^{c}	0.29	0.004
BUN, mg /dl	8.3	8.6	8.2	8.4	0.40	0.88
Total VFA, mmol/l	128.4	122.3	123.8	130.1	3.24	0.36
mol/100mol total VFA						
Acetic acid	68.2^{a}	64.1^{b}	66.2^{ab}	62.2^{b}	1.10	0.04
Propionic acid	22.0^{a}	26.1^{b}	25.5^{b}	28.6^{b}	1.02	0.02
Butyric acid	9.8	9.8	8.3	9.1	0.65	0.41
CH4 production^{A}	28.6^{a}	25.6^{b}	26.1^{ab}	23.8^{b}	0.73	0.02

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

CAP, *Centella asiatica* powder; MPP, mangosteen peel powder; CAMP, combination of CAP and MPP; SEM, standard error of the means

^{A}Calculated according to Moss *et al*. (2000) CH4 production = 0.45 (acetate)-0.275 (propionate)+0.4 (butyrate).

Table 4. Effect of *Centella asiatica* powder and mangosteen peel powder supplementation on microbial population

Items	Control	CAP	MPP	CAMP	SEM	P-Values
Direct count technique, cell/ml						
Protozoa, $\times10^5$	3.6^{a}	2.4^{ab}	2.3^{ab}	1.4^{b}	0.42	0.05
Fungal zoospore, $\times10^6$	5.4	5.4	5.4	5.5	0.03	0.23
Grouping of bacteria, CFU/ ml						
Total viable bacteria, x 10^8	5.5	4.5	62.3	5.7	0.67	0.40
Cellulolytic bacteria, x 10^8	3.5^{a}	3.7^{a}	6.2^{b}	4.2^{a}	0.57	0.05
Proteolytic bacteria, x 10^7	4.3^{a}	5.3^{ab}	4.9^{ab}	6.6^{b}	0.56	0.05
Amylolytic bacteria, x 10^7	6.3	8.2	8.1	6.4	1.07	0.48
Real-time PCR technique,						
Copies/ml of rumen content						
Total bacteria, $\times10^9$	3.88	4.07	3.45	3.76	0.38	0.72
Protozoa, $\times10^6$	2.67^{a}	2.19^{ab}	2.20^{ab}	1.92^{b}	0.17	0.05
F.*succinogenes*, $\times10^6$	2.61^{a}	2.94^{b}	2.77^{ab}	2.91^{b}	0.08	0.07
R.*flavefaciens*, $\times10^6$	1.96	2.04	2.12	2.06	0.16	0.91
R.*albus*, $\times10^6$	1.73	1.84	1.90	1.73	0.05	0.12

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

CAP, *Centella asiatica* powder; MPP, mangosteen peel powder; CAMP, combination of CAP and MPP; SEM, standard error of the means

Table 5. Effect of *Centella asiatica* powder and mangosteen peel powder supplementation on nitrogen balance and microbial protein synthesis

Items	Control	CAP	MPP	CAMP	SEM	P-Values
Nitrogen balance, g/d						
Nitrogen intake	46.6[a]	49.5[ab]	48.9[a]	52.2[b]	0.82	0.02
Fecal nitrogen	23.4	23.7	25.0	25.2	1.96	0.87
Urinal nitrogen	10.6	11.2	10.4	11.4	1.31	0.93
Nitrogen absorbed	23.2	25.8	23.9	26.9	1.46	0.34
Nitrogen retained	12.7	14.6	13.6	15.6	2.48	0.86
Microbial protein synthesis						
PD excreted, mmol/d	25.5	27.2	26.1	27.2	0.64	0.26
PD absroped, mmol/d	77.6	90.9	85.5	92.6	5.29	0.28
Microbial nitrogen supply, gN/d	56.4	66.1	62.2	67.3	3.85	0.28
EMPS, gN/kg OMDR	25.8	29.6	29.1	30.8	2.08	0.44

[a, b, c,] Means in the same row with different superscripts differed (P<0.05)
CAP, *Centella asiatica* powder; MPP, mangosteen peel powder; CAMP, combination of CAP and MPP; SEM, standard error of the means
PD, purine derivative; EMPS, efficiency of microbial protein synthesis; OMDR, organic matter digestible in the rumen

