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Effect of Superblock Supplementation to Native Grass Based Diet on Rumen Fermentation *In Vitro*

Wahyono T, Hardani SNW, Firsoni

Agricultural Division, Center of Application for Isotope and Radiation, National Nuclear Energy Agency
Jl. Lebak Bulus Raya No. 49, Cilandak, South Jakarta 12070
teguhwahyono@batan.go.id

ABSTRACT

Superblock is the new generation of feed supplement which formulated from rice bran, molasses, soybean meal, urea, salt, and lacta-mineral. *In vitro* experiment was carried out to evaluate the effect of Superblock, supplemented in native grass based diet. Treatments evaluated were: C (60% native grass + 40% rice bran) and S (C + Superblock). Experimental design of this study was completely randomized design with two treatments and six replication. Gas production measurements were performed at 0, 2, 4, 6, 8, 10, 12, 24, and 48 h. Methane and carbondioxide concentration were measured after 48 h incubation. *In vitro* organic matter digestibility (IVOMD), energy metabolism (EM) and total volatile fatty acids (TVFA) were calculated from the total gas production after 24 h incubation. Superblock supplementation in native grass based diet could increase optimum total gas production (13.37%), IVOMD (8.45%), EM (7.28%), TVFA (10.41%) and methane production (4.67%). It was concluded that Superblock supplementation to native grass basal diet increased total gas production, IVOMD, EM and TVFA production. However, methane production also increased which is unwilling result therefore more appropiate strategy is needed to overcome this problem.

Key Words: *In Vitro* Study, Rumen Fermentation, Superblock, Total Gas

INTRODUCTION

The use of feed supplement in ruminant diet is one of the potential strategy to improve the animal production, because it can optimize the rumen ecosystem as a vital digestive tract in ruminant (Haryanto 2015). Feed supplement could be probiotics, natural antibiotics from herbs (Jouany & Morgavi 2007) and the formulation from various feedstuffs. Many feed ingredients derived from agricultural and food by-products can be utilized in the feed supplement formulation. Roza et al. (2013) reported that there are many ways to produce feed supplement from by-product materials. However, the main principle of the supplement is to provide principal nutrient for microbial activity such as minerals, protein and soluble carbohydrates. Some study on feed supplement using by-product materials were reported by Roza et al. (2013), Wanapat & Khampa (2006), Suharyono et al. (2014) and Firsoni & Suharyono (2008). Indonesia national nuclear energy agency (BATAN) using tracer-radioisotope (^{32}P) technique previously reported that the formulation of agricultural and food by-products has resulted in feed supplement for ruminants. This supplements were urea molasses multinutrient block (UMMB), multinutrient feed supplement (MFS) and multinutrient feed supplement without molasses (MFSWM) (Suharyono 2009; Wahyono et al. 2010).

Superblock is the new generation of feed supplement which was formulated from agricultural and food by-product consisted of rice bran, molasses, soybean meal, urea, salt and lacta-mineral. *In vitro* experiment is needed to evaluate the effect of Superblock, supplementation in native grass based diet. *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 *in vitro* rumen fermentation product of native grass based diet suplemented with Superblock.

MATERIAL AND METHODS

Samples preparation

Native grass, rice bran and Superblock were dried at 60°C oven for three days and then ground to pass a fine particle size. Rumen fluid obtained from fistulated male buffalo fed a 60:40 concentrate:field grass. Buffalo was fed twice daily at 8.00 am and 4.00 pm. Rumen fluid was collected from middle part of the rumen.

In vitro gas production

The rumen strained and filtered through nylon net. The glassware infused with CO_2 and kept at approximately 39°C before use. Amounts of 380 mg DM samples were added with 40 ml of rumen fluid-buffer in 100 ml syringe (Fortuna model, Germany) by following the method of Menke et al. (1979) modification by Blümmel et al. (1997). The incubation was carried out in waterbath at 39°C for 48 h. All of measurements were repeated six times. Gas production measurements were performed at 0, 2, 4, 6, 8, 10, 12, 24, and 48 h. Methane and carbondioxide production were measured after 48 h incubation.

