Ruminal Methane Emissions *In Vitro* of Plants Differing in Their Main Phenolic Fractions

Anuraga Jayanegara[1,*], Muhammad Ridla[1], Erika B. Laconi[1], Nahrowi[1]
[1] Department of Nutrition and Feed Technology, Bogor Agricultural University, Indonesia
* Corresponding email: anuragaja@ipb.ac.id

ABSTRACT

Plants containing phenolics have been known to produce less ruminal methane emissions when incubated with buffered-rumen fluid *in vitro*. However, little is known concerning methane mitigation effects of plants differing in their phenolic fractions. The present study was aimed to observe such effects by using several plants, namely *Swietenia mahagoni* (SM; rich in condensed tannins), *Clidemia hirta* (CH; rich in hydrolysable tannins) and *Eugenia aquea* (EA; rich in non-tannin phenolics). The plants were incubated either individually or mixed with a control plant with negligible phenolic contents, i.e. *Carica papaya* (CP; 1:1 w/w). An amount of 200 mg dry matter from each plant sample was incubated *in vitro* with 10 ml of rumen fluid and 20 ml of buffer solution in a Hohenheim gas test syringe. Incubations were conducted in four replicates (runs), represented by one syringe per replicate. In each run, incubation was performed for 24 h and kept at 39^{o}C. Total gas and methane productions were measured after the incubation. Fermentation fluid was subjected to volatile fatty acid (VFA) and ammonia measurements as well as bacteria and protozoa counts. Data were analyzed by analysis of variance and subsequent Duncan's test. Results revealed that combining each species of plant containing phenolics with CP significantly lowered methane emissions (as percentage of total gas) ($P<0.05$). Methane emissions of CP, CP+SM, CP+CH and CP+EA were 18.6, 14.0, 14.3 and 16.1%, respectively. Total gas production of CP decreased significantly after being mixed with SM, CH and EA ($P<0.05$). Similar pattern to total gas was observed for those of total VFA and ammonia concentrations. It can be concluded that methane mitigation effects of plants rich in either condensed or hydrolysable tannins are higher than that of plant rich in non-tannin phenolics.

Keywords: phenolic, methane, rumen, fermentation, *in vitro*

INTRODUCTION

Current goals of livestock production have expanded from previously focusing on improving productivity to more system oriented approach by integrating other related aspects such as environment, quality of products, welfare, etc. With regard to environment, livestock especially ruminants have been considered to considerably contribute to global warming by emitting a green-house gas namely methane (Moss et al., 2000). Such emission is not merely related to environmental problem, but it also represents a certain proportion of energy loss from the animals; it has been estimated that the loss is around 6-10% from the gross energy intake or 8-14% loss from the digestible energy intake (Cottle et al., 2011). Therefore various attempts have been made in order to mitigate methane emissions originated from ruminant livestock (Beauchemin et al., 2008). Plants containing phenolics have been shown to produce less ruminal methane emissions, both *in vitro* and *in vivo* (Jayanegara et al., 2009; 2011; 2012). However, there is an uncertainty whether the methane mitigating properties of plants containing phenolics are retained when the plants are mixed and used together with forages containing negligible contents of the compounds. Further, little is known concerning methane mitigation effects of plants differing in their phenolic fractions since phenolics chemically are such a diverse compounds. The present study was aimed to observe such effects by using several plants, namely *Swietenia mahagoni* (SM; rich in condensed tannins), *Clidemia hirta* (CH; rich in hydrolysable tannins) and *Eugenia aquea* (EA; rich in non-tannin phenolics). The plants were mixed with *Carica papaya* (CP) which contains very low amount of phenolics.

