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IN VITRO RUMEN DEGRADATION KINETICS AND VOLATILE FATTY ACIDS PRODUCTION OF CATTLE MANURE VERMICAST SUPPLEMENTED WITH RICE STRAW

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Abstract: Different processing methods have been used to develop ingredients for ruminants' feedstuff from manure. It is important to study dynamics of rumen degradation of various potential feeds before using it to formulate diets for ruminants. The objectives of the study were to determine the effects of Rice straw treated with graded level of Cattle Manure Vermicast (CMV) on in vitro gas degradation kinetics and volatile fatty acids (VFA) production.Inclusion of varying levels of CMV has significant difference (P<0.05) on potential gas production (*b*). CMV inclusion reduces in vitro gas production, with 30 % CMV inclusion having least potential gas production of 31.13 mL/200mg. Comparing rice straw and CMV, there was significant difference (P<0.05) in *b*, CMV has the highest potential gas production (56.52 mL/200 mg) compared with rice straw (41.44 mL/200 mg). In vitro rumen effective degradability decreases with increased of CMV inclusion. Significant difference was observed on*in vitro* rumen VFA production. However, it does not correspond with an increase of CMV inclusion.There was no significant difference on acetic acid propionic acid ratio (A: P), but CMV increases butyric acid significantly (P<0.05) with corresponding increase of CMV inclusion. This shows that CMV inclusion has the potential of being used as by-pass protein.

Keywords: Cattle manure vermicast, feedstuff, *in vitro* gas production, vermicomposting

Introduction

Livestock manure is a traditional source of organic nutrients in agriculture (Szögi and Vanotti, 2003). However, it is one of the under-utilised resources (Sarwar *et al*., 2011). Among the options for utilising manure is recycling it as animal feed as well as nutrient source in aquaculture (Lytem*et al*. 2013). Leytem *et al*. (2013) opined that ruminants are particularly ideal for feeding of manure due to their rumen microbiome which enables them to utilize forage fibre, non-proteineous nitrogen and nucleic acids. With proper and acceptable processing method, cattle manure is an economic and safe source of energy, protein and minerals to livestock (Zinn *et al*., 1996). Processing methods used in treating manure includes ensiling, dehydration, single cell protein production, pelleting, deep stacking, and chemical preservation with the aim of improving its nutritive value (Sarwar*et al*. 2011). Up to thirty percent of cattle manure inclusion in silage making was recommended for optimal utilization in ruminant feeding (Hassan *et al*., 2011; Sarwar *et al*., 2011).

Another manure treatment method is vermicomposting. Vermicomposting is a mesophilic bio-oxidation and stabilisation process of biomass that involves the joint action of earthworm and microorganism.Vermicomposting process has the potential of eliminating the risk associated with feeding cattle manure as collected. These include suppression of pathogens (Edwards and Subler, 2011), increases in Nitrogen content of the substrate due to its ability to decrease Carbon Nitrogen ratio (Atiyeh *et al*., 2000; Garg *et al*., 2006; Singh *et al*., 2011). Vermicomposting also increases metabolisable energy as a result of cellulo-lignolysis(Vinceslas-Akpa and Loquet, 1997) and increases mineral content (Garg*et al*., 2006). Therefore, vermicast is an aerobically degraded and humified organic matter which has undergone chemical degradation by enzymes in the gut of earthworms and by the associated microbial population (Anand *et al*., 1995).

The *In vitro* gas production technique has been widely used for evaluation of nutritive value of feedstuffs for decades ((Menke*et al*., 1979; Menke and Steingass, 1988; Blu¨mmel and Ørskov, 1993; Rymer *et al*., 2005; Singh *et al*., 2014). Blu¨mmel and Ørskov (1993) found out that total gas production as described by exponential equation was correlated with intake, digestible dry matter intake and growth, in a multiple regression model. The objectives of this study were to evaluate the effects of Rice straw treated with graded level of Cattle Manure Vermicast (CMV) on in vitro gas degradation kinetics and volatile fatty acids (VFA) production.

Materials and Methods

Vermicomposting of cattle manure and sample preparation

Cattle manure was collected from selected farms in Kepala Batas, Penang, Malaysia (5.5172^0 N and 100.4315^0 E); the climatic condition is tropical rain forest. The manure was air dried before use. The earthworms (African night crawlers (*Eudriluseugeniae*)) used for vermicomposting of cattle manure were obtained from BP GemilangAgrobio, BalikPulau, Penang. Vermicomposting was performed according to Nasiru *et al*. (2014). CMV was harvested at six (6) week. Rice straw was collected from Ruminant research unit of Department of Animal Science at Universiti Putra Malaysia (2.9917^0 N and 101.7163^0 E). Rice straw with harvested CMV was oven dried at 60^{o}C for 48 h and milled to pass 1 mm sieve. CMV was added to rice straw at an inclusion rate of 0, 5, 10, 15, 20, 25 30 and 100%. The chemical composition of rice straw and CMV is shown in Table 1.

