

## COMPLETE RUMEN MODIFIER SUPPLEMENTATION IN CORN COB SILAGE BASAL DIET OF LAMB REDUCES METHANE EMISSION

### *Suplementasi Complete Rumen Modifier dalam Pakan Silase Tongkol Jagung untuk Menurunkan Emisi Metana pada Domba*

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Submitted 3 April 2017; Revised 10 May 2017; Accepted 18 May 2017

#### ABSTRACT

Feeding animal with fibrous materials such as corn cob will emit methane. Complete rumen modifier (CRM) is an improved feed additive comprised a mixture of *Sapindus rarak*, sesbania, albizia leaves and minerals that functions as a methane inhibitor. The study aimed to determine the effect of CRM supplementation on the feed intake, nutrient digestibility, rumen fermentation, methane emission and growth of lambs. The experiment was designed in a complete randomized block, four levels of CRM (0%, 1%, 2%, and 3%), six group of 24 male lambs per treatment based on the body weight. Basal diet used was corn cob silage *ad libitum* and concentrate (500 g/day) as a supplement. The results showed that CRM supplementation did not affect feed consumption and average daily gain, but significantly decreased the dry matter, as well as organic matter and protein digestibility. The neutral detergent fiber (NDF) and acid detergent fiber digestibility linearly decreased with increasing level of CRM. Ruminal pH, ammonia concentration and volatile fatty acid concentration were not affected by the CRM supplementation. Methane production expressed in kJ/MJ gross energy or digestible energy intake significantly decreased by 32% at the 2–3% CRM supplementation and reduced by 39% when methane production was expressed in g/kg digested NDF. It can be concluded that 2% CRM supplementation in the corn cob basal diet did not affect nutrient intake and growth rate of the lamb, as well as rumen fermentation. The study suggests that CRM is an environmentally friendly feed additive for lamb.

[**Keywords:** complete rumen modifier, corn cob, lamb, methane, silage]

#### ABSTRAK

Pemberian pakan berserat seperti tongkol jagung pada domba dapat menghasilkan gas metana sebagai salah satu gas rumah kaca. Complete rumen modifier (CRM) merupakan pakan aditif yang mengandung buah lerak, daun sesbania dan albizia, serta campuran mineral dan vitamin yang berfungsi sebagai inhibitor metana. Penelitian bertujuan mengetahui pengaruh suplementasi CRM terhadap konsumsi pakan, pencernaan nutrisi pakan, fermentasi rumen, emisi metana, dan pertumbuhan domba. Penelitian menggunakan rancangan acak kelompok lengkap dengan empat level CRM (0%,

1%, 2%, 3%) dan enam kelompok domba (masing-masing kelompok 24 ekor), berdasarkan bobot badan. Domba diberi pakan dasar silase tongkol jagung *ad libitum* dan konsentrat 500 g/hari. Hasil penelitian menunjukkan bahwa suplementasi CRM tidak berpengaruh terhadap konsumsi pakan dan pertambahan bobot badan harian domba, tetapi menurunkan pencernaan bahan kering, bahan organik, dan protein. Pencernaan serat detergen netral (NDF) dan serat detergen asam (ADF) menurun secara linier seiring dengan meningkatnya suplementasi CRM. pH rumen, konsentrasi amonia, dan VFA tidak dipengaruhi oleh suplementasi CRM. Produksi metana dalam kJ/MJ konsumsi energi kasar (GE) atau energi tercerna (DE) turun 32% pada suplementasi CRM 2–3% dan berkurang 39% jika dihitung berdasarkan g/kg serat detergen netral tercerna. Dapat disimpulkan bahwa suplementasi CRM 2% pada pakan dasar silase tongkol jagung tidak berpengaruh terhadap konsumsi pakan, pertumbuhan domba, dan fermentasi rumen. Oleh karena itu, CRM dapat disarankan sebagai pakan aditif pada domba.

[**Kata kunci:** complete rumen modifier, domba, metana, silase, suplementasi, tongkol jagung]

#### INTRODUCTION

The major constraint in improving ruminant production in Indonesia is discontinuous availability of forage feed throughout the year, particularly in a dry season when the forage sources are limited. Utilization of agricultural by-products such as corn cob is usually practiced to overcome this problem. However, corn cob is unpalatable and easily contaminated by toxic fungi such as *Aspergillus flavus*. This contamination could be solved by ensiling, which is a simple and an applicative preservation method to small scale farmers. Ensiled corn cob is very palatable as shown by a higher feed consumption of sheep fed on ensiled corn cob basal diet than that fed on grass basal diet (Yulistiani and Puastuti 2012). Although it is palatable, the fiber content of corn cob silage was high (Yulistiani et al. 2012a), which is

potential in producing methane during fermentation in the rumen.