Table 6. PCR primer sets for real-time PCR

Target species	Primer sequence
Total bacteria[A]	5′-CGGCAACGAGCGCAACCC-3′
	5′ CCATTGTAGCACG TGTGTA GCC-3′
Protozoa[C]	5'-GCTTTCGWTGGTAGTGT TT-3'
	5'-ACTTGCCCTCYAATCGTWCT-3'
F.succinogenes[A]	5′-GTTCGGAATTACTGGGCGTAAA-3′
	5′-CGCCTGCCCCTGAACTATC-3′
R.flavafaciens[A]	5′-CGAACGGAGATAATTTGAGTTTACTTAGG-3′
	5′-CGGTCTCTGTATGTTATGAGGTATTACC-3′
R.albus[B]	5′-CCCTAAAAGCAGTCTTAGTTCG-3′
	5′-CCTCCTTGCGGTTAGAACA-3′

[A] Denman and McSweeney (2006)
[B] Koike and Kobayashi (2001)
[C] Sylvester *et al*. (2004)

DISCUSSION

Feed intake and nutrient digestibility: Although feed intake was not affected by feed supplementation, fiber digestion was changed by either CAMP or control supplementation. Beauchemin *et al.* (2007) reported that adding 2% quebracho tannin extract to the diet had no effect on either DM or NDF digestibility in cattle. However, other authors have found that feeding high levels of dietary saponins and/or tannins decreased apparent digestibility (Poungchompu *et al*., 2009). The present study may suggest that selective suppression of cellulolytic bacteria by saponins and tannins did occur as reported by McSweeney *et al*. (2001a). Moreover, this variable effect could be due to the type and concentration of saponins and tannins containing in the plants. The mangosteen peel powder used in this study contained 17.7%, and 12.3% DM of condensed tannins and saponins, respectively, and was similar to the study of Kanpukdee and Wanapat (2008).

Rumen fermentation and blood metabolites: There were no effects of *Centella asiatica* powder and mangosteen peel powder supplementation on ruminal pH and temperature (P>0.05). However, ruminal pH and temperature were in the normal range at 6.5-6.6 and 38.5-38.6^{o}C, respectively. Ruminal NH_3-N and BUN values, as influenced by supplementation group, were in normal ranges, in which these values were reported for optimal microbial digestion of fiber and protein (Wanapat, 1990). According to numerous reports, the optimal level of ruminal ammonia nitrogen concentration for efficient digestion is from 5.0 to 25.0 mg/dl (Preston and Leng, 1990) and 15 to 30 mg/dl (Wanapat and Pimpa, 1999). Changes in the dietary factors will alter NH_3 concentration in the rumen (Ørskov, 1982). Preston (1996) suggested that the quantity of ammonia absorbed from the rumen was reflected in circulating BUN. Earlier studies (Poungchompu *et al*., 2009; Pilajun and Wanapa, 2011) found that NH_3-N concentration in the rumen of swamp buffalo were affected by MPP supplementation, which was similar to the data found in the present study. Moreover, Pilajun and Wanapat (2011) stated that 8% supplementation of coconut oil mixed with MPP decreased NH_3-N concentration by depressing of protein degradation. Bach *et al*. (2005) reported that an elevated concentration of blood urea–nitrogen could affect from ruminal ammonia–nitrogen concentration because up to 50% of ammonia–nitrogen produced from rumen fermentation was absorbed into the blood via the rumen wall and influenced on urea–nitrogen concentration. Although concentration of ammonia–nitrogen is directly

affected by protein degradation, it also can fluctuate by assimilation of rumen microbes (Atasoglu *et al*., 1998).

Although contradictory findings can be found in the literature, it has been reported that rumen NH_3-N concentration was not affected by any of the followings: dietary condensed tannin intake (Perez-Maldonado and Norton, 1996), Mangosteen peel powder (Kanpukdee and Wanapat, 2008), and soapberry fruit-mangosteen peel pellet (Poungchompu *et al*., 2009). However, addition of ethanol extract from soapnut (Sapindus mukorossi) onto *in vitro* rumen fermentation of feed in buffalo rumen liquor decreased NH_3-N concentration (Kamra *et al*., 2006). Moreover, Grobner *et al*. (1982) found a reduction of ammonia concentration when saponins were included at 60 mg/kg in the incubation medium. Condensed tannin in the diet has beneficial effects which are mediated by protein-tannin complexation, decreasing availability of feed protein for ruminal degradation and ammonia nitrogen release (Makkar, 2003).