Table 1: Chemical composition of rice straw and cattle manure vermicast (% Dry Matter)

	OM	CP	NDF	ADF
Cattle manure vermicast	86.18	9.24	58.9	31.49
Rice straw	87.55	3.46	67.51	46.10

OM organic matter, CPcrude protein, NDFneutral detergent fibre, ADFacid detergent fibre

In vitro gas production

The experiment was conducted in the Department of Animal Science, Universiti Putra Malaysia. Rumen fluid was collected before morning feeding from two ruminallyfistulatedkajang goats that were fed concentrate as a supplement (300 g/head/day) and 700 g/head/day of rice straw as basal diet. Water was offered *ad libitum* throughout the experiment. In vitro gas production was carried out following the procedure of Menke and Steingass (1988). Gas production was recorded at 2, 4, 6, 12, 24 and 48 h of incubation, and adjusted for rumen fluid alone. The non-linear equation $y = b(1 - e^{-ct})$ was fitted to gas production data using NEWAY program (Chen, 1996). According to Siaw *et al*. (1993) no gas is produced from unfermented feed. **Where:** y = the gas produced (mL) at time t, b = the potential gas production (mL), c = the gas production rate constant and t = the incubation time (h). Effective degradability (ED) was calculated using the equation $ED = (bc/(c + k))$, where k is the outflow rate from the rumen assumed to be either 0.02 and 0.05/h. The outflow rate of 0.02 and 0.05/h is representative for low and medium feeding levels (AFRC, 1993).

Volatile fatty acids (VFA) determination

At the end of *In vitro* incubation, fermented contents were sub-sampled for VFA determination. Approximately 6 mL of rumen liquor from cannulated goats were aliquoted into 10 mL plastic tubes and centrifuged at 6000 rpm for 20 min. Subsequently, 1 mL of supernatant was collected and 200 µL of metaphosphoric acid (H_3PO_4, 0.1 mL of 8.2%, w/w) was added into each sample and centrifuged again under the same condition. A 0.5 mL aliquot of supernatant was pipetted into a gas chromatography (GC) auto-sampler vial containing 0.5 mL of internal standard (0.75 mM of 3-methylvaleric acid); the vial was sealed and placed in an auto-sampler tray (Filípek and Dvořák, 2009). VFA concentrations (C2: acetic, C3: propionic, bC4: isobutyric, C4: butyric, C5: isovaleric, C5: valeric acids) were determined using a Hewlett Packard HP5890 Capillary Gas Chromotograph. From chromatograph VFA peak areas were identified and quantified using calibration with internal standards, according to Attwood *et al*. (1998).

Statistical analyses

Data on in vitro gas production, degradation kinetics and volatile fatty acids were analysed as completely randomized design experiment. Means were separated at 5% level of significance using Tukey test, where significance difference exist using general linear model procedure in SPSS v20.

Results and Discussion

In vitro gas degradation Kinetics

The *In vitro* gas production at 48 h, gas production parameters and effective degradability of rice straw supplemented with CMV at varying levels is presented in Table 2. Inclusion of varying levels of CMV has significant difference (P<0.05) on potential gas production (*b*). An increase in CMV inclusion increases *b*, at 10% CMV inclusion (48.87 mL/200 mg) at the point where the potential gas production is highest and start declining with increase in CMV level, though there was no significant difference (P>0.05) between 5 and 10% CMV inclusion on potential gas production. 30% CMV inclusion level has the least potential gas production *In vitro*. Comparing rice straw and CMV, there was significant difference (P<0.05) in *b*, CMV has the highest potential gas production (56.52 mL/200 mg) compared with rice straw (41.44 mL/200 mg). There was no significant difference (P>0.05) in gas production constant (*c*) due to varying level of CMV inclusion.

The values ranged from 0.010 to 0.017/h, with 15% CMV inclusion has the highest value and 5% CMV inclusion has the lowest value. There was no significant difference (P>0.05) on *c* between rice straw (0.015) and CMV (0.014). Inclusion of CMV at different level has significant (P<0.05) effect on *In vitro* effective degradability at 0.02/h outflow rate. An increase in CMV inclusion decreases *In vitro* effective degradability. Effective degradability decreases from 33.08% at 5% CMV inclusion to 23.50% at 30% CMV inclusion. There was significant difference (P<0.05) on effective degradability at 0.02/h outflow rate between rice straw (28.71 %) and CMV (14.38 %). The same pattern was observed on effective degradability at 0.05/h outflow rate. However, the values recorded were lower than what were observed at 0.02/h outflow rate. The values decreased from 13.77 % (5% CMV inclusion) to 9.86 % (30 % CMV inclusion). Significant difference (P<0.05) was observed between rice straw (11.99 %) and CMV (6.41 %) on *In vitro* effective degradability at 0.05/h outflow rate.

