The Use of *in Vitro* Gas Production Technique to Evaluate Molasses Supplementation to Mulberry (*Morus alba*) and Rice Straw Mixed Diets

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ABTRAK

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Hijauan dari murbei mempunyai potensi digunakan sebagai pakan tambahan pada pakan serat berkualitas rendah karena mengandung nilai nutrisi yang tinggi (protein, daya cerna dan degradasi dalam rumen). Tujuan dari penelitian ini adalah untuk menguji pengaruh penambahan energi terfermenasi dalam rumen pada pakan basal campuran hijauan murbei dan jerami padi. Pakan kontrol terdiri dari campuran jerami padi (yang tidak ataupun yang diberi perlakuan urea) dan hijauan murbei dengan perbandingan 60 : 40%. Pakan perlakuan diformulasi dengan menggunakan pakan kontrol yang disuplementasi dengan molases (sebagai sumber energi terfermentasi dalam rumen) pada 3 taraf pemberian (5, 10 dan 15%). Penelitian dilakukan dengan menggunakan percobaan faktorial 2x4, yang terdiri dari 2 taraf jerami padi (diberi perlakuan atau tidak diberi perlakuan) dan 4 taraf suplementasi energi (0, 5, 10 dan 15%). Pakan dievaluasi dengan menggunakan metode *in vitro* produksi gas. Fermentasi kinetik dievaluasi dengan menginkubasikan 200 mg sampel selama 96 jam. Penghitungan fermentasi kinetik berdasarkan persamaan eksponensial $P = A+ B(1-e^{-ct})$. Substrat yang sebenarnya terfermentasi dievaluasi dengan menginkubasi 500 mg sampel selama 24 jam. Hasil dari penelitian ini .menunjukkan bahwa tidak terdapat interaksi secara nyata antara perlakuan jerami dengan suplementasi molasses terhadap fermentasi kinetik dan kecernaan *in vitro*. Di pihak lain terdapat interaksi secaranya nyata ($P<0,05$) antara perlakuan jerami dengan suplementasi molases terhadap PF, produksi gas, produksi asam propionat dan perbandingan asam asetat dengan propionat. Suplementasi molases pada pakan campuran jerami yang tidak diberi perlakuan dengan hijuan murbei secara nyata dapat meningkatkan PF, produksi asam propionat dan menurunkan produksi gas dan rasio antara asam asetat dan propionat. Dari hasil penelitian ini dapat disimpulkan bahwa suplementasi molases pada pakan campuran jerami dan hijauan murbei dapat meningkatkan PF dan produksi asam propionat dan menurunkan produksi gas dan rasio asam asetat dan propionat.

Kata Kunci: Hijauan Murbei, Molases, Jerami Padi, In Vitro Produksi Gas

ABSTRACT

YULISTIANI, D., Z.A. JELAN, J.B. LIANG, H. YAAKUB and N. ABDULLAH. 2007. The use of *in vitro* gas production technique to evaluate molasses supplementation to mulberry (*morus alba*) and rice straw mixed diets. *JITV* 12(4): 255-261.

Mulberry foliages have high nutritive value (protein content, digestibility and degradability), therefore it is potential to be used as a supplement to poor quality roughages. The objective of this experiment was to evaluate the effect of addition of fermentable energy in the mixed of mulberry and rice straw basal diet. A control diet consisted of either rice straw (RS) or urea treated rice straw mixed with mulberry foliage (URS) with ratio of 60 : 40%. Treatment was formulated by supplementation of control diet with molasses (as sources of fermentable energy) at 3 levels (5, 10 and 15%). The study was conducted in a 2 x 4 factorial experiment, consisted of 2 levels rice straw (untreated and urea treated) and 4 levels molasses supplementation (control and 3 levels for molasses). Diets were evaluated using *in vitro* gas production. The fermentation kinetics was determined from the incubation of 200 mg sample during 96 hours. The calculation of the kinetics based on exponential equation $P = A+ B (1-e^{-ct})$. A shorter gas production test was carried out to determine truly degradable fermented substrates (*in vitro* true organic matter degradability/IVTOMD) by incubating 500 mg of samples 24 hours. The result showed that there was no significant interaction between rice straw treatment and molasses supplementation on fermentation characteristics, in vitro true dry matter digestibility, fermented substrate and total volatile fatty acid (VFA) production. However there was a significant interaction between rice straw treatment on partitioning factor (PF), gas produced, propionic acid production and ratio between acetic acid and propionic acid. Molasses supplementation significantly ($P<0.05$) decreased gas production and ratio of acetic to propionic acid, and increase PF, propionic acid production in untreated rice straw mulberry (RSM) basal diet. It is concluded that molasses supplementation to RSM diet decreased gas production and ratio of C2/C3, and increased PF and propionic acid production.

