DOI: http://dx.doi.org/10.14334/Proc.Intsem.LPVT-2016-p.115-121

Total Gas and Methane Evaluation of Rejected Mungbean Meal (*Vigna radiata*) with Jackfruit Leaves Addition

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ABSTRACT

Protein protection on rejected mungbean meal (RM) using tannins derived from jackfruit leaves is aplicable methods. The purpose of this study was to evaluate total gas and rumen fermentation characteristics of rejected mungbean meal with jacfruit leaves (JL) addition. Methane production was also observed to determine the efficiency of RM as feed after JL addition. Treatments consisted of RM (rejected mungbean meal), RM + 0.7% JL and RM + 1.4% JL. Experimental design was a completely randomized design with three treatments and three replicates. Gas production were measured at 0, 2, 4, 6, 8, 10, and 24 h of incubation. Methane production were measured after 24 h incubation. Rumen fluid-buffer was taken after 24 h incubation and analysed for pH, NH_3, total VFA, and protozoa population. Addition of either 0.7 and 1.4% JL significantly reduced the total gas production at 24 h incubation. RM with 0.7% JL addition reduced methane concentration (%), methane production (ml/IVOMD) and protozoa population (cell/ml). It is concluded that addition of jackfruit leaves reduced optimum total gas production, while addition of either 0.7 and 1.4% jackfruit leaves reduced methane production and protozoa population. The best dose of jackfruit leaves to protect rejected mungbean meal is 0.7%.

Key Words: Methane, Jackfruit Leaves, Rejected Mungbean Meal, Total Gas

INTRODUCTION

Agriculture byproduct are important feeds for domesticated ruminants in Indonesia. Rejected mungbean meal is one of the potential protein source that has not exploited well. Habibullah et al. (2007) reported that mungbean meal contained 20.8% crude protein (CP) and it can also fulfill the minerals deficiency. Chumpawadee et al. (2005) reported that mungbean meal contained 18.46% of CP but it has a low levels of rumen undegradable protein (RUDP) that is 34.31% and undegradable intake protein (UIP) 35.23%. It is therefore suggested that rejected mungbean meal is a potential feed source but its protein should be protected from ruminal degradation. This is because protein is an expensive ingredients so that it must be used efficiently.

Protein passing through the rumen will be degraded by rumen microbes into smaller molecules such as peptides, amino acids and ammonia. These rumen degradation products will be used for rumen microbial protein synthesis. Proteins derived from microbes has not been able to meet the ruminant nutrient requirements. Therefore it is necessary to guarantee that part of the protein in feed could pass the rumen undegraded in the small intestine (Ali et al. 2009). Availability of protein in intestine is necessary for the host, espescially for growing, pregnant, birth, and lactating animal (Klopfenstein 2006). Protected protein feeding to lactating animals leads to increasing proportion of amino acids supply for productive and reproductive purpose (Shelke et al. 2012). Various treatments have been success in protecting dietary protein from ruminal degradation such as using formaldehyde (Dutta & Agrawal 2000), chelate binding with mineral (Haryanto 2012) and

organic protectants (tannin) (Bunglavan & Dutta 2013). The use of formaldehyde and other chemical for protein protection are not enviromentally friendly way if applied in the organic farming (Bunglavan & Dutta 2013). The best alternative is using organic protection such as condensed tannins as secondary metabolites that protecting protein from rumen degradation.

Protein protection using tannins in jackfruit leaves could be more aplicable. Since jackfruit trees growing in tropical climate and locally available for use by the farmer. It was reported that jackfruit leaves (leaf + petiole) containing 130 g/kg condensed tannins (Kongmanila & Ledin 2009). *In vitro* experiment is need to evaluate JL added in RM. *In vitro* gas test method has been selected for single feed screening and has a high correlation compared with *in vivo* methods (Menke et al. 1979; Getachew et al. 2000; Hamid et al. 2007). Therefore, the purpose of this study was to evaluate total gas and rumen fermentation product of rejected mungbean meal with jacfruit leaves addition. Methane production also observed to determine the effect of tannins derived from jackfruit leaves to improve efficiency of rejected mungbean meal fermentation.

MATERIAL AND METHODS

Samples preparation and treatments

Rejected mungbean meal and jackfruit leaves were dried at 60°C for three days and then ground to pass a fine particle size. Samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash content by procedures of AOAC (2010). NDF and ADF were analyzed using Goering & Van Soest (1970) procedures. Rumen fluid obtained from fistulated male buffalo.

In vitro procedures

Amounts of 380 mg samples were weighed and transferred into 100 ml syringe (Fortuna model, Germany). The rumen fluid was collected before the morning feeding from fistulated male buffalo fed on roughage and concentrate based diets (50:50 based on DM). The rumen fluid was strained and filtered use nylon cloth. The glasswares were kept at approximately 39°C before use. The substrates were added with 40 ml of rumen fluid-buffer following the method of Menke et al. (1979) modification by Blümmel et al. (1997). The incubation was carried out at 39°C for 24 h. All of measurements were repeated three times as replicate. Gas production measurements were performed at 0, 2, 4, 6, 8, 10, and 24 h incubation time. Methane production were measured after 24 h incubation. The rumen fluid-buffer contained in syringes were sampled for analysis of pH, NH_3, total VFA, and protozoa population.

