Effect of mangosteen peel and galic powder supplementation on rumen fluid fermentation of beef cattle by using *in vitro* gas technique

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ABSTRACT: An *in vitro* study was conducted to evaluate effect of mangosteen peel (MP) and garlic powder (Gar) supplementation on rumen fermentation of beef cattle. A completely randomized design was used with ten treatments including control and supplementation with MP (3, 6, 9% DM) and Gar (0.5, 1% DM). The results were found that supplementation of Gar and MP increased gas production, VFA concentration and *in vitro* true digestibility ($P<0.05$). However, the lower level of mangosteen peel (MP3%) had more impact on rumen fluid fermentation than higher levels (MP6%, MP9%) in term of gas production, NH_3-N and VFAs in while MP6% was not different from MP9% ($P>0.05$). Garlic supplementation in supplemental mixture could not affect on rumen fluid fermentation end-products except increased propionate proportion, Moreover, disappearance of dry matter and organic matter were not significantly different between levels of Galic and MP supplementation ($P>0.05$). It could be recommended that next experiment should be less then 9% DM for mangosteen peel and 1% DM for garlic supplementations respectively.
Keywords: Mangosteen peel, garlic, gas production, rumen fermentation, beef cattle

Introduction

Manipulation of the rumen microbial ecosystem to enhance fibrous feed digestibility, reduce methane emission and reduce nitrogen excretion by ruminants such as to improve their performance are some of the most important goals for animal nutritionists (Patra et al., 2006). Currently, utilization of feed additive has proved to be a useful strategy to improve the efficiency of energy and protein utilization in the ruminant. One of possible alternatives is using the secondary compound in natural plants such as saponins, tannins, and essential oils. Mangosteen peel contains high amount of secondary compounds, especially condensed tannin (15.8%) and saponin (9.8%) (Ngamseang et al., 2006). Poungchompu et al. (2009) reported that mangosteen peel containing condensed tannin and saponin caused changes in ruminal microorganism and fermentation end-products. Garlic is an other kind of herb that has been used by humans as a source of antimicrobial agents for the gastrointestinal. Therefore, it could manipulate rumen fermentation. Busquet et al.(2005) reported that garlic supplementation decreased in the proportion of acetate and increased proportion of propionate and butyrate, inhibition of methanogenesis and decreased in the CH4:VFA ratio. However, manipulating ruminal fermentation of beef cattle by using combination of mangosteen peel and garlic still limit of data. Therefore, the objectives of this study were to determine effect of mangosteen peel and garlic supplementation on rumen fluid fermentation of beef cattle by using *in vitro* gas technique.

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Materials and methods

Experimental design and fermentation technique

The experiment was conducted at Tropical Feed Resource Research and Development Center (TROFREC), Department Animal Science, Faculty of Agriculture, Khon Kean University, Thailand in January, 2011.

An *in vitro* study to evaluate effect of mangosteen peel (MP) and garlic powder (Gar) supplementation on rumen fermentation was conducted. Completely randomized design was used with ten treatments including control and supplementation with MP or/and Garlic. Treatments (T) were; T1 = non-supplementation (control); T2 = supplemented with 3% MP; T3 = supplemented with 6% MP; T4 = supplemented with 9% MP; T5 = supplemented with 0.5% Gar and 3% MP; T6 = supplemented with 0.5% Gar and 6% MP; T7 = supplemented with 0.5% Gar and 9% MP; T8 = supplemented with 1% Gar and 3% MP; T9 = supplemented with 1% Gar and 6% MP and T10 = supplemented with 1% Gar and 9% MP.

The method used for *in vitro* fermentation based on the technique described by Menke et al. (1979). Five hundred milligrams of feed samples were weighed into 100 ml bottle. Substrates, dried MP and Gar were milled to pass through a 1mm screen. Roughage (rice straw) and concentrate (C) ration 60:40 was used as substrate. Feed and chemical compositions are shown in **Table 1**. 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, male, rumen fistulated, crossbred beef cattle (Brahman x Holstein Friesian) fed on rice straw *ad libitum* as roughage and concentrate (0.5% BW, 14,2%CP). Forty ml of buffered rumen fluid were taken into bottle containing the feed samples. The bottles were placed in an incubator at 39 °C for fermenting.

