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Influence of tropical plant sources containing plant secondary compound on rumen fermentation using *in vitro* gas fermentation technique

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ABSTRACT

The objective of this study was to investigate the effect of mangosteen peel powder (MPP) and *Centella asiatica* powder (CAP) supplementation on gas production kinetics and fermentation efficiency using *in vitro* gas production technique. Two male, rumen fistulated swamp buffaloes were used as rumen fluid donors. The treatments were arranged according to a 3×3 factorial arrangement in a completely randomized design using 3 levels of CAP supplementation (0, 5, 10 mg) and 3 levels of MPP supplementation (0, 5, 10 mg). Untreated rice straw was used as a main roughage source. Under this investigation, the results revealed that supplementation of CAP and MPP showed an effect on gas production kinetics, except for the gas production from the immediately soluble fraction (a), while treatments with combination of CAP and MPP at 5 mg, each resulted in the highest values. Supplementation of either CAP or MPP up to 10 mg reduced the gas production kinetics. In addition, *in vitro* degradability of DM (IVDMD) and OM (IVOMD) were not affected by CAP or MPP supplementation; however, there was a tendency increase on IVDMD by CAP and MPP supplementation (P=0.08). CAP and MPP supplementation reduced total volatile fatty acid and acetic acid while propionic acid and butyric acid were enhanced especially in treatment combination of CAP and MPP supplementation. Methane production were decreased with increasing level of CAP and MPP supplementation both in combination and separately. On the other hand, ammonia nitrogen (NH_3-N) concentration was not influenced by CAP and/or MPP supplementation; except at 4 h incubation, while an increasing of NH_3-N concentration was obtained by dietary supplementation. Based on the present findings, it could be concluded that supplementation of CAP and MPP and/or combination could increase gas production kinetics, IVDMD and propionic acid while methane production was suppressed. It is recommended that level of CAP and MPP supplementation was at 5 mg and the combination at ratio of 5 to 5 mg, CAP and MPP. However, *in vivo* trials should be further conducted to elucidate the effect of CAP and MPP supplementation on rumen ecology as well as ruminant production.

Key words: *Centella asiatica* powder, Methane production, Mangosteen peel powder, Rumen fermentation

Effect of, methane a greenhouse gas, is estimated to be 25 times that of CO_2 based on equal molar amounts. Ruminant animals, one of the largest sources of methane emission, produce 81–92 million tonnes/year globally, which is equivalent to 23–27% of total anthropogenic methan (IPCC 2007). Methane produced during ruminal fermentation represents a loss of 2–15% of gross energy intake and thus decreases the potential conversion of digesta to metabolisable energy (Giger-Reverdin and Sauvant 2000).

Asiatic pennywort *(Centella asiatica* (L.) urban), a stoloniferous perennial herb, commonly growing in humid areas in several tropical countries, is used as a etno-veterinary medicine for many diseases (Shrestha and Dhillion 2003, Mamedov 2005). The chemical composition of asiatic pennywort consists of triterpenoid saponins and Aglycones. It also contains amino acids, flavonoids, alkaloids, volatile oils. However, Devkota *et al.* (2010) found that asiatic pennywort contains the triterpene in which substances in this group will result in suspension of the growth of bacteria, reducing inflammation. The essential oil from *C.asiatica* grown in South Africa contains 11 monoterpenoid hydrocarbons (20.2%), 9 oxygenated monoterpenoid (5.46%), 14 sesquiterpenoid hydrocarbons (68.8%), 5 oxygenated sesquiterpenoid (3.9%) and 1 sulphide sesquiterpenoid (0.76%). The predominant constitutes were β-caryophyllene (19.08%), bicyclogermacrene (11.22%), germacrene B (6.29%) and myrcene (6.55%) (Oyedeji and Afolayan 2005).