Experimental methods and analysis

Experimental design of this study was completely randomized design with two treatments and six replicates. All treatments descripted as follows: C (60% field grass + 40% rice bran) and S (C + 3.42 mg Superblock).

Variables measured were total gas production after 0, 2, 4, 6, 8, 10, 12, 24, and 48 h incubation. Methane and carbondioxide production after 48 h incubation were determined using MRU gas analyzer®. Cumulative total gas production data were fitted to the model of Ørskov & McDonald (1979) using software NEWAY® as follows:

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

Where, P is the gas production at time t, a is the gas production from soluble fraction (ml/380 mg DM), b is the gas production from insoluble fraction (ml/380 mg DM), c is the gas production rate constant (ml/h), (a+b) is the potential gas production (ml/380 mg DM) and t is the incubation time (h).

In vitro organic matter digestibility (IVOMD) and metabolisable energy (ME) were calculated by equation of Menke et al. (1979). Total volatile fatty acids (TVFA) were calculated by equation of Getachew et al. (2000):

IVOMD (%) = 4.88 + 0.889 GP + 0.45 CP + 0.0651 ash
ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP
TVFA (mmol) = 0.0239 GP – 0.0601

GP was the nett gas production after 24 h incubation. CP and ash were crude protein and ash in g/100 g DM. ME and TVFA were converted to kcal/kg DM and mM. Effect of treatment was analyzed using SPSS 16.00 based the test of variance (ANOVA).

RESULTS AND DISCUSSION

Total gas production

Total gas production from the fermentation of samples are presented in Table 1. Total gas production at 2, 4, 24, and 48 h incubation time were highly significant (P<0.01). Total gas production at 6, 10, and 12 h incubation time were significant (P<0.05). However, Adding Superblock were not significantly increase total gas production at eight hours incubation. In present study, the increase pattern in total gas showed that Superblock could improve substrate digestibility in the early and late hours of incubations.

Table 1. *In vitro* total gas production of diet based fieldgrass

Treatment	Incubation time (h)							
	2	4	6	8	10	12	24	48
C	6.420	11.980	17.870	24.720	29.070	36.640	51.680	61.620
S	8.740	14.560	20.250	26.910	33.560	39.440	56.800	69.190
SEM	0.463	0.536	0.580	1.058	1.023	0.677	1.034	1.451
Significant	**	**	*	NS	*	*	**	**

C: 60% field grass + 40% rice bran; S: C + 3.42 mg Superblock; SEM: Standard error of mean; NS: Non-significant; *Significant at P<0.05; **Significant at P<0.01

Total gas production from *in vitro* fermentation reflects the extent of feed fermentation and digestibility (Getachew et al. 2000). *In vitro* total gas production increase by Superblock supplementation. This is caused by Superblock increase the microbes activity and feed degradability in rumen. Some of feed ingredients on Superblock is source of minerals, urea and molasses that can improve microbes activity. Minerals are required for microbial chemical reactions. Supplementation urea as soluble nitrogen in rumen ecosystem increase ammonia concentration (NH_3) which is required for microbial protein synthesis (Haryanto 2015). Molasses also has characteristics of rapidly fermented substrate, and high total gas production associate with sucrose concentration (Ferraro et al. 2009). This mechanism could increase degradability which are represented in total gas production. Fazaeli et al. (2012) reported that variations in gas production is the result of variations in chemical composition. The increase of total gas production not yet represented the efficiency of feed substrate utility. The feed efficiency could be observed from variable of gas characteristics (Table 2).

Table 2. *In vitro* gas characteristics of diet based field grass after 48 h incubation

Treatment	Treatment		SEM	Significant
	C	S		
a + b (ml/380 mg DM)	64.60	73.24	1.697	**
c (ml/h)	0.069	0.063	0.002	NS
CH_4 (%)	14.75	14.84	0.067	NS
CO_2 (%)	58.21	59.34	1.092	NS
CH_4 (ml/IVOMD)	4.92	5.15	0.053	*
CO_2 (ml/IVOMD)	19.43	20.58	0.393	NS
CO_2:CH_4	3.95	4.00	0.081	NS