Key Words: Mulberry Foliage, Molasses, Rice Straw, In Vitro Gas Production

INTRODUCTION

Rice straw is abundantly available in South East Asia where 90% of the world rice produced (VAN SOEST, 2006) and traditionally used as feed for large ruminants. As feed, rice straw have several limitations such as low in digestibility and protein content and imbalance mineral composition, these condition limit its intake (DRYHURST and WOOD, 1998). Ammoniation derived from urea could increase digestibility of rice straw, however, feeding only urea treated rice straw caused weight loss of sheep (ELSEED, 2004). Several supplementation strategies have been practiced to overcome this low productivity. The supplement must supply nutritive elements which are deficient in low quality roughage such as nitrogen, mineral, and vitamin, and increase nutrient available for intestinal digestion (DEVENDRA and SEVILLA, 2002).

Mulberry foliage is suitable to be used as supplement in low quality roughage ration such as rice straw (JELAN and SADDUL, 2004) because it has high content of protein (24.7%) (SADUL *et al*., 2005). However, its protein degradability in the rumen was high (87.3%) (SADDUL *et al*., 2005) and the rapid fermentation of N caused rapid production of ammonia. The excess of ammonia is absorbed from the rumen (BEVEER *et al*., 1986). The urea will be recycled in the gut, however majority of urea produced will lost as urinary urea (EGAN and ULYATT, 1980). The high rate of rumen ammonia production requires a matching energy supply from rumen fermentable carbohydrate (RFC) to improve microbial protein production (CORBETT, 1987). In order to enhance microbial growth rate and efficiency of nutrient utilization, nitrogen and energy must be simultaneously available for utilization (SNIFFEN and ROBINSON, 1987).

Molasses together with mineral and urea is common component in multi-nutrient block which is used as feed supplement for low quality roughage in tropical countries (LENG, 1997). Molasses contains sugar as predominant carbohydrate content (SUDANA and LENG, 1986) which is readily fermentable energy (OBARA *et al*., 1981) can be used to match the high ammonia availability from nitrogen degradation of mulberry. KIM *et al*. (1999) reported that increasing levels of sucrose and urea by intraruminal infusions in wethers fed grass silage supplemented with urea resulted in significant increase of microbial synthesis. This indicates the increased efficiency of rumen ammonia utilization, when readily fermentable carbohydrate is provided.

The gas production technique has been proved to be potentially useful technique for feed evaluation (BLUMMEL and ORSKOV, 1993). The *in vitro* gas production allowed to measure rate and extent of nutrient degradation (CONE *et al*., 1997). The gas produced indicated how much substrate was used for the formation of short chain fatty acid (SCFA) and gasses. This due to gas production and SCFA are very closely stochiometrically (BLUMMEL and ORSKOV, 1993).

The high protein degradation of mulberry possibly could be used efficiently for rumen microbial production if it is supplemented with rumen fermentable carbohydrate. The objective of this experiment was to evaluate the effect of the addition of fermentable energy to the mixed mulberry and of rice straw basal diet using *in vitro* gas production technique.

