Effect of *Centella asiatica* powder and mangosteen peel powder using *in vitro* gas production technique

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Abstract: The objective of this study was to determine the effect of *Centella asiatica* powder (CAP) and mangosteen peel powder (MPP) supplementation using *in vitro* gas production technique. The experimental design was a 3×3 factorial arrangement in a Completely randomized design (CRD). Factor A was 3 levels of *Centella asiatica* powder (CAP) (0, 5, 10 mg) and factor B was 3 levels of mangosteen peel powder (MPP) (0, 5, 10 mg). The dietary treatments were analyzed for *in vitro* ruminal gas production, *in vitro* degradability and ammonia nitrogen. It was found that supplementation of CAP and MPP affected on 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) ($P>0.05$). Cumulative gas production (96 h) was higher by different level of supplementation ($P<0.05$) and were found different in CAP 5 mg with 5 mg of MPP than in other level. However, *in vitro* degradability both IVDMD was significantly higher in supplementation of CAP at 5 mg with 5 mg of MPP ($P<0.01$) when compared with other treatments , while IVOMD was not significantly different among treatment ($P>0.05$). NH_3-N was higher in CAP 10 mg with 5 mg of MPP. It cloud be suggests that the level of CAP supplementation at 5 mg with 5 mg of MPP increased gas production and other fermentation parameters. However, further work in *in vivo* trials using CAP and MPP should be conducted.

Keywords: *Centella asiatica* powder, mangosteen peel powder, fermentation, *in vitro* gas production.

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Introduction

Methane is a greenhouse gas whose effect is estimated to be 25 times that of CO_2 based on equal molar amounts. Ruminant animals are one of the largest sources of methane emission with 81–92 million tons produced per year globally which is equivalent to 23–27% of total anthropogenic methane (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). A reduction in methane emissions could increase body weight gain of growing cattle or milk production of dairy cows, based on the energy balances reported by Nkrumah et al. (2006).

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 (Shrestha and Dhillion, 2003; Mamedov, 2005). The chemical composition of asiatic pennywort consists of several groups is Triterpenoid saponins and Aglycones. It also contains other groups, including amino acids, flavonoids, alkaloids, volatile oils (Department of Medical Sciences, 2007). However, Devkota et al. (2010) found that in the asiatic pennywort containsthe triterpene rather which substances in this group will result in suspension of the growth of bacteria, reducing inflammation. In ruminants this material will result in the degradation of carbohydrates (starch and sugar) (Department of Medical Science, 2007). 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 b-caryophyllene (19.08%), bicyclogermacrene (11.22%), germacrene B (6.29%) and myrcene (6.55%) (Oyedeji and Afolayan, 2005).

There have been reports of decreased methane emission by ruminants consuming plant secondary compounds. Feeding 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). The above studies agreed with Guo et al. (2008) who concluded that tannin has effect 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). Thus, the objective of the present study was to investigate effect of mangosteen peel powder (MPP) and *Centella asiatica* powder (CUP) supplementation on fermentation end-products in *in vitro* gas technique using rumen fluid of cattle.

Materials and Methods

Experimental design

An *in vitro* study was conducted to evaluate effect of mangosteen peel powder (MPP) and *Centella asiatica* powder (CAP) supplementation on fermentation end-products. Completely randomized design was used with 9 treatments (T) including control and supplementation with CAP (0, 5, 10 mg) and/or MPP (0, 5, 10 mg); therefore, treatments were as follows:

T1 = No-supplementation

T2 = Supplementation of CAP at 5 mg

T3 = Supplementation of CAP at 10 mg

T4 = Supplementation of MPP at 5 mg

T5 = Supplementation of MPP at 10 mg

T6 = Supplementation of MPP at 5 mg and CAP at 5 mg

T7 = Supplementation of MPP at 5 mg and CAP at 10 mg

T8 = Supplementation of MPP at 10 mg and CAP at 5 mg

T9 = Supplementation of MPP at 10 mg and CAP at 10 mg

Gas production technique

The method used for *in vitro* fermentation based on the technique described by Menke et al. (1979). Two hundred milligrams of feed samples 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 two 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 the method of 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 incubater at 39°C for 1 hour. Thirty 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.

Sample collection and analysis

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

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

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 and MPP 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). *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 percent loss in weight was

determined and presented as IVDMD percentages. The dried feed sample and residue left above was ashed at 550 °C for determination of IVOMD percentages.

Statistical analysis

Data were analyzed by using the General Linear Models (GLM) procedure (SAS Inst. Inc., Cary, NC). Data were analyzed using the model $Yij=\mu+Ti+\varepsilon ij$ where Yij, observation from treatment i, j, the replication; μ, the overall mean, Ti, the mean of treatment and εij, the residual effect. Mean separations with a significant *F* ($P<0.05$) for treatments were statistically compared using the orthogonal contrast.