Methane emission by ruminants has negative effects on the animal and causes greenhouse effect to the environment. Methane which is an end product of fermentation process of feed is formed through methanogenesis. Methanogenesis cause 2–12% energy loss of the digested energy depending on diet type (Johnson and Johnson 1995). Methane emission from enteric fermentation contributes 25% to the total global greenhouse gases from agriculture (Oliver et al. 2005).

Previous studies showed that saponin feed additive is effective to inhibit methanogenesis through defaunation mechanism on protozoa population. Saponin is a plant bioactive compound and can be obtained from *Sapindus* spp. (Hess et al. 2003), tea (Mao et al. 2010; Zhou et al. 2011), *Carduus*, *Knautia*, sesbania leaves and fenugreek seed (Goel et al. 2008), *Albizia lebbek* (Sirohi et al. 2014), and mangosteen peel (Sineenart et al. 2016). Saponin in *Sapindus rarak* was effective as a defaunator and methanogenesis inhibitor (Thalib 2004; Wina et al. 2005). Supplementation of crude extract of *S. rarak* in the diet increased average daily gain (ADG) of sheep by 40% (Wina et al. 2005b).

Complete rumen modifier (CRM) is a mixed feed additive formulated from *S. rarak*, sesbania and albizia with the addition of minerals and vitamins to promote microbial growth. The formulae have been developed at the Indonesian Research Institute for Animal Production (Thalib et al. 2010) based on the results of serial experiments. As a single feed additive, *S. rarak* (seed pericarp) was able to reduce protozoa population and increase the growth of sheep by 44% (Thalib et al. 1996). It also decreased methane production and protozoa population, but enhanced propionate production (Thalib 2004). Those findings indicated that saponin from *S. rarak* is effective as a protozoal defaunator and methanogenesis inhibitor.

A mixture of *S. rarak* with minerals and vitamins (microbial growth factors, MGF) decreased protozoa population and increased bacterial population and rice straw digestibility (Thalib et al. 1998). In *in vivo* study, supplementation of *S. rarak* and MGF mixture in grass basal diet increased the growth of sheep and feed digestibility (Thalib 2002). To increase protein and saponin contents of ground *S. rarak* seed pericarp, the finely ground sesbania and albizia leaves, respectively, containing 8.4% and 12.9% saponin and 26.3% and 24% protein are added (Thalib et al. 2010). This mixture was then added with vitamin and minerals to formulate CRM. Minerals and vitamins have a function as a bacterial growth factor, fiber digestion stimulator and CO<sub>2</sub> anti-reductance. Therefore, CRM serves as a defaunator,

methanogenesis inhibitor, bacterial growth factor, fiber digestion stimulant and CO<sub>2</sub> anti-reductance.

The use of CRM as a feed additive in rice straw basal diet was reported to reduce enteric methane production, increase average daily gain and improve feed efficiency in sheep (Thalib et al. 2010). In dairy cow fed on grass basal diet, CRM supplementation reduced methane production by 14% and improved milk quality (Thalib et al. 2011). In dairy goat diet, supplementation of CRM mixed with *Calliandra* increased milk production and production efficiency of Etawah cross bred goat and reduced methane emission (Sukmawati et al. 2011). Utilization of CRM as a methane inhibitor and stimulator of fiber digestibility also occurred on grass and rice straw basal diet. *In vitro* study on rumen fluid of sheep fed on corn cob basal diet supplemented with CRM showed that protozoa population and methane production were lower than those of the control (Yulistiani et al. 2012b). Currently, no information is available on *in vivo* study of CRM supplementation in corn cob basal diet for sheep and its effect on methane emission and growth of lamb. The objective of this study was to determine the effect of CRM supplementation on nutrient digestibility, rumen fermentation, methane emission, and growth of lamb fed on corn cob silage basal diet.

## MATERIALS AND METHODS

The study consisted of two experiments. The first experiment was conducted in the research station of the Indonesian Research Institute for Animal Production (IRIAP), Bogor, to measure the effect of CRM supplementation in corn cob basal diet on growth of lamb, nutrient digestibility and rumen fermentation. The second experiment was conducted in the field laboratory of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang to measure methane production of lamb fed on corn cob basal diet and supplemented with CRM.

### Feeding Trial and Growth Study

#### Feed Preparation

Corn cob was obtained after the grain was removed from corn field in Sukabumi, West Java. The cob was ground into 1 cm mesh (similar size to corn grain) and stored in plastic bags. Corn cob silage was prepared by mixing 100 kg corn cob (94% dry matter, DM) with 2 kg finely ground maize (2% of ground corn cob DM) as a source of fermentable carbohydrate, then sprayed with water at 60% of corn cob DM to obtain a mixture DM of 30–40%.