MATERIALS AND METHODS

A control diet consisted of either rice straw or urea treated rice straw mixed with mulberry foliage (RSM) in ratio DM of 60:40. Urea treated rice straw was prepared by diluting 50 g of urea in 1l of water and sprayed to 1 kg of dry rice straw. The straw was then kept in a plastic bag for 3 weeks. Treatment diets were formulated by supplementation of control diet with molasses (as sources of fermentable energy) to a control diet at 4 levels (0.5, 10 and 15% of DM). The study was conducted in a 2 x 4 factorial experiment, consisted of 2 levels rice straw treatment (untreated and urea treated) and 4 levels of molasses supplementation (0, 5, 10 and 15%). Diets were evaluated using in vitro gas production (MENKE and STEINGAS, 1988). The fermentation kinetics were determined from 96 hours incubation of 200 mg substrate in triplicates into 100 ml calibrated glass syringes, fitted with plungers as describe by MENKE and STEINGASS (1988) and modified as described by BLUMMEL and ORSKOV (1993). A total medium containing 10 ml rumen fluid and 20 ml mixture of bicarbonate, mineral, and distilled water was injected into the syringes. Triplicates of blank containing only medium and standard samples known gas production were also incubated. The gas production was recorded at 2, 4, 6, 8, 10, 24, 48, 72 and 96 h incubation at 39°C. The calculation of the kinetics based on exponential equation $P = A + B(1-e^{-ct})$ from ORSKOV and MCDONALD (1979).

A shorter gas production test was carried out to determine truly degradable fermented substrates (*in vitro* true organic matter degradability/IVTOMD) at 24 h. In this incubation, 500 mg sample was incubated in 40 ml of medium. The medium was prepared according to MAKKAR *et al*. (1995). Gas volume was recorded at 2, 4, 6, 8, 10 and 24 h incubation. After terminating the incubation at 24 h, five ml of supernatant from each syringes was taken for volatile fatty acid(VFA) analysis, prior the residue in the syringe was transferred into a 600 ml spoutless beaker. The syringe was washed with a total of 70 ml of NDS solution. The procedure of VAN SOEST (1994) was then applied by refluxing the incubation residue for 1.0 h and filtering the undigested matter on pre-tared filter crucibles. Truly digested matter was calculated as the weight of substrate incubated minus the weight of the residue after NDS treatment. The partitioning factor (PF) value at a given time of incubation was calculated as OM truly degraded (mg) divided by gas volume produced (ml). The PF was computed to give an estimate of variation in short chain fatty acid (SCFA) per microbial cell yield per unit substrate truly degraded. The PF values were determined after 24 h of incubation. The total VFA and molar proportions of acetic, propionic and butyric acids were determined by gas chromatography (Model G1540N, Agilent Technologies, USA). Rumen fluid was obtained from rumen-cannulated cattle maintained on roughage concentrate diet at the ratio of 60 : 40 DM.

Data collected were analyzed using general linier model procedure in SAS package version 6.12. Means were compared using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

There was no significant interaction between rice straw treatment and molasses supplementation on fermentation characteristics. Constant A, which reflects gas produced from soluble and readily available fraction was significantly (P<0.05) increased by molasses supplementation (Table1). This suggests that molasses supplied soluble and readily available carbohydrate as reported by CONE and VAN GELDER (1999) that the initial gas production derived from the fermentation of the water soluble components, such as sugar and protein. The rate of gas production (c) from the fermentation of insoluble but fermentable fraction in molasses supplemented diet was significantly higher. This indicates that the soluble carbohydrate enhanced cellulolytic activity of the microbes (GETACHEW *et al*., 2005). The rate of gas production (c) was significantly (P<0.05) higher in mixed urea treated rice straw mulberry diets than untreated diets which indicates that urea treatment increased the fermentability of rice straw.

There was a significant interaction (P<0.05) between rice straw treatment and supplementation on gas production (GP) and partitioning factor (PF) (Figure 1). The IVTOMD was significantly (P<0.05) affected by rice straw treatment and supplementation but no interaction between these factors (Table 2). However, substrate truly fermented was not affected by any factors (Table 2). Molasses supplementation to rice straw and mulberry mixed diet significantly reduced gas production at all levels of supplementation (Figure 1a).