There are reports of decreased methane emission by ruminants consuming plant secondary compounds. Feeding

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condensed tannin-containing plants to ruminants reduces methane emissions (Carulla *et al.* 2005, Puchala *et al.* 2005). Supplementation of pellets containing condensed tannins and saponins (mangosteen peel powder and soapberry fruit) influenced rumen ecology by significantly lowering methane concentration in rumen atmosphere and reduced methanogen population (Poungchompu *et al.* 2009). Similarly, Guo *et al.* (2008) concluded that tannin affect by inhibiting protozoa and presumably lowering methanogenic activity of protozoal-associated methanogens. However, although protozoal population were decreased but calculated methane production did not affect with supplementation of plant-containing condensed tannin and saponin (mangosteen peel powder) (Ngamsaeng *et al.* 2006).

Currently, researchers try to reduce methane production in the rumen by using feed additives to inhibit methanogenesis. Meanwhile, plants produce a diverse array of plant secondary metabolites to protect against microbial and insects attacks (Wallace 2004). These natural plant eco-chemicals such as essential oils (EO), saponins, tannins and organosulphur compounds have been shown to selectively modulate the rumen microbial populations (Wallace 2004, Patra and Saxena 2009a), resulting in an improvement of rumen fermentation and nitrogen metabolism, and a decrease in methane production and nutritional stress such as bloat or acidosis, thus improving the productivity and health of animals (Kamra *et al.* 2006, Rochfort *et al.* 2008). Several studies discussed the potential of plant bioactives as modifiers of rumen microbial fermentation and ruminant production (Wallace 2004, Hart *et al.* 2008, Calsamiglia *et al.* 2007, Patra and Saxena 2009b). Thus, the objective of the present study was to investigate effect of MPP and CAP supplementation on fermentation end-products in *in vitro* gas production technique using rumen fluid of cattle.

MATERIALS AND METHODS

Mangosteen peel and *Centella asiatica* were collected around region, and sun-dried for 2 days before oven-drying at 60°C for 2 days. Dried mangosteen peel and *Centella asiatica* were then ground pass through a 1-mm screen into powder form. Rice straw was used as main roughage source. The chemical composition of MPP, CAP and rice straw are given in Table 1. Two rumen fistulated swamp buffaloes were used as sources of rumen inoculum. The animals were fed rice straw base. The animals were placed on a routine for at least 21 days before sampling for rumen fluid where they were fed twice a day with water freely available and with access to a mineral block lick A 3×3 factorial arrangement in a completely randomized design (CRD), with 3 replications per treatment including triplicates of blank (medium only) in 3 incubation runs was used to evaluate effect of MPP and CAP supplementation on gas production and fermentation efficiency in buffalo rumen fluid in *in vitro* gas production system. The dietary treatments were combination of 3 CAP levels (0, 5, 10 mg) and 3 MPP levels (0, 5, 10 mg) supplementation to dietary substrate rice straw based. There were 9 dietary treatments combination and were as follows:

Table 1. Chemical composition of rice straw, mangosteen peel powder and *Centella asiatica* powder used in the experiment

Item	Rice straw	MPP[1]	CAP[2]
Chemical composition			
DM,%	90.6	93.1	88.5
	……..% of DM ……		
OM	86.5	96.4	87.2
CP	3	21.2	16.7
NDF	85.6	56.3	37.79
ADF	53.2	52.1	24.26
Condensed tannin	-	17.7	12.11

[1]MPP, mangosteen peel powder; [2]CAP, *Centella asiatica* powder

T_1, no-supplementation; T_2, supplementation of CAP at 5 mg; T_3, supplementation of CAP at 10 mg; T_4, supplementation of MPP at 5 mg; T_5, supplementation of MPP at 10 mg; T_6, supplementation of MPP at 5 mg and CAP at 5 mg; T_7, supplementation of MPP at 5 mg and CAP at 10 mg; T_8, supplementation of MPP at 10 mg and CAP at 5 mg; T_9, supplementation of MPP at 10 mg and CAP at 10 mg.

The method used for *in vitro* fermentation based on the technique described by Menke *et al.* (1979). Dietary substrate (200 mg) were weighed into 60 ml bottle. Buffered mineral solution was prepared and placed on a magnetic stirrer at 39°C under continuous flushing with CO_2. Rumen fluid was collected before the morning feeding from 2 ruminally fistulated cattle fed with rice straw as roughage. Rumen fluid was taken from the rumen and transferred into pre-warmed thermos flasks, then filtered through one-layer of cheesecloth and flushed with CO_2. Preparation of artificial saliva was done according to Menke and Steingass (1988). The artificial saliva and rumen fluid was mixed in a 2:1 ratio to a serum inoculums mixed. The serum bottles with the mixture of substrate treatments were pre-warmed in an incubator at 39°C for 1 h; 30 ml of rumen inoculums mixed were taken into bottle containing the feed samples. The bottles were placed in an incubator at 39°C for fermenting.