The mixture was thoroughly mixed and transferred into black plastic bags. The bag (15 kg treated straw per bag) was carefully trampled to remove the air, tightly sealed and stored for at least 3 weeks. After the curing period, the corn cob silage was ready for feeding.

CRM was prepared using *S. rarak* seed pericarp, sesbania and albizia leaves (Thalib 2004). The seed pericarp was separated from seed, dried under sunshine, finely ground and kept for treatments. Similarly, sesbania and albizia leaves were dried and finely ground. The CRM was formulated by mixing finely ground *S. rarak*, sesbania and albizia leaves with some microminerals and vitamins. The 1%, 2% and 3% CRM contained saponin of about 4.56%, 9.12% and 13.68%, respectively.

## Animals and Diets

A digestibility study was conducted using 24 male lambs of Sumatra composite breed sheep with an average body weight of  $14.8 \pm 1.66$  kg. The lambs were divided into four groups in a completely randomized design. The lambs were kept in individual pen during the experimental period (13 weeks) and fed corn cob silage as a basal diet with four supplemental treatment diets. The diet treatments were the level of CRM supplementation in concentrate diet which consisted of 0% (control), 1%, 2%, and 3% CRM. The concentrate was formulated in iso-nitrogenous and iso-energetic containing calculated crude protein (CP) of 18% and metabolizable energy (ME) of about 9 MJ/kg. The concentrate was offered at 500 g/head/day while corn cob silage offered *ad libitum*. The concentrate consisted of coconut meal, rice bran, ground corn grain, soy bean meal, molasses, urea, salt and mineral mix. Water was available at all time. The chemical composition of feed ingredients used in experiment is presented in Table 1.

## Digestibility Trial and Growth Study

The growth trial was conducted for 13 weeks included one week adaptation period. Lambs were weighed on weekly basis to observe the growth rate. Feed offered

and refusal were weighed daily before morning feeding to measure daily feed intake. Feed conversion ratio was calculated from daily feed intake divided by average daily gain (ADG). Protein conversion ratio was calculated from daily protein intake divided by ADG. The energy conversion ratio is the daily gross energy intake divided by ADG. At the end of feeding trial, the lambs were placed in metabolic crates for digestibility measurement.

The digestibility trial consisted of 14 days for adaptation period, 7 days for feces and urine collection, and 1 day for sampling of rumen fluid. During the collection period, daily feed intake and refusal, fecal and urine output of the individual lamb were measured. Urine and fecal samples were separated by the separator attached below each metabolic crate. Daily fecal output was collected from individual lamb before morning feeding. Each representative portion (10% of total fecal production) of fecal sample was oven-dried at 60°C for 48 hours. At the end of collection period, the feces were pooled for individual lamb and 10% was sub-sampled, ground passed through 1 mm sieve and stored in the freezer for analyses. Total urine produced daily per lamb was collected in a plastic bucket containing 100 ml of 10% sulfuric acid to maintain the pH below 3 to inhibit microbial activity and N losses. The sample was collected every morning and after recording the volume, the urine was mixed thoroughly. A representative of urine sample was collected and kept in the freezer and pooled for each animal at the end of collection period for urine-N analyses.

On the final day of digestibility trial, rumen fluid from individual lamb was sampled using a stomach tube at 0 and 4 hours after morning feeding. Rumen fluid pH was measured immediately after sampling using a portable pH meter. One drop of concentrated sulfuric acid was then added (for stopping microbial activity) and the fluid was later centrifuged at 3000 g for 10 minutes. After centrifugation, 10 ml of each supernatant was kept in air tight container and stored at -20°C for further analyses of rumen ammonia N ( $\text{NH}_3\text{-N}$ ) and volatile fatty acid (VFA).

