Effects of polyphenol rich bamboo leaf on rumen fermentation characteristics and methane gas production in an in vitro condition

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Received:10-05-2017 Accepted:30-11-2017

ABSTRACT

The aim of this study was to test the effect of bamboo leaf (BL) on rumen methane gas production and rumen fermentation characteristics, in vitro. Different amounts of BL; CON (0 %), Low BL (LBL, 10 %), Medium BL (MBL, 15 %) and High BL (HBL, 25 %) of replacement with alfalfa hay (AH) in substrate (50 % concentrate + 50 % AH) were mixed with 30 millilitre (mL) of buffered rumen liquor for 48 h of incubation. Total gas production (mL/250 mg DM) was not affected (P>0.05) among BL treatment groups at different times of incubation. Production of methane gas (mL/250mgDM) decreased at a declining rate (P<0.05) with higher BL levels. Methane gas inhibitory effects of BL treatment groups as compared with CON were; 29%, 35% and 62% for LBL, MBL and HBL, respectively. The ratio of acetic/propionic was lowest (P<0.05) for HBL (1.67) as compared to CON (2.09).

Key words: Bamboo leaf, Fermentation, In vitro gas production, Methanogenesis, Rumen.

INTRODUCTION

Methane as a greenhouse gas is known for a giver to the worldwide temperature alteration (Bhatta et al. 2013). Therefore, decreasing the methane produced by ruminants is viewed as a critical objective as it reduces greenhouse gas emissions and improves feed utilization (Bouchard et al. 2015). However, until the present day much effort has been made to recognize the potential feed material maximizing rumen fermentation characteristics while decreasing rumen methane production in ruminants. Potential solutions include supplementing plants containing secondary metabolites as rumen modifiers, due to the fact that they occur naturally and are thus environmentally friendly and therefore are more widely accepted when it comes to food safety (Bhatta et al. 2012).

Bamboo, a group of commonly found large grasses, looks more promising in this regard (Halvorson et al. 2010). Bamboos are mixed with the custom and culture of country and tribal populations which have been called as “The Cradle to Coffin Plant,” “The Poor Man’s Timber,” “Friend of the People,” “Green Gasoline,” “The Plant with Thousand Faces,” and “The Green Gold (Chongtham et al. 2011). Bamboo has been given to cattle (“Bos” spp.), sheep (“Ovis” spp.), yaks (“Bos grunniens”), gayals (“Bos frontalis”), dairy cattle and buffalo (“Bubalus” spp.) in different parts of the globe (Halvorson et al. 2010). It is likely that bamboo leaves contain significantly higher concentrations of components like protein and non-structural carbohydrates that are important nutritionally, along with some major minerals, in comparison with other plant parts (Li et al. 1998). In a study by Hoyweghen et al. (2012) on 12 selected bamboo species, it was reported that the major phenolic compounds present in the bamboo leaf (BL) were flavonoid glycosides and phenolic acids which are the main antioxidants. In an animal trial study, BL could improve growth performance, feed conversion efficiency, and meat quality in broiler chickens (Kim et al. 2011). In another animal study, it was reported that BL contains sufficient nutrients for maintenance of adult goats (Halvorson et al. 2010).

By considering the above facts in view and absence of enough study in literature about the effect of BL on rumen fermentation characteristics, this study aimed to determine the effect of BL on rumen methanogenesis and rumen fermentation characteristics in an in vitro condition. The results could also be useful for the further rational development of BL-based food additives and food supplements in ruminant’s nutrition.

MATERIALS AND METHODS

Experimental plant materials and animals used for in vitro study: The experiment was directed at the research farm of University Putra Malaysia (UPM). The Institutional Animal Care and Use Committee (IACUC) of Faculty of Veterinary Medicine, University of Putra Malaysia approved the experimental protocol (AEC FPV 05/2015). Samples of BL were harvested from the farm exists in the UPM. Four

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rumen flstulated goats (Kajang crossbred) that weighed averagely of 39±0.70 kg were used for collecting rumen fluid. The goats were fed twice daily with alfalfa hay (AH) and concentrate (50:50, w/w). Samples of rumen liquor were taken from four goats before morning feeding at 08:30am and immediately placed in warm insulated flasks at 39±1°C under anaerobic conditions. Samples were equally apportioned then strained through four layers of cheesecloth under anaerobic conditions and used promptly in the laboratory.