There were no significant differences (P>0.05) on TVFAs production and proportions of butyric acid among treatments. The concentration of propionic acid was significantly increased, while proportion of acetic acid was decreased by supplementation with saponin rich tropical fruit (Poungchompu *et al*., 2009). This finding agrees with previous *in vitro* study (Norapoke *et al*., unpublished) in which CAP and MPP supplementation decreased ruminal acetic acid proportion, whilst increased propionic acid proportion. It is likely that in studies with defaunation resulted in increased propionate concentration (like the present experiment), the defaunating agent inhibited bacteria and protozoa that are not involved in propionate production, thus indirectly promoted the growth of species such as *S. ruminantium* that are central to propionic acid production in the rumen. However, Macheboeuf *et al*. (2008) reported that dose-response effects of different essential oil on methane inhibition and VFA production. However, Ngamsaeng *et al*. (2006) found no significant effects of MPP feeding level on either total VFA or individual VFA concentration. Moreover, cattle fed on forage-based diet supplemented with quebracho tannin extract did not exhibit a changed proportion of propionic acid, although acetic acid percentage was linearly decreased (Beauchemin *et al*., 2007). As reported by Ørskov (1987) that propionate was an essential glucogenic compound and synthezed via gluconeogenesis in the liver of the ruminants. The expected shift in the VFA profile from acetate to propionate was associated with an increased fiber digestion.

Calculated methane production by using volatile fatty acid as a variable and was different among treatments. Moreover, Moss *et al*. (2000) reported that prediction of methane production by using proportion of volatile fatty acid could elucidate the changing of hydrogen itinerary in rumen ecology. Supplementation with CAMP reduced estimated CH_4 production which is in agreement with our previous *in vitro* study (Norapoke *et al*. unpublished) and other study (Kongmun *et al*., 2009). The results were also in agreement with Beauchemin *et al*. (2007) who also failed to reduce enteric methane emission from growing cattle by feeding the dietary DM as quebracho tannin extract. In addition, methane emission was decreased by mangosteen peel and soapberry fruit pellet supplementation (Poungchompu *et al*., 2009) in a dairy heifer diet. McAllister *et al*. (2005) reported that condensed tannins extracted from different plants vary greatly in their capacity to bind carbohydrates and proteins. Thus, it is possible that MPP tannin is a less effective methane suppressant than other condensed tannin sources. Nevertheless, the methane suppressing-effects of saponins were presumably a direct action against rumen microbes involved in methane formation including methanogens and protozoa (Sliwinski *et al*., 2002b). Tavendale *et al*. (2005) suggested two mechanisms whereby condensed tannins reduced methane emission from ruminants: indirectly through a reduction in fiber digestion, which decreases hydrogen production, and directly through an inhibition of the growth of methanogens. Previous studies have reported that feeding condensed tannin-containing plants to ruminants reduced methane emission (Puchala *et al*., 2005). Moreover, Guo *et al*. (2008) concluded that saponins appeared to reduce methane production by inhibiting protozoa and presumably lowering methanogenic activity of protozoal-associated methanogens. The lack of methane reduction in the present study could be due to either plant species and/or the quantity that the animals received; likewise, the calculated methane production may not be accurate due to the tannins and saponins having a direct toxic effect on methanogens as mention before.

Rumen microorganism population: Ruminal protozoa count was reduced when CAMP was supplemented at 50 g/kg of dry matter intake. The general mode of action of tannins and saponins on microorganisms is their interaction with the sterol moiety, which is present in the membrane of protozoa (Ando *et al*., 2003). In addition, ruminal protozoa counts were reduced through the addition of either CAP or MPP in agreement with earlier in vivo study (Kongmun *et al*., 2009). On the other hand, Poungchompu *et al*. (2009) found that populations of protozoa and fungi were dramatically decreased when dairy heifers were fed with plants containing condensed tannins and saponins. Moreover, the ethanol extract of soapnut (Sapindus mukorossi) seed pulp completely inhibited *in vitro* methane emission along with a significant reduction in protozoa count (Agarwal *et al*., 2006). The sensitivity of protozoa towards plant secondary compounds may be explained by the presence of sterols in cell membranes (Newbold *et al*., 1997).