## Methane Emission Measurement

Measurement of methane emission was conducted in the field laboratory of Diponegoro University, Semarang. Sixteen lambs with an average body weight of  $15.0 \pm 1.53$  kg were kept in individual pen. The lambs were divided into four groups in which each lamb in each group was fed one of the diet treatments. The diet treatments were the same composition to feeding trial study. Lambs were

**Table 1. Chemical analysis of the feed used in the experiment.**

Feed ingredient	Chemical composition (% dry matter)					GE (kcal/ kg)
	CP	OM	NDF	ADF	Lignin	
Corn cob silage	2.77	97.6	79.9	40.60	8.1	4186
Concentrate	19.20	90.0	27.2	17.53	7.8	3994

CP = crude protein; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; GE = gross energy.

adjusted to the diet treatment for 2 weeks to achieve dry matter intake (DMI) with a proportion of concentrate and corn cob silage similar to feeding trial study. During the adaptation period, feed offering and refusal were recorded daily to measure feed intake. At the end of adaptation period, methane emission of the lamb was measured using face mask methods as described by Kawashima et al. (2001). The face mask was connected to a methane analyzer (Horiba, Japan) for determining methane concentration and airflow meter for measuring total air volume. The measurement was done for 10 minutes at 3-hour intervals for 2 days. Methane emission values were expressed in several units, as L/day, L/kg DMI, kJ/MJ GE intake, kJ/MJ DE intake, g/kg digested NDF. The value of digested NDF was calculated by multiplying NDF intake in methane study and NDF digestibility obtained from digestibility trial. Similarly, methane production efficiency was calculated from methane production (L/day obtained from methane measurement study) divided by ADG (g/day, obtained from growth trial). The factor used for converting units of methane production GE (kJ/MJ) and DE (kJ/MJ) were 1L methane = 0.716 g = 39.54 kJ (Ramin and Huhtanen 2013).

### Chemical Analyses

Feeds, residues and feces were analyzed for DM, OM and crude protein (CP) contents according to AOAC (1990). The fiber components (NDF and ADF) were

determined according to van Soest et al. (1991).  $\text{NH}_3\text{-N}$  was determined by Conway method (Conway and Byrne 1933). Concentration of rumen VFA was determined using gas chromatography (GC-14A, Shimadzu Corporation, Japan, Tokyo) fitted with a flame ionization detector. Separation was carried out using a stainless steel column packed with GP 107, SP 1,000/L %  $\text{H}_3\text{PO}_4$  on Chromosorb WAW (100/120 mesh).

### Statistical Analysis

Data were analysed using general linear model (GLM) for randomized complete block design (SAS 2004) and differences among means were compared using Duncan's multiple range test. All data were analysed for linear (L), quadratic (Q) and cubic (C) responses to CRM level using Orthogonal Polynomial contrast of SAS 9.1 (SAS 2004).

## RESULTS

### Feed Consumption, Lamb Growth and Nutrient Digestibility

Feed consumption and ADG of lambs are presented in Table 2. DM consumption was not significantly different between treatments. The average of total DMI (g/day) and DMI per body weight were 698.6 g/head/day and 3.81%, respectively. CP consumption was cubically affected by CRM supplementation. CP consumption at 1% CRM

**Table 2. Feed consumption, average daily gain and feed conversion of lambs fed on silage basal diet supplemented with different levels of complete rumen modifier (CRM).**

Parameter	CRM levels (%)				S.E.M	Effects		
	0	1	2	3		L	Q	C
Total DMI (g/head/day))	640.2	732.9	696.4	724.8	33.459	NS	NS	NS
DMI/BW (%)	3.78	3.92	3.72	3.84	0.158	NS	NS	NS
Silage intake (g/head/day)	246.0	316.6	283.1	308.6	30.868	NS	NS	NS
Proportion of silage intake (%)	38.15	42.64	39.83	41.85	2.581	NS	NS	NS
CP intake (g/head/day)	73.95 <sup>b</sup>	83.32 <sup>a</sup>	70.6 <sup>b</sup>	82.21 <sup>a</sup>	2.061	NS	NS	*
CP intake/BW <sup>0.75</sup> (g/kg BW <sup>0.75</sup> )	8.92 <sup>a</sup>	9.45 <sup>a</sup>	8.18 <sup>b</sup>	9.47 <sup>a</sup>	0.241	NS	NS	*
GE intake (kkal/day)	2757.7 <sup>b</sup>	3369.6 <sup>a</sup>	3222.1 <sup>a</sup>	3341.9 <sup>a</sup>	124.244	*	NS	NS
GE intake / BW <sup>0.75</sup> (kg BW <sup>0.75</sup> )	330.9 <sup>b</sup>	379.0 <sup>a</sup>	370.2 <sup>a</sup>	381.9 <sup>a</sup>	10.279	*	NS	NS
ADG (g/head/day)	84.417	92.2	87.867	97.4	6.773	NS	NS	NS
Feed conversion	7.85	7.97	8.2	7.47	0.489	NS	NS	NS
CP conversion ratio	0.925	0.907	0.837	0.851	0.065	NS	NS	NS
Energy conversion ratio	34.43	36.66	38.02	34.52	2.553	NS	NS	NS

Different superscripts in one row indicate significantly different ( $P < 0.05$ ); L= linear; Q= quadratic; C= cubic; NS= non significant; \* significant effect at  $P < 0.05$ ; SEM = standard error mean; CRM = complete rumen modifier; DMI = dry matter intake; BW = body weight; CP = crude protein; GE = gross energy; ADG = average daily gain.