Table 1. The mean values of mean factor effect of rice straw treatment and molasses supplementation on constant of fermentation characteristics of the dietary treatments in *in vitro* gas production model $P = A + B(1-e^{-ct})$

	A	B	C	A+B
Means of rice straw Treatment				
Untreated	6.77	50.7	0.044[b]	57.5
Urea treated	6.79	50.5	0.051[a]	57.3
S.E.M	0.267	0.787	0.002	0.856
Significance	n.s	n.s	0.01	n.s
Means of molasses supplementation				
0	4.47[c]	52.1	0.044[c]	56.6
5	5.67[b]	51.2	0.047[bc]	56.8
10	7.95[a]	48.7	0.051[a]	56.6
15	9.05[a]	50.4	0.048[ab]	59.4
S.E.M	0.378	1.11	0.003	1.21
Significance	0.01	n.s	0.01	n.s

Different superscript in the same column indicate significance different (P<0.05)

S.E.M = standard error of treatment mean

ns = non significant

A = A considered as gas produced from the fermentation of immediately soluble materials

B = gas produced from the fermentation insoluble fraction, but fermentable

C = the rate of gas production of B fraction

A+B = extent gas production

On the other hand, molasses supplementation to urea treated rice straw and mulberry significantly ($P<0.05$) increased gas production at levels of 5 and 10% molasses supplementation. When the supplementation further increased the gas production was significantly decreased (Figure 1a). The increased of gas production in urea treated rice straw mulberry mixed diet when it was supplemented with molasses was due to the synchronization of the availability of rapidly fermentable energy from molasses (CONE and VAN GELDER, 1997) and rapidly fermentable nitrogen from mulberry (JELAN and SADUL, 2004). This stimulate the fermentation of the diets, in addition, urea treatment to rice straw caused saponification of the lignin-cellulose-hemicellulose linkages resulted cellulose and hemicellulose become available for digestion by rumen microbes (HARTLEY and JONES, 1978). At the same time, urea treatment supplied nitrogen needed by rumen microorganism (van Soest, 2006). Therefore, the gas production of urea treated rice straw mulberry mixed diet was higher than untreated rice straw mulberry mixed diet. The IVTOMD of urea treated diets was significantly higher ($P<0.05$) than that of untreated rice straw diets. Molasses supplementation significantly ($P<0.05$) increased IVTOMD at 24 h (Table 2). *In vitro* gas production and true degradability are highly correlated (BLUMMEL and ORSKOV, 1993). However, there was an inverse relationship between gas volume and microbial biomass (BLUMMEL *et al*., 1997). The use of the *in vitro* gas production measured could indicate that the high gas production results in low microbial biomass production. This is due to the substrate being use to yield high short chain fatty acid (SCFA). This disadvantage can be overcome by combining gas measurement with determination of the truly degradable substrate (IVOMD) (BLUMMEL *et al*., 1997). The combination of this measurement can be used to measure efficiency of microbial production (EMP). Truly degradable substrate indicated the amount of substrate available for fermentation and that the gas volume indicated the proportion of the substrate used to produce SCFA. The proportion of substrate which, contributed to the biomass production, can be obtained from PF. The higher PF is an indication of higher microbial biomass production. BLUMMEL *et al.* (2003) reported that there was a significant relationship between PF measurement at $t_{1/2}$ (a time at which half-maximum gas production was achieved) and EMP *in vivo*. It is desirable in ruminant nutrition to produce proportionally high conversion of rumen degraded feed into microbial biomass, i.e. a high EMP may lead to efficient feed N and C utilization (BLUMMEL *et al*., 1997).