The potential gas production 'b' decreases with an increase of CMV inclusion. This is due to the influence of CMV on nutrients degradability which depressed ruminal degradability by inhibiting microbial activity. Presence and or inclusion of the following reduce *In vitro* rumen degradability: tannins (Camacho *et al*., 2010; Getachew *et al*., 2008; Hervás *et al*., 2003; Mbugua *et al*., 2008), monensin and vegetable oil (Kim *et al*., 2014; Weimer *et al*., 2011) and vanillin (Patra and Yu, 2014). The rate of gas production 'c' observed from this study was lower than what was reported by Ndagurwa and Dube (2013) for mistletoes and woody species browsed by goats. This is due to the humification nature of the CMV. The effective degradability at the outflow rate of 0.05/h ($K_{0.05}$) reported in this study was within the range reported by Tahir *et al.* (2013) for starch rich feed fraction. Effective degradability decreases with increase in CMV inclusion, the reason for that is the lower degradability of CMV.

Table 2: *In vitro* gas production (IVGP) (gas production volume after 48 h of incubation, mL/200 mg DM), potential gas production (b), gas production rate constant (c) and effective degradability (at two passage rates) of rice straw supplemented with CMV at varying level

		Cattle manure vermicast inclusion level (%)							
	RS	5	10	15	20	25	30	CMV	SE
$IVGP_{48}$	29.33[b]	35.00[a]	30.67[b]	30.00[b]	27.33[bc]	24.00[c]	23.67[c]	11.00[d]	3.03
Degradability constant									
B	41.44[bc]	43.25[b]	48.87[b]	38.39[bc]	36.10[bc]	35.73[bc]	31.13[c]	56.52[a]	6.26
C	0.015	0.010	0.014	0.017	0.016	0.011	0.012	0.014	0.01
Effective degradability									
$K_{0.02}$	28.71[a]	33.08[a]	30.78[a]	30.83[a]	26.94[ab]	22.83[b]	23.50[b]	14.38[c]	3.61
$K_{0.05}$	11.99[ab]	13.77[a]	12.79[a]	12.97[a]	11.36[ab]	10.68[b]	9.86[b]	6.41[c]	1.55

Different superscript in the same row is significantly different (P<0.05). CMV: cattle manure vermicast; RS: rice straw; SE: standard error

Constants b and c are described by the equation $y = b(1 - e^{-ct})$, where y = the gas produced at time t, b = the potential gas production, c = the gas production rate constant and t = the incubation time (h). Effective degradability (ED) was calculated using the equation $ED = (bc/(c + k))$, where k is the outflow rate from the rumen assumed to be either 0.03 or 0.05/h.

Table 3: *In vitro* volatile fatty acids production as influenced by CMV inclusion on rice straw

		Cattle manure vermicast inclusion level (%)							
	RS	**5**	**10**	**15**	**20**	**25**	**30**	**CMV**	**SE**
pH	6.73	6.67	6.80	6.76	6.73	6.80	6.70	6.80	0.08
TVFA	59.65[a]	61.85[a]	58.68[b]	54.25[c]	55.29[c]	58.98[b]	53.51[cd]	60.57[a]	1.28
Acids proportion (%)									
Acetic	33.78	33.58[ab]	34.28[a]	35.22[a]	32.87[b]	32.33[b]	32.24[b]	32.05	1.47
Iso-butyric	2.93	2.67	2.67	2.79	2.65	2.80	3.57	2.96	0.46
Butyric	22.87[d]	23.08[c]	23.47[bc]	23.70[bc]	24.94[bc]	25.32[b]	29.76[a]	22.91[d]	0.82
Propionic	32.01[b]	31.96[b]	30.58[c]	29.72[c]	30.05[c]	29.93[bc]	21.95[d]	33.62[a]	0.72
Valeric	3.59	3.43	3.46	3.39	3.59	3.81	4.29	3.33	0.99
Iso-valeric	4.82	5.28	5.53	5.17	5.91	5.82	8.17	5.13	0.38
A:P	1.00	1.00	1.00	1.00	1.00	1.00	1.33	1.00	0.16

Different superscript in the same row is significantly different (P<0.05); CMV: cattle manure vermicast; RS: rice straw; SE: standard error; TVFA: total volatile fatty acids (mM/ML); A:P :acetic to propionic ratio