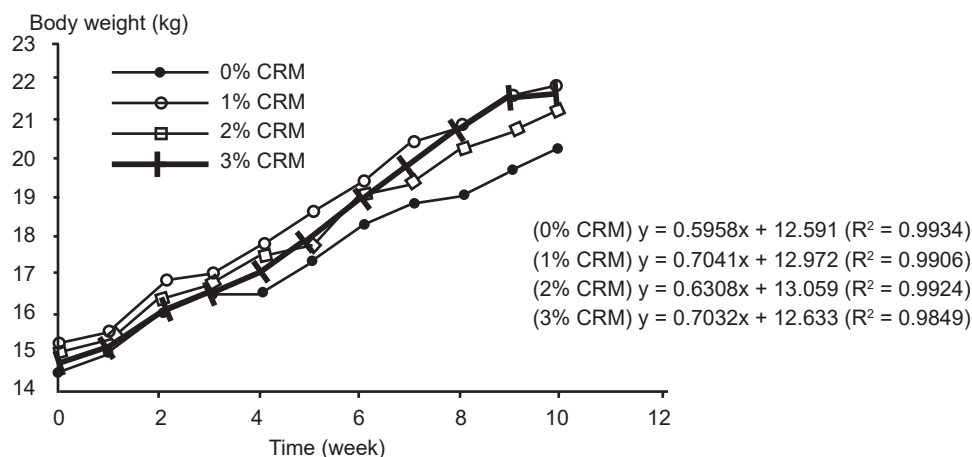


was similar to control, then decreased at 2% CRM and increased again at 3% CRM. CP intake of lambs at 2% CRM was significantly lowest. On the other hand, energy consumption linearly increased with an increasing level of CRM supplementation. GE consumption of control was significantly lower compared to that of 1%, 2% and 3% CRM rates. However, there were no significant differences among 1%, 2% and 3% CRM rates. The ADG was not significantly affected by CRM supplementation with the average ADG of 90 g/head/day. Similarly, feed conversion ratio (DMI/ADG), protein conversion ratio (CP intake/ADG) and energy conversion ratio (energy intake/ADG) were not significantly different between treatments with average values of 7.87, 0.88 and 35.91, respectively.

Growth pattern of lambs during experiment is presented in Figure 1. The growth of lambs was still in linear stage, in which the growth increased with an increasing time of feeding. From the equation of lamb growth pattern, it shows that the slopes of 1% and 3% CRM rates were

higher than those of 0% and 2% CRM rates. This indicates that the growth rates of lambs received 1% and 3% CRM were faster than those with 0% and 2% CRM supplementation.

Nutrient digestibility of lambs fed on corn cob silage basal diet supplemented with CRM is presented in Table 3. DM digestibility significantly decreased at 3% CRM supplementation and OM digestibility linearly decreased with increasing levels of CRM supplementation. However, the decreased OM digestibility at 3% CRM supplementation was only significantly lower than the control. The decreased OM digestibility was about 12%. On the other hand, CP digestibility was significantly lower at 2% and 3% CRM supplementation than that of the control and 1% CRM supplementation. There was no significant difference between 2% CRM and 3% CRM treatments on CP digestibility. Energy digestibility linearly decreased with an increasing level of CRM supplementation. The lowest energy digestibility was at 3% CRM supplementation.



**Fig.1.** Growth pattern of post-weaning male lambs fed on corn cob basal diet supplemented with different levels of complete rumen modifier (CRM).

**Table 3.** Means of nutrient digestibility of lambs fed on silage basal diet supplemented with different levels of complete rumen modifier (CRM).

Parameter	CRM levels (%)				S.E.M	Effects		
	0	1	2	3		L	Q	C
Dry matter (%)	55.48 <sup>a</sup>	51.019 <sup>ab</sup>	51.90 <sup>ab</sup>	49.23 <sup>b</sup>	1.531	NS	NS	NS
Organic matter (%)	57.14 <sup>a</sup>	52.86 <sup>ab</sup>	54.00 <sup>ab</sup>	50.38 <sup>b</sup>	1.507	*	NS	NS
Crude protein (%)	57.36 <sup>a</sup>	54.18 <sup>a</sup>	47.74 <sup>b</sup>	52.12 <sup>b</sup>	2.736	NS	NS	NS
Neutral detergent fiber (%)	45.80 <sup>a</sup>	47.83 <sup>a</sup>	45.26 <sup>a</sup>	38.53 <sup>b</sup>	1.614	*	NS	NS
Acid detergent fiber (%)	39.23 <sup>a</sup>	33.54 <sup>ab</sup>	27.45 <sup>b</sup>	31.72 <sup>b</sup>	2.196	*	NS	NS
Energy (%)	56.59 <sup>a</sup>	51.6 <sup>ab</sup>	53.22 <sup>ab</sup>	49.88 <sup>b</sup>	1.501	*	NS	NS