Chemical analyses: For the experiment, fresh BL samples were analyzed for the chemical composition (dry matter, crude protein, ash and ether extract) according to AOAC (1990). Determination of neutral detergent fiber (NDF) was without sodium sulfite and with the use of a heat stable α amylase. Both NDF and acid detergent fiber (ADF) were expressed with residual ash (Van Soest et al., 1991). The concentrate consisted of corn (25.44%), soybean meal (20%), palm kernel cake (35.87%), rice bran (11.69%), palm kernel oil (5%), ammonium chloride (1%) and a vitamin and mineral mixture (1%). The chemical composition of substrates used for the in vitro incubations has been shown in Table 1.

### Determination of phenolic compounds in bamboo leaf:
A modified Folin–Ciocalteu method using polyvinyl-polypyrrolidone (Sigma-Aldrich GmbH, Steinheim, Germany) was used to determine the phenolic compound (total phenol (TP), total tannin (TT), condensed tannin (CT), hydrolyzed tannin (HT)) in BL. The method was conducted as described by Jafari et al. (2016). The values were calibrated against a gallic acid standard (Sigma-Aldrich) and expressed as gallic acid equivalents. Hydrolysable tannins (HT) were estimated as the difference between CT and TT.

### In vitro incubation:
Strained rumen liquor as described earlier was diluted 1:2 with two kinds of buffers; phosphate buffer and bicarbonate buffer according to Fievez et al. (2007). A total of 250 mg DM of sample was incubated with 30 mL buffered rumen fluid in 100 mL calibrated syringes. Concentrations of BL were; CON (0 %, concentrate + alfalfa hay (50:50 %)), low BL (15% of AH in substrate replaced by BL), medium BL (MBL, 25% of AH in substrate replaced by BL) and high BL (HBL, 50% of AH in substrate replaced by BL). Incubation of syringes was conducted at 39°C at different times of 0, 2, 4, 6, 8, 10, 12, 24 and 48 h. The gas produced was calculated by subtracting gas produced in blank syringe (containing only buffered rumen fluid) from the syringes containing buffered rumen fluid plus samples.

### Post incubation analysis: After 48 h of incubation, 1mL of the head-space gas phase was analyzed by gas - liquid chromatography (Agilent 5890 Series Gas Chromatograph, Wilmington, DE, USA) equipped with a flame ionization detector to determine methane production. Using standard methane prepared by Scotty Specialty Gases (Supelco, Bellefonte, PA, USA) calibration was completed. Each of the procedures was repeated three times. The pH was determined by a pH electrode (Mettler-Toledo Ltd., England). Then, 25% metaphosphoric acid was used to cease fermentation and samples were centrifuged (10 min, 4°C at 15,000×g) and filtered to determine volatile fatty acid (VFA) and NH₃N. The determination of VFA was made by using Agilent 7890A gas-liquid chromatography (Agilent Technologies, Palo Alto, CA, USA). Internal standard (4-methyl-n-valeric acid) was used for VFA determination. NH₃N (mg/dL) concentration was found using the method as described by Jafari et al. (2016).

Statistical analysis: All fermentation data were analyzed using the MIXED procedure of SAS (2003) ver. 9.1 (SAS Institute Inc., Cary, N.C., USA). Sampling at different times was added to the model and analyzed using repeated measures. Means were separated using the “pdiff” option of the “lsmeans” statement of the MIXED procedure. Polynomial contrasts were also used. Values of P < 0.05 were considered significant. The data were checked for normality using PROC UNIVARIATE of the SAS ver. 9.1.

### RESULTS AND DISCUSSION

#### Phenolic compounds in bamboo leaf:
In the current study, the phenolic compounds in BL were; TP (19.38 mg/g), TT (20.48 mg/g), HT (10.33 mg/g) as gallic acid equivalent and CT (10.21 mg/g) as catechin equivalent (Table 2).

As an important category of phytochemicals, plants containing phenolic compounds have been considered to have high antioxidant ability and free radical scavenging capacity (Maisarah et al. 2014). In a study conducted by Bhatta et al. (2012) on tannin-containing leaves from tropics (Ficus religosa, Ficus racemosa, Leucaena leucocephala, Moringa oleifera, Sesbania grandiflora), TP, TT, CT and HT averaged 28.60, 20.11, 11.47 and 11.45 (mg/gDM), respectively, for fresh leaves.