Experimental methods and data analysis

Experimental design of this study was a completely randomized design with three treatments and three replicates. All treatments were described as follows: (1) RM (rejected mungbean meal); (2) RM + 0.7% jackfruit leaves (DM); and (3) RM + 1.4% jackfruit leaves (DM).

Variables measured were total gas production after 0, 2, 4, 6, 8, 10, and 24 h incubation. Methane and carbondioxide production after 24 h incubation were determined using MRU gas analyzer®. Measurement of pH was determined using Hanna instruments pH digital. Measurement of total VFA was done using AOAC (2010). NH_3 measurement

was conducted using Conway microdifusion methods. Protozoa population measurement was carried out according to the method of Ogimoto & Imai (1981). Cumulative total gas production data were fitted to the model of Ørskov & McDonald (1979) using software NEWAY® as follows:

$$P = a + b\ (1\text{-}e^{-ct})$$

P: The gas production at time t; a: The gas production from soluble fraction (ml/380 mg DM); b: The gas production from insoluble fraction (ml/380 mg DM); c: The gas production rate constant (ml/h); (a+b): The potential gas production (ml/380 mg DM); t: The incubation time (h)

Effect of treatment was analyzed using SPSS 16.00 based the test of variance (ANOVA) and differences between treatment were analyzed using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Total gas production

Total gas production from the fermentation of rejected mungbean meal are presented in Figure 1. Total gas production at 2, 4, 6, 8 and 10 h incubation time were not significant. However, Adding both 0.7 and 1.4% jack fruit leaves (JL) significantly ($P<0.05$) reduced total gas production at 24 h incubation. in present study, JL could decreased total gas production after 10 h incubation. This result could be caused by the long time to protect protein. Ruminal microbes need adaption time (lag phase) before degrading the insoluble particle (Sofyan et al. 2015). However, potential gas production (a+b fraction) of RM treatment still the highest one (Table 1).

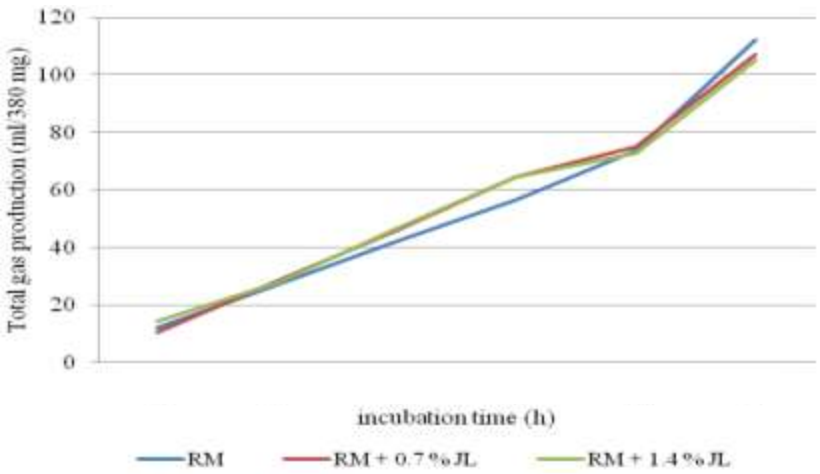

Figure 1. Total gas production of rejected mungbean meal with jackfruit leaves addition

The similar result was also reported by Serensinhe et al. (2012), that supplementation of condensed tannin could reduced forage fermentability as indicated by total gas production. Mohammadabadi & Chaji (2011) using 30 g (DM) tannin extracted from oak leaves and pistachio. Low concentration of tannin addition (0.5 mg/ml rumen fluid-buffer) from mimosa and *quebracho* extract decreased total gas production of hay diet (Jayanegara et al. 2009). the reduced total gas production as affected by tannin could be due to inhibition mechanism on microbes enzym activity. The negative effect of tannins on digestion is not just the result of their interaction with macromolecules in the feeds, but

also interference with fibrolytic and proteolytic enzymes. Tannins can reduce the nutritive value of feedstuffs and they can affect the health of the animal as well (Gürbüz et al. 2008). Gürbüz et al. (2008) also reported that gas is produced mainly when substrate is fermented to acetate and butyrate, therefore relatively lower gas production is associated with propionate production.