C: 60% field grass + 40% rice bran; S: C + 3.42 mg Superblock; a+b: Potential gas production; c: Gas production rate; DM: Dry matter; IVOMD: *In vitro* organic matter digestibility; SEM: Standard error of mean; NS: Non-significant; *Significant at P<0.05; **Significant at P<0.01

In vitro gas characteristics

In vitro gas characteristics shown in Table 1. The potential total gas production (a+b) of S treatment was significantly 13.37% higher than C treatment (P<0.01). However, CH_4 production (ml/IVOMD) of S treatment also higher than C treatment (P<0.05). There is no significant difference between C and S treatment in gas production rate (c), CH_4 concentration (%), CO_2 concentration (%), CO_2 production (ml/IVOMD) and CO_2:CH_4 rate. Sofyan et al. (2015) reported that fermentation evaluation by *in vitro* gas production indicated with kinetics gas parameters. Higher value of potential gas production in S treatment indicated that superblock can increase the microbial activity in the rumen.

The increase of microbial activity followed by increasing in protozoa growth population. It could be seen from the higher value of methane production in S treatment. Some of feed ingredients on superblock is molasses and minerals. Molasses ingredient in supplement may increase protozoa population (Suharyono et al. 2010). Suharyono & Widiawati (2007) also reported that molasses is easily digested carbohydrates which are major feed for protozoa. Bhatta et al. (2015) also reported that methane production was related to the protozoa population. Methane was generated by methanogens bacteria that consumed hydrogen. Supplementation of 3.42 mg superblock increase methane production and indicated the low efficiency of energy use of feed by microbial activity. Energy lost was represented by high methane emission. This present study contradiction to Se-Young et al. (2011) that using glycerol as supplement decreased methane production and increased substrate efficiency. Prasetiyono et al. (2007) also reported that supplementation of protein supplement (SPN) in rice straw and bran based ration can decrease methane production. This difference perhaps due to the differences of ingredients in each supplement, which superblock contains molasses that can increase easily digested substrate for protozoa.

Rumen fermentation products

Calculation of *in vitro* rumen fermentation products of diet based fieldgrass are presented in Table 3. The IVOMD, EM and TVFA results were higher in S treatments than C treatment (P<0.01). The increase of IVOMD, EM and TVFA were a representation from *in vitro* total gas production after superblock supplemented (Table 1). Minerals, urea and molasses that contained in superblock can support microbial growth and improve fermentation activity.

Table 3. Calculated *in vitro* rumen fermentation product of diet based fieldgrass

Treatment	IVOMD (%)	EM (kcal/kg DM)	TVFA (mM)
C (60% field grass + 40% rice bran)	55.29	2322.29	29.38
S (C + 3.42 mg Superblock)	59.96	2491.39	32.44
SEM	0.932	33.902	0.618
Significant	**	**	**

IVOMD: *In vitro* organic matter digestibility; EM: Energy metabolisable; TVFA: Total volatile fatty acid; SEM: Standard error of mean; **Significant at P<0.01

The production of TVFA in present study was 29.38 and 32.44 mM. This value was lower than the range of TVFA production in rumen. The range of TVFA in the rumen are 50-100 mM (forages fed) and 80-150 mM (concentrate fed) (Owen & Goetsch 1988;

Jayanegara & Sofyan 2008). The lower value in TVFA production from present study due to the conditions of closed fermentation system. It could be give negative effect for microbial activity (Jayanegara & Sofyan 2008). The lower TVFA value is a reflection from the changes of rumen microbial population (Pamungkas et al. 2006). The increasing IVOMD after Superblock supplementation will be followed with the increasing of EM. This is caused by the improved of balance microbial activity in rumen ecosystem as Superblock contained molasses as higher fermentable carbohydrates and urea as available nitrogen source. The higher fermentable carbohydrates and available nitrogen source make a better nutrient availability for rumen microbial activity that represented in IVOMD and ME (Ahmed et al. 2007).

CONCLUSION

Supplementation of superblock increase total gas production, IVOMD, EM and TVFA production from diet based field grass. However, methane production also increased, so there is still need more appropriate strategy to overcome this problem.

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