Different superscripts in one row indicate significantly different ( $P < 0.05$ ); L = linear; Q = quadratic; C = cubic; NS = non significant; \* significant effect at  $P < 0.05$ ; SEM = standard error mean; CRM = complete rumen modifier.

### Rumen Fermentation

Rumen pH and rumen ammonia ( $\text{NH}_3\text{-N}$ ) and VFA concentration of rumen fluid taken at 0 and 4 hours after feeding are shown in Table 4. The rumen pH either taken at 0 or 4 hours after feeding was not significantly different between treatments. The rumen pH decreased at 4 hours after feeding. Similarly, rumen ammonia ( $\text{NH}_3\text{-N}$ ) concentration was not significantly different between treatments either at 0 or 4 hours after feeding. In contrast to rumen pH, the rumen  $\text{NH}_3\text{-N}$  concentration increased at 4 hours after feeding, from 7.04 mg/100 ml at 0 hour to 17.50 mg/100 ml at 4 hours after feeding.

Total VFA production at 0 and 4 hours after feeding was not significantly different between treatments, except for acetic acid production at 4 hours after feeding, linearly and quadratically affected by CRM supplementation. At 1% CRM supplementation, acetic

acid proportion increased, while at 2% and 3% CRM supplementation, its proportion decreased but it was not significantly different to the control.

### Methane Emission

Methane emission either in L/day or in L per DMI was not significantly affected by CRM supplementation rates (Table 5). However, methane emission expressed in kJ/MJ energy intake (GE or DE) at 2% CRM supplementation showed the significantly lowest value. Similarly methane emission in g/kg digested NDF of 2% CRM supplementation was significantly the lowest.

### DISCUSSION

The DMI was not affected by CRM supplementation with the average of 3.8% of body weight (BW) (Table 2). This

**Table 4. Means of rumen pH, ammonia ( $\text{NH}_3\text{-N}$ ) concentration, and volatile fatty acid (VFA) taken at 0 and 4 hours after feeding from lambs fed on corn cob basal diet supplemented with complete rumen modifier (CRM).**

Parameter	Time (h)	CRM levels (%)				SEM	Effects		
		0	1	2	3		L	Q	C
pH	0	7.16	6.94	7.12	7.03	0.008	NS	NS	NS
	4	6.22	6.11	6.24	6.15	0.018	NS	NS	NS
$\text{NH}_3\text{-N}$ (mg/100 ml)	0	6.71	7.88	6.89	6.68	0.546	NS	NS	NS
	4	18.97	19.65	15.90	15.48	3.600	NS	NS	NS
VFA (mmol)	0	123.60	103.30	105.60	110	7.964	NS	NS	NS
	4	119.50	146.90	122.40	116.30	8.864	NS	NS	NS
Molar proportion (%)									
Acetate	0	0.723	0.743	0.726	0.725	0.006	NS	NS	NS
	4	0.648 <sup>b</sup>	0.665 <sup>a</sup>	0.621 <sup>b</sup>	0.618 <sup>b</sup>	0.009	*	*	NS
Propionate	0	0.148	0.158	0.161	0.164	0.007	NS	NS	NS
	4	0.204	0.200	0.222	0.204	0.011	NS	NS	NS
Butyrate	0	0.087	0.072	0.073	0.080	0.009	NS	NS	NS
	4	0.122	0.114	0.119	0.152	0.017	NS	NS	NS
Others VFA	0	0.041	0.026	0.039	0.031	0.003	NS	NS	NS
	4	0.025	0.019	0.037	0.025	0.008	NS	NS	NS
Acetate:propionate ratio	0	3.93	4.58	3.64	4.42	0.223	NS	NS	NS
	4	3.25	3.22	3.02	2.48	0.129	NS	NS	NS

Different superscripts in one row indicate significantly different ( $P < 0.05$ ); L = linear; Q = quadratic; C = cubic; NS = non significant; \* significant effect at  $P < 0.05$ ; SEM = standard error mean; CRM = complete rumen modifier; VFA = volatile fatty acid; others VFA included isobutyric, valeric and isovaleric acid.