Gas production kinetics: During the incubation, the gas production kinetics were recorded at 0, 1.5, 3, 6, 9, 12, 24, 36, 48, 72 and 96 h. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follows:

$$Y = a + b\,(1\text{-}e^{(-ct)})$$

where a, the gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction (b); t, incubation time; (a+b), the potential extent of gas production; and y, gas produced at time "t".

Nutrient compositions of CAP, MPP and rice straw were analyzed according to the standard methods (AOAC 1990,

Van Soest *et al.* 1991). Inoculum's ruminal fluid was collected at 0, 2, 4 and 6 h post inoculations. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into 2 portions; the first portion was centrifuged at 16,000×*g* for 15 min and the supernatant was stored at –20°C before NH_3–N analysis using the micro-Kjeldahl methods (AOAC 1990) and VFA analysis using HPLC (Samuel *et al.* 1997).

In vitro degradability was determined after termination of incubation, the contents were filtered through pre-weighed gooch crucibles and residual dry matter was estimated. The per cent loss in weight was determined and presented as *in vitro* DM degradability (IVDMD) percentages. The dried feed sample and residue left above was ashed at 550 °C for determination of *in vitro* OM degradability (IVOMD) percentages. Content of condensed tannins in CAP and MPP were analysed by using the modified vanillin-HCl method based on Burns (1971). Calculation of ruminal methane (CH_4) production using VFA proportions according to Moss *et al.* (2000) as follows:

CH_4 production = 0.45 (acetate)-0.275 (propionate)+0.4 (butyrate)

Data were analyzed by using the General Linear Models (GLM) procedure (SAS 1998). Data were analyzed using the model Yij=ì+Ti+åij where Yij, observation from treatment i, j, the replication; ì, the overall mean, Ti, the mean of treatment and åij, the residual effect. Mean separations with a significant *F* (P<0.05) for treatments were statistically compared using the orthogonal contrast.

RESULTS AND DISSCUSSION

Fig. 1. Gas kinetic was different among treatments (P<0.05; Table 2). Supplementation of CAP and MPP affected on cumulative gas production (96 h), gas production for the insoluble fraction (b), gas production rate (c) and potential extent of gas production (a+b) (P<0.05), but did not affect on gas production from the immediately soluble fraction (a) (P>0.05). Supplementation of MPP highly affected on gas kinetic when compared with control (P<0.05). However, supplementation of MPP at 5 mg resulted in the highest potential of gas production especially when combined with 5 mg CAP. Gas kinetic was changed, while digestibility of feed was not affected by MPP supplementation. While, Min *et al*. (2005) reported that quebracho condensed tannin consistently decreases the rate of ruminal gas and methane production. The ability of plant CT to inhibit the growth of microorganisms (Min *et al*. 2003) and reduced the rate of ruminal gas production (Frutos *et al.* 2004) is well known. Similary with Anantasook and Wanapat (2012) who reported that gas production and gas kenetic were increased with increasing level of condensed tannin 0 to 12%. However, the use of high level of condensed tannin should be taken into consideration regarding rumen ecology and animal health problems when applied to the *in vivo* system.