### Table 1: Chemical composition of substrates used for the in vitro incubations.

<table>
<thead>
<tr>
<th>(g/kg DM)</th>
<th>AH</th>
<th>Concentrate</th>
<th>BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>90.70</td>
<td>91.00</td>
<td>450.60</td>
</tr>
<tr>
<td>CP</td>
<td>203.00</td>
<td>167.00</td>
<td>145.30</td>
</tr>
<tr>
<td>NDF</td>
<td>517.01</td>
<td>244.03</td>
<td>688.10</td>
</tr>
<tr>
<td>ADF</td>
<td>334.00</td>
<td>117.00</td>
<td>423.02</td>
</tr>
<tr>
<td>EE</td>
<td>9.40</td>
<td>18.01</td>
<td>22.50</td>
</tr>
<tr>
<td>ASH</td>
<td>80.00</td>
<td>50.34</td>
<td>115.00</td>
</tr>
</tbody>
</table>

| alfalfa hay; AH; Bamboo leaf, BL; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber, ADF, acid detergent fiber; EE, ether extract |
respectively. Jayanegara et al. (2009) also reported the phenolic compounds from the leaves of medicinal plants (Iris lacteal, Sterrella chamaejasme, Toraxacum officinale, Delphinium elatum, Salsola laricifolia), TP, TT and CT ranged from (22.20 to 65.30 mg/gDM), (7.10 to 32.00 mg/ gDM) and (0.70 to 14.30 mg/gDM). Our results obtained in the current study are also consistent with the mentioned studies.

**Effect of bamboo leaf on total gas and methane gas production:** Increases in levels of BL did not result in negative gas production (mL/250 mg DM) at different times of incubation (Figure 1) and also rate of gas production or (c) constant (Table 3) in BL treatment groups compared to the CON. Methane production as shown in Figure 2 decreased at a decreasing rate with increasing levels of BL. Methane production (mL/250mg DM) at 48 h of incubation was: 8.92, 6.27, 5.75 and 3.34 for CON, LBL, MBL and HBL, respectively. Treatment groups; LBL, MBL and HBL showed respectively 29%, 35% and 62% reduction of methane production as compared to CON. Reduction of rumen methane production in this study could be due to the presence of secondary metabolites in BL as it is shown in Table 3.

It has been shown that tannin containing feed materials suppress rumen methanogenesis (Kushwaha et al. 2011). Leaves of papaya inhibited rumen methane production until 38% as compared with control at 24 h of incubation in an in vitro study conducted by ourselves (Jafari et al. 2016). Our in vitro results confirm that all tannins containing feed materials have different effect on rumen methanogenesis. However, plant tannin effectiveness changes with the tannings’ source, type and content (Bhatta et al. 2013). Znora et al. (2013) indicated that Mentha piperita leaves at different concentration as substrate mitigated rumen methane production at 24 h of incubation by changing the rumen microbial populations with no effect on digestibility of dry matter.

**Effect of bamboo leaf on rumen fermentation characteristics:** The results of effect of the addition of different levels of BL on rumen fermentation characteristics have been shown in Table 4. Generally, rumen fermentation parameters were improved by the use of BL in this experiment. The pH in the current study ranged from 7.35 to 7.38 without much difference between CON group and BL treatment groups (LBL, MBL and HBL). The ratio of acetic/propionic was highest in CON (2.28) compared to HBL (1.67) and MBL (2.07) and LBL (2.21). Total VFAs and molar proportions of propionic and butyric acids were not significantly (P>0.05) affected in current study by BL. Interestingly, the molar proportion of acetic acid was affected (P<0.05) by the BL addition in which LBL (51.83 mol/100mol), MBL (44.74 mol/100mol) and HBL (43.49 mol/100mol) had lower acetic acid concentration as compared with CON (51.57 mol/100mol). Rumen NH₃N (mg/mL) concentration was highest (P<0.05) for MBL (23.44) and LBL (23.05) compared to HBL (16.99) and CON (18.23).

**Table 3:** Effect of the addition of different levels of BL on fractional rate of gas production (h⁻¹) or constant (c), after in vitro incubation with rumen liquor from goats.