However, as suggested by Williams and Coleman (1992), the effect of defaunation on propionate concentration is likely not to be a direct effect of decreasing protozoal counts, but an indirect effect of the defaunating agent on ruminal bacteria.

The population of fungal zoospores was not affected by CAP, MPP or CAMP supplementation. In contrast, the number of fungal zoospores was decreased by soapberry fruit-mangosteen peel pellet supplementation in dairy heifers (Poungchompu *et al*., 2009). Protozoa and methanogens interact with each other, including extracellular adhesion and intracellular symbiosis (Ushida and Jouany, 1996), whether inside or outside of protozoa. Methanogens serve as scavengers removing the hydrogen produced by protozoa and ruminal fungi have been related to fiber digestion (Bauchop, 1979) which leads to hydrogen production for methanogenesis. Thus, reduction of the protozoal population could be a cause of the decreasing fungal population due to the lack of an electron sink.

A previous *in vitro* study (Norapoke *et al*., unpublished) found that numbers of total bacteria were increased by MPP supplementation. However, that was not shown in the present study. This could be due to the differences in methodology used to determine total bacteria population. The roll-tube technique used in this study was able to investigate only bacteria that can grow in *in vitro*. Proteolytic bacteria was increased by CAMP supplementation which may be related to the increase of NH_3-N concentration in the rumen. Hungate (1966) reported that microbes in the rumen, especially bacteria, are the primary influence on feed digestion of host ruminants. Although either CAP or CAMP supplementation by themselves did not affect on cellulolytic bacteria, a combination of CAP and MPP could improve digestibility of swamp buffalo. However, McSweeney *et al*. (2001a) found that cellulolytic bacteria in the rumen of sheep was depressed by the condensed tannins presented in calliandra (Calliandra calothyrsus). A reduction in the cellulolytic bacteria population could be explained by direct inhibition of the micro-organisms through tannin interactions with the cell wall and secreted catabolic enzymes (Jones *et al*., 1994), and/or by reduced substrate availability due to complexing of tannin with nutrients. The absence of any effect in this study may be due to adaptation of the microbes by alteration in the feeds (Makkar, 2003). Similar to *in vitro* studies, the results have also demonstrated that some rumen bacteria have developed mechanisms to prevent these specific interactions (tannin tolerance) (Jones *et al*., 1994). As the results showed, the population of protozoa was lowest in CAMP supplementation, while total bacteria, *R.flavefaciens* and *R.albus* were not affected. Similarly to Poungchompu *et al*. (2009) who reported that MPP and soapberry fruit remarkably reduced rumen protozoas. Moreover, plants containing tannins could reduce protozoas as reported by Bhatta *et al*. (2009). In addition, this study agreed with Chen and Weimer (2001) who reported that the most predominant species of cellulolytic bacteria in the rumen was *F. succinogenes*.

N utilization and efficiency of microbial protein synthesis: Effect of CAP and MPP in swamp buffaloes on N utilization is shown differently among treatments in terms of N intake, while no differences were found on N urine, N feces, N absorption, N retention and total N excretion (Table 5). Although microbial populations were changed by feed supplementation, nitrogen balance and microbial protein synthesis were not different among treatments. McSweeney *et al*. (2001a) also reported that supplementation with the tropical forage calliandra which is rich in condensed tannins did not change microbial protein synthesis in the rumen of sheep. Similar results were reported by McNeill *et al*. (1998) who found that microbial flow from the rumen was not inhibited by tannins in *Leucaena leucocephala* (7.3% condensed tannin). However, Makkar *et al*. (1995d) has shown *in vitro* that tannins can either reduce or increase the efficiency of microbial protein synthesis. It may be the reason that the *in vivo* situation can not mimick or the effects are too small to be detected in animal studies. In addition, the absence of an effect of feed addition on microbial protein synthesis may have resulted from higher recycling of plasma PD than in cattle (Pimpa *et al*., 2003) which is one of the nitrogen conservation systems of ruminants.

Conclusions: Supplementation of CAMP resulted in increased propionic acid concentration. The protozoal population was dramatically decreased with dietary supplementation, while fungal zoospore numbers and proteolytic bacteria were also increased when CAMP was supplemented. CAP and MPP have specific effect on rumen fermentation however; combination of the sources significantly further improved rumen fermentation by enhancing on microbial fermentation, population and nutrient digestibilities while mitigating rumen methane emission in the rumen of swamp buffalo when fed with rice straw.

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