IVDMD was significantly highest in the combined

Table 2. Effect of feed supplementation on gas production kinetic and degradability from *in vitro* incubation with rumen fluid

Treatments	Gas kinetics[1]				Gas (96 h) ml/0.2 g DM substrate	*In vitro* degradability,%	
	a	b	c	a+b		IVDMD	IVOMD
Control	–1.79^{ab}	51.80^{b}	0.038^{a}	50.01^{c}	48.13^{c}	35.51^{c}	46.40
CAP 5 mg	–1.16^{ab}	67.54^{a}	0.031^{ab}	66.38^{a}	63.27^{a}	37.18^{c}	50.03
CAP 10 mg	–0.94^{a}	62.92^{a}	0.027^{b}	61.98^{b}	56.80^{bc}	55.20^{ab}	63.01
MPP 5 mg	–2.74^{ab}	66.00^{a}	0.030^{ab}	63.26^{ab}	59.87^{ab}	44.20^{abc}	55.69
MPP 10 mg	–1.53^{ab}	64.58^{a}	0.028^{b}	63.06^{ab}	58.77^{ab}	46.87^{abc}	58.71
CAP 5 mg+MPP 5 mg	–1.36^{ab}	67.60^{a}	0.033^{ab}	66.24^{a}	64.20^{a}	60.31^{a}	60.67
CAP 5 mg+MPP 10 mg	–2.20^{ab}	57.66^{ab}	0.031^{ab}	55.46^{bc}	51.80^{bc}	40.15^{bc}	52.78
CAP 10 mg+MPP 5 mg	–2.47^{ab}	65.33^{a}	0.030^{ab}	62.86^{ab}	59.40^{ab}	44.25^{abc}	58.71
CAP 10 mg+MPP 10 mg	–2.88^{b}	57.42^{ab}	0.031^{ab}	54.55^{bc}	50.50^{bc}	34.79^{c}	51.63
SEM	0.54	3.29	0.003	3.09	3.11	5.12	7.82
Contrast							
Control vs Supp	ns	**	**	**	**	0.08	ns
Control vs CAP	ns	**	**	**	**	ns	ns
Control vs MPP	ns	**	**	**	**	ns	ns
Control vs CAP MPP	ns	**	*	*	*	ns	ns
CAP vs MPP	0.05	ns	ns	ns	ns	ns	ns
CAP 5mg vs CAP 10mg	ns	**	ns	**	**	ns	ns
MPP 5mg vs MPP 10mg	ns *	ns *	* *	ns			

[1] a, the gas production from the immediately soluble fraction, b, the gas production from the insoluble fraction, c, the gas production rate constant for the insoluble fraction (b), a+b, the potential extent of gas production

CAP 5mg, supplementation of *Centella asiatica* powder at 5 mg, CAP 10 mg, supplementation of *Centella asiatica* powder at 10 mg, MPP 5 mg, supplementation of mangosteen peel powder at 5 mg, MPP 10 mg, supplementation of mangosteen peel powder at 10 mg, Supp, supplementation groups, CAP MPP, combination of CAP and MPP

*P<0.05, **P<0.01, ns=Non-significant, SEM=Standard error of the mean

Table 3. Effect of feed supplementation on volatile fatty acid and methane (CH_4) production

Treatments	TVFA[1] mmol/L	C_2	C_3 ——% TVFA——	C_4	CH_4 production[2] (mM/L)
Control	100.24^{a}	65.44^{a}	23.32^{b}	11.24^{c}	27.53^{a}
CAP 5 mg	95.46^{ab}	59.82^{b}	27.69^{a}	12.49^{c}	24.30^{b}
CAP 10 mg	89.03^{c}	57.62^{bc}	27.76^{a}	14.61^{ab}	24.14^{b}
MPP 5 mg	94.61^{ab}	56.83^{bc}	29.19^{a}	13.99^{b}	23.14^{b}
MPP 10 mg	92.43^{bc}	57.42^{bc}	28.34^{a}	14.25^{ab}	23.74^{b}
CAP 5 mg+MPP 5 mg	96.21^{ab}	56.49^{c}	29.09^{a}	14.43^{ab}	23.19^{b}
CAP 5 mg +MPP 10 mg	84.68^{c}	55.77^{c}	28.87^{a}	15.36^{a}	23.30^{b}
CAP 10 mg +MPP 5 mg	90.49^{bc}	54.62^{c}	29.54^{a}	15.85^{a}	22.79^{b}
CAP 10 mg +MPP 10 mg	84.29^{c}	54.17^{c}	30.06^{a}	15.77^{a}	22.42^{b}
SEM	2.26	0.88	1.08	0.65	0.76
Contrast					
Control vs Supp	**	**	**	**	**
Control vs CAP	*	**	*	*	*
Control vs MPP	0.07	**	**	**	**
Control vs CAP MPP	**	**	**	**	**
CAP vs MPP	ns	ns	ns	ns	ns
CAP 5mg vs CAP 10 mg	**	ns	ns	ns	ns
MPP 5mg vs MPP 10 mg	*	0.09	ns	ns	ns