MATERIALS AND METHODS

General: All plants used in the present experiment, i.e. CP, SM, CH and EA were collected from the area of Bogor, Indonesia. These plants are used either as ruminant feeds in rural areas or as traditional veterinary medicinal plants across the country. Approximately 3 kg fresh matter of each plant species was collected. Shortly after the collection, the samples were air-dried in a greenhouse for two days and then subsequently oven-dried at 50°C overnight. Prior to further steps, all samples were ground to pass a 1 mm sieve. The plants were characterized for their chemical compositions, including crude nutrients, Van Soest's fiber fractions and phenolic fractions. The plants were incubated *in vitro* in rumen-buffer mixture by following the procedure of Menke and Steingass (1988). Incubation of the plants was conducted either individually or mixed with a control plant with negligible phenolic contents, i.e. CP (1:1 w/w). An amount of 200 mg dry matter from each plant sample was incubated *in vitro* with 10 ml of rumen fluid and 20 ml of buffer solution in a Hohenheim gas test syringe, performed for 24 h and kept at 39°C. The syringes used have two outlets; the first outlet is used for filling and emptying the liquid phase and the other one is used for sampling of the gas phase, i.e. methane. Total gas, organic matter digestibility and methane production were measured after the incubation as previously described in Jayanegara et al. (2011). Fermentation fluid was subjected to volatile fatty acid (VFA) and ammonia measurements as well as bacteria and protozoa counts.

Statistics: Allocation of the plants into experimental units were based on a randomized complete block design. Incubations were conducted in four replicates (runs), represented by one syringe per replicate. The block employed was the rumen content used in each run. Data obtained were analyzed by a two-way analysis of variance. When the experimental plants showed significantly different at $P<0.05$ for a particular parameter, the statistical analysis was continued with a post-hoc test namely Duncan's multiple range test.

RESULTS AND DISCUSSION

Total phenol (TP) contents of CP, SM, CH and EA were 2.5, 20.7, 21.6 and 16.9% DM, respectively, whereas total tannin (TT) contents of these plants were 0.8, 13.8, 21.2 and 6.7% DM, respectively. The main phenolic fractions in SM, CH and EA were condensed tannins (41.6% from TP), hydrolysable tannins (93.5% from TP) and non-tannin phenolics (60.4% from TP), respectively.

Table 1. Total gas production, organic matter digestibility (OMD), methane emission and microbial population of the incubated experimental plants

Plant	Total gas (ml)	OMD (%)	CH_4 (% gas)	CH_4/OMD (ml/g DM)	Bacteria (log cell/ml)	Protozoa (log cell/ml)
CP	44.1^{e}	76.9^{f}	18.6^{e}	53.5^{e}	9.45	4.40^{bc}
SM	22.1^{b}	43.5^{c}	9.4^{a}	24.2^{a}	9.40	4.20^{a}
CH	21.1^{b}	41.7^{b}	11.5^{b}	29.1^{b}	9.35	4.28^{ab}
EA	10.8^{a}	29.6^{a}	11.7^{b}	21.8^{a}	9.31	4.50^{c}
CP+SM	32.4^{d}	59.5^{e}	14.0^{c}	38.3^{c}	9.48	4.41^{bc}
CP+CH	33.0^{d}	59.6^{e}	14.3^{c}	39.7^{cd}	9.39	4.23^{ab}
CP+EA	27.5^{c}	53.2^{d}	16.1^{d}	41.9^{d}	9.41	4.25^{ab}
P-value	<0.001	<0.001	<0.001	<0.001	0.338	0.008

CH, *Clidemia hirta*; CP, *Carica papaya*; DM, dry matter; EA, *Eugenia aquea*; SM, *Swietenia mahagoni*

Combining each species of plant containing phenolics with CP significantly lowered methane emissions (as percentage of total gas) than that of CP only ($P<0.05$; Table 1); mixing SM, CH or EA with CP decreased such methane emissions by 24.7, 23.1 or 13.4%, respectively. Further, the relationship between total phenol (TP) or total tannin (TT) contents in plants and *in vitro* ruminal CH_4 emissions (% total gas) is presented in Figure 1. This finding supports previous studies that reported the potentiality of phenolics in mitigating ruminal methane emissions (Jayanegara et al., 2009; 2011). The mechanisms on how phenolics could lower the methane emissions are due to a decline in nutrient digestibility, direct inhibitory effect on methanogens and defaunation effect on protozoa where part of the methanogens are symbiotically living together (Jayanegara et al., 2012). The decline of digestibility by adding plants containing phenolics was also observed in the present experiment. The results also suggest that methane mitigation effects of plants rich in either condensed or hydrolysable tannins are higher than that of plant rich in non-tannin phenolics. Total gas production and OMD of CP decreased significantly after being mixed with SM, CH and EA ($P<0.05$). Similar pattern to total gas was observed for those of total VFA and ammonia concentrations (Table 2).