There was a significant ($P<0.05$) interaction between rice straw treatment and supplementation on molar proportion of propionic acid and ratio acetic acid to propionic acid (Figure 2). However, there was no interaction between rice straw treatment and supplementation on total VFA, proportion of acetic and butyric acid (Table 3). Total VFA and proportion of

Table 2. The mean values of mean factor effect of rice straw treatment and molasses supplementation on *in vitro* true organic matter digestibility (g/kg) and substrate truly fermented (mg) of the diets after incubated in the rumen buffer solution for 24 hours

	Digestibility (%)	Substrate fermented(mg)
Means of rice straw treatment		
Untreated	69.8^{b}	311.8
Urea treated	72.3^{a}	326.5
S.E.M	0.60	4.03
Significance	0.01	n.s
Means of molasses supplementation		
0	67.3^{b}	313.1
5	70.9^{a}	309.7
10	72.4^{a}	324.3
15	73.6^{a}	329.5
S.E.M	0.85	5.7
Significance	0.01	n.s

Values in the same column with different superscript is significantly different ($P<0.05$)
S.E.M = standard error of treatment mean
n.s = non significant

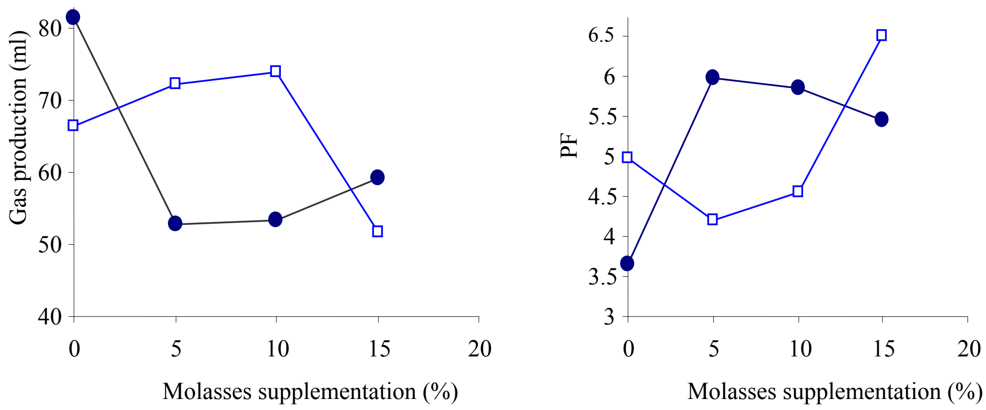

Figure 1. The interaction effect of molasses supplementation and rice straw treatment (●untreated; □urea treated) on gas production (a) and partitioning factor(PF) (b) of sample incubated for 24 h