[1]TVFA, total volatile fatty acid; C_2, acetic acid; C_3, propionic acid; C_4, butyric acid. [2]Calculated according to Moss *et al.* (2000) CH_4; production, 0.45 (acetate)-0.275 (propionate) + 0.4 (butyrate). CAP 5 mg, supplementation of *Centella asiatica* powder at 5 mg; CAP 10 mg, supplementation of *Centella asiatica* powder at 10 mg; MPP 5 mg, supplementation of mangosteen peel powder at 5 mg; MPP 10 mg, supplementation of mangosteen peel powder at 10 mg; Supp, supplementation groups; CAP MPP, combination of CAP and MPP. *P<0.05, NS, non-significant; SEM, standard error of the mean.

supplementation of CAP at 5 mg with 5 mg of MPP (P<0.01) among treatments. This could be due to a corresponding increase of microbes which then increased degradability. In contrast, IVOMD was not significantly different among treatments (P>0.05; Table 2). Beauchemin *et al.* (2007) also found that adding 2% quebracho tannin extract to the diet had no effect on DM or NDF digestibility in cattle. In addition, it was reported that feeding high levels of dietary saponins and/or tannins decreased apparent digestibility (Klita *et al.* 1996, Carulla *et al.* 2005, Ngamsaeng *et al.* 2006, and Poungchompu *et al.* 2009). This could be due to type and concentration of saponins and tannins contained in the plants. This suggested that selective suppression of cellulolytic bacteria by saponins and/or tannins (McSweeney *et al.* 2001a) may not occur in the present study.

Supplementation with MPP at 5 mg in combination with 10 mg CAP resulted in the highest ammonia nitrogen (NH_3–N) concentration (Table 3). The NH_3–N concentration ranged from 14.6 to 29.4 mg/dl. This result could be due to the effects of tannins contained in both CAP and MPP which protected crude protein from degradation by the formation of tannin-protein complexes in the rumen, thereby increasing metabolizable protein supply to the duodenum (Mueller-Harver 2006, Waghorn 2008). However, the present level of supplemention was similar to the level reported as an optimal to improve rumen ecology, digestibility and intake (Wanapat 1990) (15–30 mg/dl). Manipulating rumen fermentation through treatment of roughage and strategic supplementation with high quality feed block, tannin-containing plants, especially cassava hay and other local feed resources, could improve rumen efficiency by maintaining higher pH, optimum NH_3-N and increasing microbial protein synthesis and essential VFAs, and thus, enhanced ruminant productivity in the tropics (Wanapat 2000).

Total volatile fatty acid (TVFA) concentrations and proportions of acetic acid (C_2), propionic acid (C_3), and butyric acid (C_4) were affected by feed supplementation (P<0.05; Table 3). CAP and MPP supplementation decreased total VFA production and acetic acid proportion, while propionic acid and butyric acid were increased (P<0.05). High level of CAP and MPP highly impacted on VFA -production than using lower level (P<0.05). However, high level of both sources resulted in reducing of C_3 proportion. Regarding, MPP effect, earlier work reported that condensed tannins and saponins have a variable effect on ruminal VFA concentration. This could be due to the shift of hydrogen from the methane pathway made it available to be used to produce propionate. Effects of tannins on increased propionate and reduced acetate to propionate ratio have been found to vary with diets and applications. A similar shift in propionate production has been reported by Bhatta *et al.* (2009). However, Poungchompu *et al.* (2009) found that the concentrations of total VFA and C_3 were significantly increased, while proportion of C_2 was decreased by supplementation of MPP and soapberry fruit

Table 4. Effect of feed supplementation on ammonia-N at different time of incubation