**Table 5. Methane emission of lambs fed on corn cob silage basal diet supplemented with complete rumen modifier (CRM).**

Parameter	CRM levels (%)				S.E.M	Effects		
	0	1	2	3		L	Q	C
Methane L/day	33.49	31.44	26.31	32.25	2.721	NS	NS	NS
Methane L/kg DMI	50.76	46.49	39.31	48.49	3.780	NS	NS	NS
Methane kJ/MJ GEI	84.17 <sup>a</sup>	77.40 <sup>a</sup>	57.03 <sup>b</sup>	80.17 <sup>a</sup>	5.064	NS	NS	NS
Methane kJ/MJ DEI	159.10 <sup>a</sup>	141.40 <sup>a</sup>	107.20 <sup>b</sup>	154.10 <sup>a</sup>	5.870	NS	NS	NS
Methane g/kg digested NDF	197.37 <sup>ab</sup>	177.50 <sup>b</sup>	124.50 <sup>c</sup>	222.60 <sup>a</sup>	11.459	NS	NS	NS

Different superscripts in one row indicate significantly different ( $P < 0.05$ ).

value was comparable to a previous study reported by Yulistiani et al. (2012a) where total feed consumption of lambs fed on corn cob silage basal diet and supplemented with concentrate was 3.8% of BW. This value was higher than the total feed consumption supplemented with CRM for lambs fed on fermented rice straw basal diets, with DMI of 3.5% (Thalib et al. 2010). The nonsignificant effect of CRM supplementation on DMI in the current study indicated that CRM supplementation did not affect palatability of the diet. The implication is saponin in CRM derived from *S. rarak*, sesbania and albizia did not affect palatability of the diet. Similar results of mangosteen peel saponin supplementation, containing 11.3% crude saponin, did not affect DMI either mixed with or without garlic to rice straw basal diet (Manasri et al. 2012). In addition, Aazami et al. (2013) reported that DMI was not affected by commercial saponin extracted from *Quillaja saponaria*. Although energy intake was significantly increased by CRM supplementation (Table 2), the energy digestibility significantly decreased at 3% CRM supplementation. This might be due to the decrease in fiber digestibility at 3% CRM diet (Table 3).

Compared to the control treatment, only a higher rate of CRM supplementation (3%) reduced NDF digestibility, whereas ADF digestibility decreased at 2% CRM supplementation (Table 3). Reduction of fiber digestibility (NDF and ADF) could be due to the decrease in protozoa population as reported by Yulistiani et al. (2012b) where protozoa population decreased linearly with increasing CRM supplementation rates. Protozoa excreted enzyme to digest fiber, therefore, fiber digestibility in the rumen decreased if protozoa population decreased (Wina 2012). Furthermore, Wang et al. (2000) reported that steroidal saponins inhibited rumen cellulolytic bacteria and ruminal fungi thereby decreasing fiber digestibility. Similar findings were reported by Wina et al. (2005a) in *in vitro* study using *S. rarak* extract and by Manasri et al. (2012) using mangosteen supplementation containing saponin where NDF and ADF digestibility decreased. On the other hand, saponin supplementation did not affect nutrient digestibility on tropical grass hay basal diet (Abreu et al. 2004; Wang et al. 2009) and on fermented rice straw basal diet (Thalib et al. 2010). Results from various studies showed that the effect of saponin supplementations on nutrient digestibility, particularly fiber digestibility was not consistent, depending on saponin plant sources and types of basal diets.

The growth of lambs was not affected by CRM supplementation in diets. The ADG of lambs was 90.5 g/head/day (Table 2). However, other studies showed the increase in growth rate of lambs fed on CRM supplemented diet (Thalib et al. 2010) and the increase in growth rate of kids fed on tea seed or tea seed saponin

extract supplemented diet (Kumar et al. 2016). The ADG of lambs in the current study was higher (90.5 g/head/day) than ADG (71.4 g/head/day) reported by Thalib et al. (2010) in lamb fed on fermented rice straw basal diet supplemented by CRM. This difference might be due to the difference in diet qualities and feed consumption. Similar to the current study, Mao et al. (2010) did not detect any significant increase in body weight gain of lambs fed on Chinese wild rye basal diet supplemented by tea saponin either alone or mixed with soybean oil.

Ruminal pH in all diets either at 0 or 4 hours after feeding was not affected by CRM supplementation. Although at 4 hours after feeding the ruminal pH decreased which was caused by diet just after feeding, the pHs of all diets were the ideal range for rumen microbial growth and activity (pH 6-7). Similar to ruminal pH, ruminal ammonia ( $\text{NH}_3\text{-N}$ ) concentration was not affected by CRM supplementation. These results were supported by previous study by Thalib et al. (2010) on CRM supplementation in fermented rice straw basal diet, and Hess et al. (2004) on *Sapindus saponaria* supplementation in tropical grass hay basal diet.