Table 1 Ingredients and chemical compositions of concentrates and rice straw used in the experiment

Item	Concentrates	Rice straw
Ingredients, %		
Cassava chip	65.0	
Rice bran	8.0	
Coconut meal	8.0	
Palm meal	3.0	
Soy bean meal	14.0	
Molasses	0.5	
Sulfur	0.5	
Mineral premix	0.5	
Salt	0.5	
Chemical compositions ----------(%/DM)----------		
DM	89.0	94.0
OM	93.3	87.2
CP	14.3	2.4
NDF	24.6	72.3
ADF	11.1	59.6

Sample collection and analysis

The gas production was recorded at 0, 2, 4, 6, 8, 12, 24, 36, 48, and 72 of incubation. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as follow: $y = a + b (1-e^{(-ct)})$. 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, y = gas produced at time 't'. The fermentation kinetics degradable was estimated using a computer package programme called Fitcurve (Neway program, Cambridge, UK). The fermenter contents were sampled at 0, 3 and 6 h of fermenting, fixed in 1M of H_2SO_4 for NH_3-N analyze according to AOAC (1990) and VFAs analyze using HPLC (Samuel et al. 1997). At 24h post inoculation a set of sample was determined *in vitro* true digestibility according to Van Soest and Robertson (1985).

Statistical analysis

Data were analyzed by using the General Linear Models (GLM) procedures (SAS Inst. Inc., Cary, NC). Using the model $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} is observation from treatment *i*, replication *j*; μ, the overall mean; T_i the mean of treatment; and ε_{ij} the residual effect. Multiple comparisions among treatment means were performed by Duncan's New Multiple Range Test (DMRT). Comparison between control and supplements, Gar 0.5% and Gar1%, MP3% and MP6, MP9%, MP6% and MP9% was tested by orthogonal contrast.

Results and discussion

Gas production, kinetic analysis of gas production

Cumulative gas production for each of the substrate treatments was presented as gas production curves **Figure 1** and values for the estimated parameters obtained from the kinetics of gas production are given in **Table 2.** This figure shown that addition of 3%MP and 0.5% Gar was highest in gas production while control was lowest.

Supplementation of MP and Gar significantly increased DM and OM disappearance ($P<0.01$) when compared with control group ($P<0.05$), although there were not significant difference levels of Gar and levels of MP supplementation. It was agreed with Kongmun et al. (2010) who found that disappearance of dry matter and organic matter were not significantly different between 8 and 16mg/200mg subtract of garlic supplementation.

The intercept value of fraction (a) and (c) were not significantly different between control and supplemental treatments, between levels of Gar and among levels of MP supplementation among treatments, while fraction (b) and (a+b) were significantly different between control and supplemental treatments ($P<0.05$), however there was not significantly different from levels of Gar and levels of MP supplementation. It was found that cumulative gas was decreased when level of MP supplementation was increased, whereas the Gar and MP supplementation had positive effects in gas production ($P<0.05$).

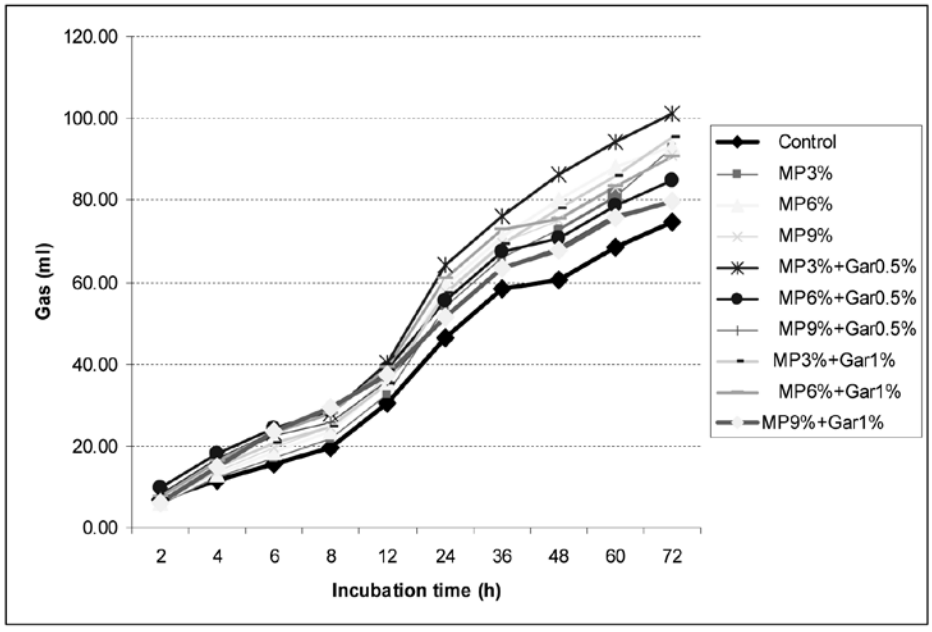

Figure 1 Effect of feed supplementation on cumulative gas production Volatile fatty acid (VFA) and ammonia nitrogen (NH_3-N)