Table 2. Volatile fatty acid (VFA) and ammonia (NH_3) concentration of the incubated experimental plants

Plant	Total VFA (mM)	C_2 (mM)	C_3 (mM)	C_4 (mM)	C_2:C_3	NH_3 (mM)
CP	73.9^{e}	51.6^{e}	12.7^{d}	6.7^{d}	4.15^{a}	28.0^{d}
SM	50.8^{b}	38.3^{b}	8.1^{b}	3.4^{a}	4.73^{b}	8.3^{a}
CH	49.7^{b}	36.4^{b}	7.8^{b}	4.5^{b}	4.71^{b}	8.9^{a}
EA	42.7^{a}	32.0^{a}	6.1^{a}	3.8^{a}	5.24^{c}	11.0^{ab}
CP+SM	62.3^{d}	45.5^{d}	10.1^{c}	5.3^{c}	4.49^{ab}	13.5^{bc}
CP+CH	59.8^{cd}	43.2^{cd}	10.0^{c}	5.2^{c}	4.34^{ab}	14.0^{bc}
CP+EA	57.9^{c}	41.9^{c}	9.3^{c}	5.1^{c}	4.51^{ab}	15.8^{c}
P-value	<0.001	<0.001	<0.001	<0.001	0.003	<0.001

C_2, acetate; C_3, propionate; C_4, butyrate; CH, *Clidemia hirta*; CP, *Carica papaya*; DM, dry matter; EA, *Eugenia aquea*; SM, *Swietenia mahagoni*

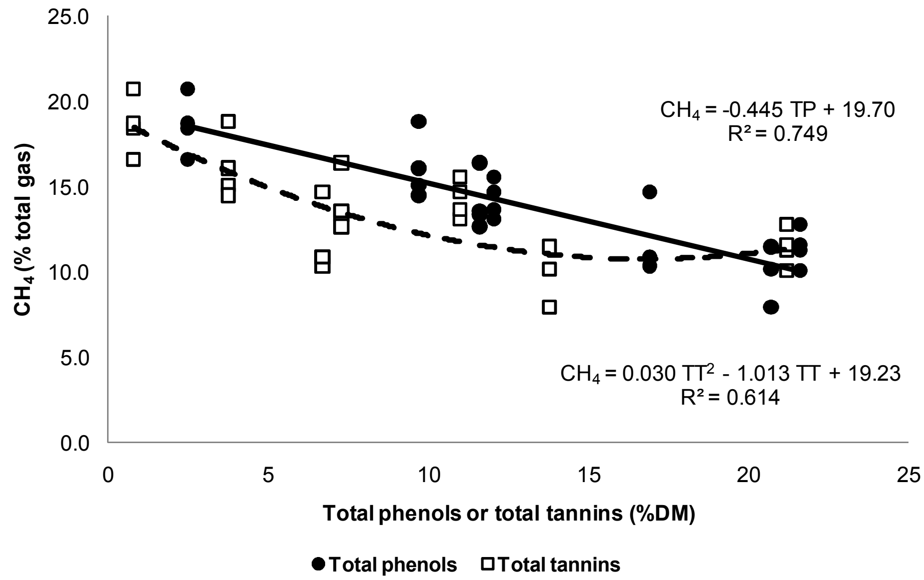

Figure 1. Relationship between total phenol (TP) or total tannin (TT) contents in plants and *in vitro* ruminal CH_4 emissions (% total gas)

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