Results and Discussions

Gas production and *in vitro* degradability

Gas production in each time of measuring was presented as a graph (Figure 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) ($P>0.05$). Mangosteen peel powder supplementation had highly affected on gas kinetic when compared to the control ($P<0.05$). However, supplementation with MPP at 5 mg resulted in highest potential of gas production especially when combined with 5 mg CAP (64.2 ml). IVDMD was significantly higher in supplementation of CAP at 5 mg with 5 mg of MPP ($P<0.01$) when compared with other treatments , while IVOMD was not significantly different among treatments ($P>0.05$) as shown in Table 2.

Supplementation with MPP at 5 mg of substrate resulted in highest ammonia nitrogen (NH_3–N) concentration when combined with 10 mg CAP as shown in Table 3. The NH_3–N concentration was ranged from 14.6-29.4 mg/dl (Table 3.) and was similar to as the report of Wanapat (1990) (15-30 mg/dl).

Conclusion and Recommendations

Based on this study it could be concluded that supplementation of CAP at 5 mg with 5 mg of MPP increased gas production and *in vitro* degradability, while NH_3-N was highest at CAP 10 mg with 5 mg of MPP supplementation. Combination of CAP and MPP had pronounced effect than those of single supplementation. Suplementation by high level of CAP or MPP did not show positive effects. However, further research should be investigated on rumen microorganism diversity and in *in vivo* feeding trials.

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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	37.8
ADF	53.2	52.1	24.3
CT	-	17.7	12.1

[1]MPP = mangosteen peel powder; [2]CAP = *centella asiatica* powder

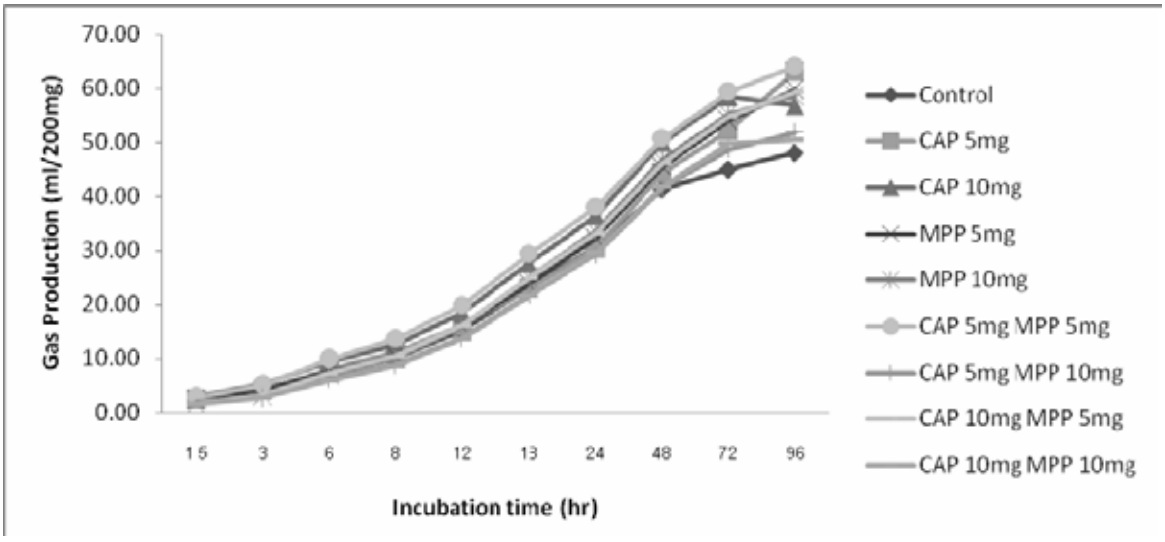

Figure 1. Effect of feed supplementation on cumulative gas production at different times of incubation**.**

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

Treatments	Gas kinetics[1]				Gas (96 h)	*In vitro* degradability, %	
	a	b	c	a+b	ml/0.2 g DM substrate	IVDMD	IVOMD
Control	-1.79^{ab}	51.80^{b}	0.038^{a}	50.01^{c}	48.13^{c}	35.51	46.40
CAP 5 mg	-1.16^{ab}	67.54^{a}	0.031^{ab}	66.38^{a}	63.27^{a}	37.18	50.03
CAP 10 mg	-0.94^{a}	62.92^{a}	0.027^{b}	61.98^{b}	56.80^{bc}	55.20	63.01
MPP 5 mg	-2.74^{ab}	66.00^{a}	0.030^{ab}	63.26^{ab}	59.87^{ab}	44.20	55.69
MPP 10 mg	-1.53^{ab}	64.58^{a}	0.028^{b}	63.06^{ab}	58.77^{ab}	46.87	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	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	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	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	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 5mg, CAP 10mg=supplementation of *centella asiatica* powder at 10mg, MPP 5mg= supplementation of mangosteen peel powder at 5mg, MPP 10mg= supplementation of mangosteen peel powder at 10mg, 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 ammonia-N at different times of incubation**.**

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

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

*P<0.05, ns=non-significant, SEM=standard error of the mean