In the present study, total VFA production was not affected by CRM supplementation, either at 0 or 4 hours after feeding. This result was similar to previous studies (Pen et al. 2006, 2007; Guo et al. 2008; Holtshausen et al. 2009). On the other hand, Lovet et al. (2006) reported the reduction of total VFA production due to saponin supplementation.

CRM supplementation did not affect molar proportion of VFA which its value was similar in all diets except for acetic acid at 4 hours after feeding. The effect of saponin supplementation on VFA production is diet dependent. Supplementation of saponin from *Y. schidigera* in barley grain diet increased total VFA production, while in lucerne hay diet it reduced VFA production (Wang et al. 2000). Moreover, Cardozo et al. (2005) reported that in high concentrate diet, ruminal pH affected VFA profile due to saponin supplementation. At pH 5.5 saponin supplementation increased molar proportion of propionate and reduced proportion of acetate, whereas at pH 7 no effect was observed. In the current study, either total VFA or VFA proportion was not affected by CRM supplementation. This indicated that CRM supplementation did not have an adverse effect on rumen fermentation. Similar results have been shown by Thalib et al. (2010) where CRM supplementation in fermented rice straw basal diet increased proportion of acetate. Results of Thalib et al. (2010) and the present study disagreed to most studies which concluded that saponin supplementation increased proportion of propionate and reduced acetate, butyrate, and branched chain fatty acid (Castro-Montoya et al. 2011; Pilajun and Wanapat

2011; Anantasook et al. 2014; Norrapoke et al. 2014). It seems that saponin was able to change the pattern of VFA production by increasing proportion of propionic acid and decreasing ratio of acetic to propionic acids. The higher proportion of propionic acid might be due to the lower production of acetic and butyric acids, which are protozoal fermentation products (Wina 2012). Patra and Saxena (2012) reviewed the effect of saponin on VFA composition and concluded that the inconsistency of saponin effects among studies was attributed by the type of diets and saponin sources.

In the present study, CRM supplementation in *in vivo* study did not significantly reduce methane production when expressed as production L/day. However, energy loss through methane relative to GE and DE intake significantly decreased at 2% CRM supplementation by 32.2% and 32.6%, respectively. Similarly methane released relative to digested NDF significantly decreased by 36.9% at 2% CRM supplementation (Table 5). Results of the present *in vivo* study confirmed the finding of *in vitro* study (Yulistiani et al. 2012a) that reduction in protozoa population reduced methane production. Thalib et al. (2010) also reported that CRM supplementation (combination of *S. rarak* and *Acetoanaerobium noterae*) reduced methane production by 24% in sheep fed on fermented rice straw basal diet. The results of the current study and previous studies reported by Sukmawati et al. (2011), Thalib et al. (2004; 2010) and Wina et al. (2006) indicated that saponin from *S. rarak* was able to be used as a methane inhibitor. Similar to *S. rarak*, tea saponin could also decrease methane production by 27.7% in growing lambs (Mao et al. 2010).

Methane is an end product of carbohydrate fermentation in the rumen which can be reduced by promoting a shift of fermentation products into propionate production. However, methane production cannot be completely eliminated without any adverse effect on ruminant production (Moss 2000). Therefore, Moss (2000) suggested that increasing ruminant production would be the most effective means of reducing methane production. Increased animal products (meat or milk) would mean declining methane production per unit of animal products. In this study, if methane production was calculated into per unit of ADG (methane production/ADG; L/g), methane production decreased by 14.0%, 28.5% and 21.9% for 1% CRM, 2% CRM and 3% CRM, respectively compared to control. These values had a significant meaning when it was observed in macro-environment, especially in Indonesia where ruminant feeding was based on agricultural by-products containing high fiber.

## CONCLUSION

Complete rumen modifier (CRM) supplementation in corn cob basal diet did not affect nutrient intake, lamb growth rate and rumen fermentation, but decreased methane emission. Supplementation of 2-3% CRM in the diets decreased nutrient digestibility and methane production. It is suggested that 2% CRM supplementation in the corn cob basal diet of lambs can be recommended to reduce glasshouse methane emission.

## ACKNOWLEDGEMENT

The authors would like to thank the Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture, Republic of Indonesia, for financial support. We thank to undergraduate students, Faculty of Animal and Agriculture, Diponegoro University for their assistance in measuring methane production.

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