Table 2 Effect of feed supplementation on gas production kinetic, dry matter and organic matter disappearance

Treatments	a	b	c	a+b	DM Disap	OM Disap
Control	2.31	77.77	0.04	80.08	58.00	50.14
MP3%	0.76	105.27	0.03	106.04	62.30	51.22
MP6%	-0.89	100.81	0.04	99.93	57.00	50.00
MP9%	0.65	94.47	0.04	95.12	62.80	52.62
MP3%+Gar0.5%	1.51	105.59	0.04	107.10	64.50	56.04
MP6%+Gar0.5%	3.48	89.56	0.06	93.03	63.30	53.64
MP9%+Gar0.5%	3.38	86.88	0.04	90.26	61.50	52.35
MP3%+Gar1%	2.65	94.98	0.03	97.63	65.20	56.06
MP6%+Gar1%	1.62	93.58	0.04	95.20	63.40	54.57
MP9%+Gar1%	0.84	87.38	0.03	88.22	62.00	51.85
SEM	1.32	6.85	0.01	7.31	1.94	1.02
Contrast						
Con vs Supp	ns	*	ns	*	*	**
Gar0.5vs Gar1%	ns	ns	ns	ns	ns	Ns
MP3 vs MP6, MP9%	ns	*	ns	0.06	ns	0.06
MP6% vs MP9%	ns	ns	ns	ns	ns	Ns

*P<0.05, **P<0.01, ns=non-significant, SEM=standard error of the mean

Table 3 Effect of feed supplementation on VFA production (mmol/l) and (NH_3-N) (mg/dl)

Treatments	NH_3-N	tVFA	C2	C3	C4
Control	14.7	53.10	40.35	8.40	4.40
MP3%	17.1	61.80	40.50	14.75	6.55
MP6%	17.4	62.25	40.82	13.25	8.20
MP9%	17.25	59.20	38.41	13.05	7.75
MP3%+Gar0.5%	19.5	71.20	47.01	16.97	7.20
MP6%+Gar0.5%	18.25	62.50	40.45	14.59	7.50
MP9%+Gar0.5%	17.65	67.20	43.80	15.05	8.40
MP3%+Gar1%	17.8	76.30	49.71	18.73	7.85
MP6%+Gar1%	14.85	77.60	51.02	18.59	7.95
MP9%+Gar1%	16.35	71.10	46.75	15.83	8.50
SEM	0.73	2.97	2.20	1.60	0.43
Contrast					
Con vs Supp	ns	*	*	**	**
Gar0.5 vs Gar1%	*	ns	ns	*	ns
MP3% vs MP6%, MP9%	*	ns	ns	ns	*
MP6% vs MP9%	ns	ns	ns	ns	ns

*P<0.05, **P<0.01, ns=non-significant, SEM=standard error of the mean

Ammonia nitrogen and VFA concentration are presented in **Table 3**. NH_3-N concentration was not affected by MP and Gar supplementation during 0-6 hours incubation, and ranged from 14.7 to 19.50 ml/dl (6h). It was optimal range for microbial protein synthesis in ruminant fed on low-quality roughage (Wanapat and Pimpa, 1999). However, NH_3-N concentration in MP3% was significant higher than in MP6% and MP9% (P<0.05). On the other hand, NH_3-N concentration was reduced when garlic supplementation increased (P<0.05). This result was similar with Cardozo et al, (2004) who reported that garlic oil supplementation reduced ammonia N due to inhibit deamination.

Mangosteen peel and garlic powder supplementation increased total VFA concentration and individual VFAs production during 0-6h incubation compared to control, although there was not significant difference from two levels of garlic supplementation (P>0.05) excepted C3 in 6h, Gar supplementation increased C3 proportion in 6 h incubation (P<0.05). Butyrate proportion in MP3% was lower then these in others. As reported by Pilajun et al. (2011) that supplementation of 30g/kg mangosteen peel powder in *in vivo* study in Swamp buffaloes increased in total VFA; however, Wanapat et al (2008) reported that increased supplementation of garlic powder in diets resulted in reduction total VFA and C2 proportion, but increased in C3 and C4 proportion.

Conclusions and recommendations

Supplementation of mangosteen peel and garlic powder had increased gas production and VFA concentration and *in vitro* true digestibility.

However, the lower level of mangosteen peel had more impact on rumen fluid fermentation than higher levels, and the effect of levels of garlic could not be different in fermentation, but increased propionate with higher level of garlic supplementation, It could be recommended that next experiment should be less then 9% DM for mangosteen peel and 1% DM for garlic supplementations respectively.

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