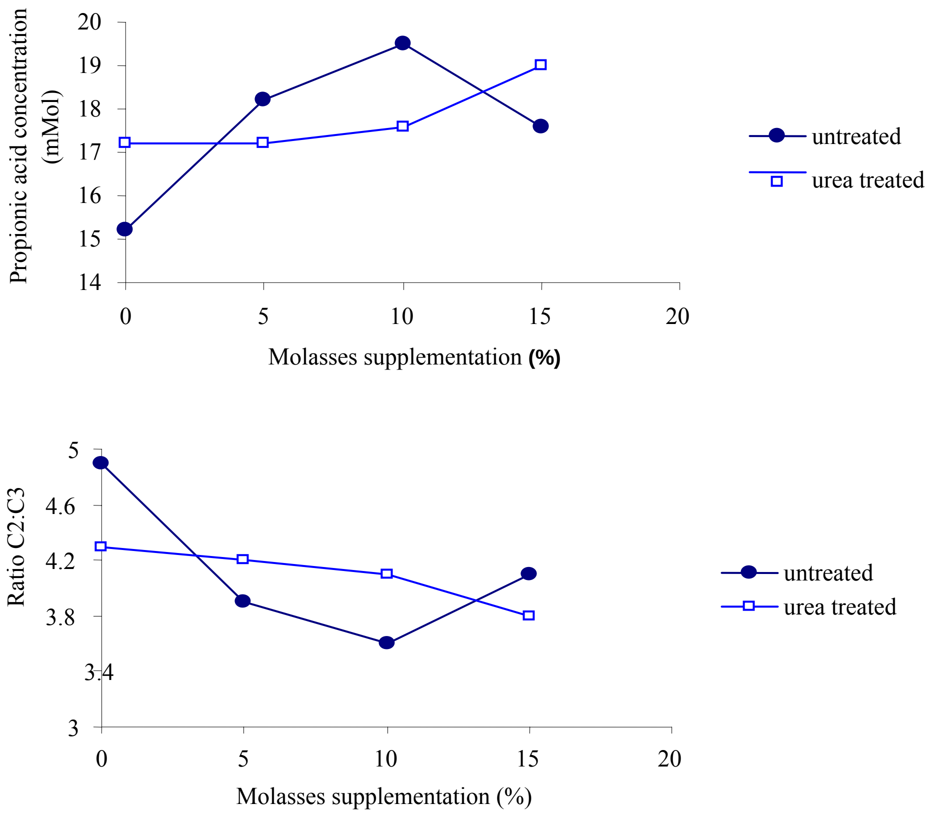

Figure 2. The interaction effect of molasses supplementation and rice straw treatment on propionic acid proportion (a) and ratio of acetic to propionic acid proportion (b) of sample after incubated for 24 h

Table 3. The mean values of mean factor effect of rice straw treatment and molasses supplementation on total volatile fatty acid production (mMol) and molar proportion of acetic (AA), and butyric acid

	Total VFA (mMol)	AA	Butyric
Means of rice straw treatment			
Untreated	55.5[b]	71.1	7.86[a]
Urea treated	71.1[a]	72.4	7.09[b]
S.E.M	1.97	0.57	0.168
Significance	0.01	n.s	0.01
Means of molasses supplementation			
0	66.5[a]	73.8[a]	6.83[c]
5	62.8[ab]	72.2[ab]	7.37[bc]
10	58.0[b]	70.1[c]	8.00[a]
15	67.3[a]	71.4[bc]	7.51[ab]
S.E.M	2.78	0.86	0.341
Significance	0.05	0.01	0.01

Values in the same column with different superscript is significantly different ($P<0.05$)

S.E.M = standard error of treatment mean
n.s = non significant
VFA = volatile fatty acid
AA = Acetic acid

butyric acid was affected by rice straw treatment and molasses supplementation. On the other hand the AA production was only significantly affected by molasses supplementation. Molasses supplementation to untreated rice straw mulberry mixed diet significantly increased propionic acid proportion (Figure 2a). Level of supplementation at 5% already increased propionic acid proportion, supplementation to higher level did not show further significantly increase of propionic proportion. Supplementation to urea treated rice straw mulberry mixture, however, did not affect molar proportion of propionic acid. The effect of supplementation on the ratio of acetic to propionic acid followed a similar pattern to propionic proportion (Figure 2b). In this study, molasses supplementation to untreated rice straw mulberry mixed diet significantly increase molar proportion of propionic acid at all levels of supplementation, as a result ratio of C2/C3 decreased at all level of supplementation. In the diet untreated rice straw mulberry mixed supplemented with molasses, the lower GP (Figure 1a) was followed by the increase of propionic acid (Figure 2a). This could be caused by the fermentation of rapidly fermentable substrate such as molasses would probably yield higher proportion of propionate, which led to a lower gas volume per-unit VFA generated (BLUMMEL and ORSKOV, 1993).

Ruminants synthesized glucose from propionic acid and amino acids. Therefore the higher proportion of propionate is desirable, since the propionate is a precursor for tissue synthesis. This glucose appears to be an essential metabolite for a number of tissues particularly the red blood cell and the brain (PRESTON and LENG, 1987). In roughage diet, propionate is very important because it contributes to 80-90% of glucose synthesis (PRESTON and LENG, 1987). The higher propionate concentration in the rumen as well as higher propionate to acetate ratio resulted higher growth rate. Therefore, molasses supplementation to urea treated rice straw mulberry diet provides beneficial effect by producing higher propionic acid proportion.

CONCLUSION

Molasses supplementation to urea treated mulberry mixed diet increased gas production, molar proportion of propionic acid, and decreased ratio acetic to propionic acid. Supplementation at 5% was sufficient in improving the parameters observed, higher supplementation did not give further benefit.

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