Table 1. Gas characteristics of rejected mungbean meal with jackfruit leaves addition

Variables	RM	RM + 0.7% JL	RM + 1.4% JL	SEM
a + b (ml/380 mg DM)	133.180[a]	115.850[b]	114.740[b]	3.599
c (ml/h)	0.083[b]	0.116[a]	0.110[a]	0.006
CH_4 (%)	13.980[a]	9.860[b]	13.610[a]	0.694
CO_2 (%)	52.560	60.280	59.900	2.059
CH_4 (ml/IVOMD)	4.960[a]	3.610[b]	4.640[a]	0.231
CO_2 (ml/IVOMD)	18.710	22.160	20.400	0.956
$CO_2 : CH_4$	3.770[b]	6.120[a]	4.430[ab]	0.390

a+b: Potential gas production; c: Gas production rate; DM: Dry matter; RM: Rejected mungbean meal; IVOMD: *In vitro* organic matter digestibility; JL: Jackfruit leaves; SEM: Standard error of mean; Number with different superscript in the same row, significantly different with $P<0.05$

Gas characteristics

Gas charecteristics evaluated by *in vitro* gas production were showed in Table 1. RM had higher potential total gas production (a+b) ($P<0.05$) compared to the other. However, gas production rate (c) of RM treatment was the lowest compared to the others. RM with 0.7% JL addition reduced both CH_4 concentration (%) and CH_4 production (ml/IVOMD) ($P<0.05$) but had no effect on CO_2 concentration (%) and CO_2 production (ml/IVOMD). RM with 0.7% JL addition also had the highest efficiency of C utilization for CO_2 production ($CO_2 : CH_4$ parameters) ($P<0.05$).

Fermentability evaluated by *in vitro* gas production was indicated by kinetics gas production parameters (Sofyan et al. 2015). Adding 0.7 and 1.4% JL could reduce potential gas production from RM fermentation in the rumen. Pure tannin can reduce total gas production from insoluble fraction (b fraction) (Mohammadabadi & Chaji 2011). Energy lost was reflected by high methane emission. Adding 0.7% JL could reduce methane emission from RM fermentation in the rumen that might be caused by tannins derived from JL. Tannins decrease the population of the principal actor of methanogenesis in the rumen (methanogens) (Jayanegara et al. 2015). Decrease in methane production also could be mediated through decrease in protozoal number (Table 2). Methanogens bacteria and protozoa have symbiotic relationship in the rumen (Morgavi et al. 2010). Bhatta et al. (2015) also reported that methane production was closely related to the protozoa number. Methane was generated by Archaea bacteria that consumed hydrogen. This bacteria activity have symbiotic with protozoa. The mechanism of effect of condensed tannin on methanogenesis is not completely understood. Tannin may be directly inhibit methanogen microbes growth in the rumen. Tannin also indirectly decreasing the nutrients availability for rumen microbes. Another possibility is that tannin is hydrogen acceptors and reduces the hydrogen availability for producing CH_4 (Naumann et al. 2013).

Rumen fermentation products

In vitro rumen fermentation products were presented in Table 2. The pH values, NH_3 concentration and TVFA concentration indicated that there were no significant different. However, adding 0.7% JL could reduce protozoa population ($P<0.05$). The NH_3 results were higher than optimal standard for closed-system culture microbes fermentation. The higher value caused by cumulation from RM fermentation product. Optimal concentration of NH_3 for microbes fermentation in closed-system culture is 5 mg/100 ml, but it depends on the rate of feed fermentability (Wanapat & Rowlison 2007; Wanapat et al. 2013).

Table 2. *In vitro* rumen fermentation product of rejected mungbean meal with jackfruit leaves addition

Treatment	pH	NH_3 (mg/100 ml)	TVFA (mM)	Protozoa ($\times 10^6$ cell/ml)
RM	6.940	25.000	62.000	2.580^{a}
RM + 0.7% JL	6.840	35.000	49.330	1.420^{b}
RM + 1.4% JL	6.950	25.000	58.670	2.420^{ab}
SEM	0.042	4.772	2.944	0.236

RM: Rejected mungbean meal; SEM: Standard error of mean; TVFA: Total volatile fatty acid

Adding JL had no effect on TVFA production. In contrast, El-Waziry et al. (2007) studied that TVFA decrease significantly in soybean meal added with pure tannin. The addition of 0.5 mg/ml pure tannin on the substrate of haydiet can reduce TVFA production by 5.7-11.7%. Tannin have a strong binding capacity for protein component, so it can protect from rumen degardation process (Cortes et al. 2009). Lower TVFA value is a reflection from the changes of rumen microbial population (Pamungkas et al. 2006). The contrast value from present study caused by the effect from the difference in the level of purity from tannin substrate. Protozoa population was reduced because the protozoa number was closely related with methane emission (Sofyan et al. 2015) (Table 2). Methanogens bacteria and protozoa have symbiotic relationship in the rumen (Morgavi et al. 2010).

CONCLUSION

Addition of jackfruit leaves reduced maximum total gas production. Addition of 0.7% jackfruit leaves also reduced methane production and protozoa population. Addition of 0.7% JL has greater effects than addition at 1.4%.

ACKNOWLEDGEMENT

The author wish to thank Shintia Nugrahini Wahyu Hardani, AMd., Dedi Ansori, Mar'atus Sholihah and all members in Animal Nutrition Laboratory. Gratitude is also expressed to Mr. Dinardi and others who helped in the field during this project.

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