Effect of CMV inclusion on In vitro volatile fatty acids (VFA) production

Table 3 shows the effects of varying CMV inclusion on rice straw on pH and volatile fatty acids production. There was no significant difference (P>0.05) due to varying CMV inclusion level on *In vitro* rumen pH. Increasing CMV inclusion significantly (P<0.05) reduces total volatile fatty acids (TVFA). The reduction was up to 15 % level of CMV inclusion (54.25 mm/mL), and then it increases with additional increase of CMV inclusion, then it declined at 30 % CMV level (53.51 mm/mL). The highest TVFA value was at 5 % CMV inclusion (61.85 mm/mL). There was no significant difference (P>0.05) between rice straw (59.65 mm/mL) and CMV (60.57 mm/mL) on TVFA production. Varying level of CMV inclusion significantly (P<0.05) affected Acetic acid proportion.

There was no definite pattern due CMV inclusion on acetic acids proportion, the highest value was observed at 15 % CMV (35.22%) and the least was at 30% CMV inclusion (32.24%). No significant difference (P<0.05) was observed on acetic acid proportion between rice straw (33.78%) and CMV (32.05%). An increase of CMV inclusion level on rice straw increases *In vitro* butyric acids proportion significantly (P<0.05). Butyric acid increases from 23.08 % (5 % CMV inclusion) to 29.76 % (30 % CMV inclusion). However, no significant difference (P>0.05) was observed on *In vitro* butyric acid proportion between rice straw (22.87 %) and CMV (22.91 %). Additional inclusion of CMV on rice straw decreases *In vitro* propionic acid proportion significantly (P<0.05). It was observed that 5 % CMV has the highest value of propionic acid proportion (31.96 %) and the least was 30 % CMV inclusion with 21.95 %. Significant difference (P<0.05) on *In vitro* propionic acid proportion was observed between rice straw and CMV, with CMV having the highest value (33.62 %) and rice straw had the least value (32.01%). CMV inclusion on rice increases valeric and isoveleric, although the changes observed were not significant (P>0.05).

Volatile fatty acids are end-products of rumen microbial fermentation and represent the main supply of energy for ruminants (Phakachoed *et al*., 2013). The values recorded for total volatile fatty acids (TVFA) production in this study was low. But the values are within the range reported by Durmic *et al*. (2010) for Australian woody perennial plant; Muetzal*et al*. (2009) for continuous rumen incubation and Weimer *et al*. (2011) for mixed rations adopted by monensin microbes. An increase of CMV inclusion on rice straw reduces TVFA values. Durmic*et al*. (2010) and Kim *et al* (2014) reported monensin addition inhibit VFA production. Patra and Yu (2014) observed linear decrease in TVFA concentration with increase in vanillin dosages. The reason is monensin and vanillin have strong antimicrobial activities against a number of bacteria, yeasts, and molds therefore, had potent inhibitory effects on gas production and VFA(Davidson and Naidu, 2000; Durmic *et al*., 2010; Kim *et al*., 2014; Patra and Yu, 2014). This is applicable to CMV. Another reason for low VFA production is pH values obtained in the study. pH values near 7.0, might have resulted from a combined effect of slow fermentation and continuous buffer infusion, and reflected low VFA concentration (Salem *et al*., 2013).CMV inclusion on rice straw increases butyric acid proportion. This finding agrees with the findings of Patra and Yu (2014), Kim *et al*. (2014) and Weimer *et al*. (2011) who reported that vanillin and monensin decreased proportion of propionate and increased proportion of butyrate and valarate. This results from lower degradability of the feed substrate. This is caused by shift in microbial population and or changes in fermentation pathways of the ruminal culture (Patra and Yu, 2014).

Conclusion and Recommendation

Evaluation through *In vitro* gas production degradability shows that additional increase of CMV in ruminant diet decreases rumen degradability and based on this CMV has the potential of being used as a medium of obtaining by-pass protein. It can be concluded that inclusion of CMV increase nutrients effective rumen degradability by up to 40%. *In vitro* method of feed evaluation suggested that inclusion of up to 20% CMV in the diet can be degraded effectively in the rumen.

Due to the low degradability of CMV observed during the study period, it shows CMV has the potential of being used as bypass protein. This also indicates that vermicomposting as a processing method can be used with the aim of producing bypass protein.

To determine the suitability of using cattle manure vermicast as a feed for small ruminant finishingproduct of standard quality, more experiments are needed to assess the intake, digestibility, blood chemistry and haematological counts as well as effect of CMV on meat quality.

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Conflict of Interests

The authors declared that they have no conflict of interest.

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