Treatments	NH_3-N, hour of incubate				
	0	2	4	6	Total
Control	14.57	18.77	22.28[a]	22.70	19.58
CAP 5 mg	15.13	17.93	24.66[ab]	20.87	19.65
CAP 10 mg	19.19	22.00	23.12[a]	17.79	20.52
MPP 5 mg	16.53	19.33	25.08[ab]	19.47	20.10
MPP 10 mg	16.67	19.47	25.64[ab]	20.03	20.45
CAP 5 mg+ MPP 5 mg	16.11	18.91	25.64[ab]	16.39	20.11
CAP 5 mg+ MPP 10 mg	17.51	20.31	26.62[ab]	21.58	21.51
CAP 10 mg +MPP 5 mg	16.39	19.19	29.42[b]	21.44	21.61
CAP 10 mg +MPP 10 mg	16.95	19.75	27.74[b]	21.30	21.44
SEM	1.08	1.01	1.45	1.22	0.68
Contrast					
Control vs Supp	ns	ns	0.07	ns	ns
Control vs CAP	ns	ns	ns	0.09	ns
Control vs MPP	ns	ns	ns	ns	ns
Control vs CAP MPP	ns	ns	*	ns	ns
CAP vs MPP	ns	ns	ns	ns	ns
CAP 5mg vs CAP 10mg	0.09	0.07	ns	ns	ns
MPP 5mg vs MPP 10mg	ns	ns	ns	ns	ns

CAP 5 mg, supplementation of *Centella asiatica* powder at 5 mg; CAP 10 mg, supplementation of *Centella asiatica* powder at 10 mg; MPP 5mg, supplementation of mangosteen peel powder at 5 mg; MPP 10 mg, supplementation of mangosteen peel powder at 10 mg; Supp, supplementation groups;CAP MPP, combination of CAP and MPP. *P<0.05; ns, nonsignificant; SEM, standard error of the mean.

pellet. Moreover, Hristov *et al.* (1999) and Hess *et al.* (2003) found a significant increase in propionate production both in *in vivo* and *in vitro* trials, respectively. While, Ngamsaeng *et al.* (2006) found no significantly effects of feeding level of MPP on total VFA and individual VFA concentration. The high proportion of propionic acid was caused by a decreased methane production due to tannins contained in MPP and CAP. The expected shift of hydrogen from the methane pathway made it available to be used to produce propionic acid. Effects of tannins on increased propionic acid and reduced acetic to propionic ratio have been found to vary with diets and applications. A similar shift in propionic acid production has been reported by Bhatta *et al*. (2009). Moreover, Chanthakhoun *et al*. (2011) reported that feeding *Phaseolus calcaratus* hay (2.8% CT) at 600 g/hd/d in swamp buffalo resulted in increased production of propionic acid.

Calculation of ruminal methane (CH_4) production using VFA proportions, according to Moss *et al.* (2000), showed that methane production was affected by feed supplementation and the lowest was (P<0.05) in high level of CAP and MPP supplementation. Methane production was also decreased with MPP supplementation, and this result agreed with Poungchompu *et al.* (2009) who found that methane emission was depressed by inclusion of MPP and soapberry fruit pellet in dairy heifer diet. Similarly with Bhatta *et al.* (2009) who reported that tannins suppress methanogenesis by reducing the protozoal population *in vitro*. Tavendale *et al.* (2005) proposed two mechanisms whereby condensed tannins reduced methane emissions from ruminants: (i) indirectly through a reduction in fiber digestion, which decreases H_2 production, and (ii) directly through an inhibition of the growth of methanogens. Various studies have reported that feeding condensed tannin-containing plants to ruminants reduced methane emissions (Waghorn *et al.* 2002, Woodward *et al.* 2004, Pinares-Patino *et al.* 2003, Puchala *et al.* 2005).

Based on this study, it could be concluded that supplementation of CAP and MPP affected on gas production and fermentation efficiency, but at high level of CAP or MPP supplement did not show positive effects. Therefore, this study suggested that combination of CAP supplementation at 5 mg with MPP (5 mg) increased gas production, *in vitro* degradability, fermentation efficiency end-products especially propionic acid while methane production was decreased. However, further research should be investigated to study on the effect of CAP and MPP supplement in *in vivo* feeding trials.

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