<table>
<thead>
<tr>
<th>Experimental diets</th>
<th>Gas production constant (c)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>1.15</td>
<td>0.13</td>
<td>0.72</td>
</tr>
<tr>
<td>LBL</td>
<td>1.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBL</td>
<td>1.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBL</td>
<td>1.09</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P: linear and quadratic respectively. CON: control (50% concentrate + 50% AH), LBL: 15% replacement of AH substrate by BL, MBL: 25% replacement by BL, HBL: 50% replacement by BL, SEM: standard error of mean, BL: bamboo leaf; ns: non significant.
Table 4: Effect of the addition of different levels of BL on rumen fermentation parameters after in vitro incubation with rumen liquor from goats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental diets</th>
<th>CON(^1)</th>
<th>LBL(^3)</th>
<th>MBL(^3)</th>
<th>HBL(^4)</th>
<th>SEM</th>
<th>Linear</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>7.38*</td>
<td>7.37*</td>
<td>7.36*</td>
<td>7.35*</td>
<td>0.007</td>
<td>0.01</td>
<td>0.58</td>
</tr>
<tr>
<td>NH(_3) (Mg/dL)</td>
<td></td>
<td>18.23*</td>
<td>23.05*</td>
<td>23.44*</td>
<td>16.99*</td>
<td>1.27</td>
<td>0.009</td>
<td>0.17</td>
</tr>
<tr>
<td>Acetic (Mol/100mol)</td>
<td></td>
<td>51.57*</td>
<td>51.83*</td>
<td>44.74*</td>
<td>43.49*</td>
<td>2.31</td>
<td>0.04</td>
<td>0.20</td>
</tr>
<tr>
<td>Propionic (Mol/100mol)</td>
<td></td>
<td>22.65</td>
<td>23.49</td>
<td>22.27</td>
<td>26.12</td>
<td>1.88</td>
<td>0.21</td>
<td>0.84</td>
</tr>
<tr>
<td>Butyric (Mol/100mol)</td>
<td></td>
<td>2.26</td>
<td>2.23</td>
<td>2.18</td>
<td>2.36</td>
<td>0.10</td>
<td>0.58</td>
<td>0.97</td>
</tr>
<tr>
<td>Total VFA (mM/L)</td>
<td></td>
<td>79.49</td>
<td>77.55</td>
<td>69.20</td>
<td>71.98</td>
<td>3.68</td>
<td>0.06</td>
<td>0.48</td>
</tr>
<tr>
<td>Acetic/Propionic ratio</td>
<td></td>
<td>2.09*</td>
<td>2.21*</td>
<td>2.07*</td>
<td>1.67*</td>
<td>0.13</td>
<td>0.92</td>
<td>0.43</td>
</tr>
</tbody>
</table>

\(^1\)CON: control (50% concentrate + 50% AH), \(^3\)LPL: 15% replacement of AH substrate by BL, \(^4\)MBL: 25% replacement by BL, \(^5\)HBL: 50% replacement by BL, SEM: standard error of mean, BL: bamboo leaf; \(^*\) different letter in each row denotes significant difference at P<0.05.

The difference in rumen pH is an indication of varying buffering capacity and rate of degradation of different nutrients. According to the pH results it seems that the addition of BL could not have negative effect on buffering capacity and nutrient degradation. The highest ratio of acetic/propionic was reported for CON (2.28) as compared to HBL (1.67) and MBL (2.07) and LBL (2.21). The ratio of acetic/propionic is considered as an important parameter in the methane production because propionic acid provides the main alternative sink for metabolic H\(_2\) during rumen fermentation, which means that lower acetic/propionic ratio could modulate methane production. The molar proportions of propionic, butyric acids and total VFAs were not significantly (P>0.05) affected in current study by BL. Polyphenol rich BL's effect on total VFA (TVFA) is less than methane's, an indication that the methane suppression was mainly because of antimethanogenic activity rather than lowered fibre digestibility. Moreover, VFAs are the main source of energy for ruminants and reduction in production of VFAs would be nutritionally negative for the ruminants (Busquet et al. 2006). The higher concentration of NH\(_3\)N seen among LBL and MBL compared to CON (18.23 mg/dL) might have resulted from an increase in the breakdown of protein (Benchaaar et al. 2008; Gunal et al. 2014). The higher concentration of NH\(_3\)N in LBL and MBL compared to CON could be due to increased dietary levels of carbohydrate and nitrogen, resulting in an increase in NH\(_3\)N concentration levels (Paengkoum et al. 2017). Lower NH\(_3\)N in HBL (16.99 mg/dL) compared to other treatment groups could be a result of properties of tannins content in BL which binds the protein, and has been reflected at higher inclusion rate of BL.

**CONCLUSION**

The present study’s data indicated that BL affected the fermentation characteristics and reduced rumen methanogenesis without affecting the total production of gas at 48 h of incubation. Methane gas suppression was accompanied by a fermentation pattern shift towards a reduction in the acetic acid production without any effect on total VFA production among BL treatment groups. However, comprehensive in vivo animal studies have to be done to evaluate the sustainability of BL supplementation for mitigating methanogenesis and modulating rumen fermentation characteristics without negative effects on the whole animal.

**ACKNOWLEDGMENT**

The authors are thankful to the Faculty of Veterinary Medicine, Faculty of Agriculture, University Putra Malaysia.

**REFERENCES**


