

## The Effect of Condensed Tannin and Saponin in Reducing Methane Produced during Rumen Digestion of Agricultural Byproducts

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### ABSTRACT

Saponin and tannin have been considered as chemical compounds that can reduce methane enteric fermentation. The magnitude of the effect of the compounds on reducing methane are various depend on the levels and sources of the compounds. Some local plants that contained high condensed tannin and saponin are Acacia leaves and Lerak fruit. Feed available for ruminants mostly come from agricultural and plantation byproducts that characterized by high NDF content and low digestibility that promote methane production. The aim of study was to investigate the effect of saponin and condensed tannin extracts in reducing methane resulted during rumen fermentation of rice straw, maize straw, sugarcane-top and palm oil leaves. The *in vitro* method was used to evaluate 24 feedtuffs that were incubated for 48 hours. Saponin and condensed tannin extracts were added individually at the level of 2.5% of DM for each feed sample. Variables measured were chemical composition of feeds, total gas and methane production. Total cumulative gas of sugarcane-top and palm oil leaves fermentation increased when saponin ( $P < 0.01$ ) or condensed tannin ( $P < 0.05$ ) was added, but saponin or condensed tannin had no effect on rice straw and maize straw. Due to saponin and condensed tannin, the proportion of methane to total gas was reduced by 16.41 and 17.36% for sugarcane-top, 4.75 and 11.20% for palm oil leaves, 4.26 and 6.38% for maize straw and 11.68 and 12.98% for rice straw, respectively. The conclusions are that the two agents can reduce methane produced during the rumen fermentation of feedstuffs, and the effect of saponin and condensed tannin was different among the feedstuffs investigated, the condensed tannin has bigger effect on reducing methane production than saponin.

**Key Words:** Saponin, Condensed Tannin, Methane, Agricultural Byproducts

### INTRODUCTION

Methane gives the strongest effect of greenhouse gas than other gasses. The effect of methane was 20-50 times higher than carbondioxide (Beauchemin & McGinn 2005). Methane emitted from livestock comes from feed digestion in the rumen as enteric fermentation. The amount of methane emitted from enteric fermentation is influenced by the fibre content of the feed, in particular NDF content. Increasing in NDF content of feed would increases methane produced during the fermentation (Kulivand & Kafilzadeh 2015). The main feed used in ruminant production in most tropical regions are roughages characterized by high in fibre content. Nowadays, ruminants consume agricultural and plantation byproducts as the main components of their diets. The feeds are characterized by high NDF content and low digestibility (Maryono & Khrisna 2009). Therefore, it is expected that they will produce high methane when digested in the rumen.

Owing to this concern on global warming, many scientists, particularly in livestock production system, conducted many studies in order to identify techniques to mitigate methane emissions. Some techniques are through feed additives (Thalib 2004; Widiawati et al. 2013; Thalib 2008; Thalib et al. 2010; 2011), supplementation of low quality feed by legumes or concentrate feed (Widiawati 2004; Pramote et al. 2006), formulating and providing balance diet (Widiawati 2013; Liang et al. 2013). Decreased methane emitted from enteric fermentation was reported to up to 12.2-16.3% when Complete Rumen

Modifier (CRM) was added to sheep diet. A complete feed consisted of legume leaves and agricultural byproducts was reported to reduce methane emission from enteric fermentation by 10-15% (Widiawati et al. 2013).

Two plant secondary compounds proven to decrease methane production are saponin and tannin (Kamra et al. 2006; Jayanegara et al. 2011). Tannin extracted from some tannin containing plants, such as *chestnut*, *mimosa*, *quebracho* and *sumach* significantly reduced methane produced from grass hay in *in vitro* experiment (Jayanegara et al. 2010). Tannin extracted from *chestnut* and *sumach* was hydrosable tannin, while tannin extracted from *mimosa* and *quebracho* was condensed tannin. The two types of tannin can reduce methane produced significantly (Jayanegara et al. 2011). Saponin extracted from *Sapindus rarak* was reported to reduce methane production up to 31% in *in vitro* experiment. However, the effect was not recorded in the *in vivo* experiment (Thalib 2004). However, when saponin was combined with *Acetoanaerobium noterae*, methane production decreased up to 24% when offered to sheep (Thalib et al. 2010). Moreover, the methane mitigating property of tannin and saponin in the rumen depend on the level and sources of tannin and saponin (Jayanegara et al. 2014). Two local plants as tannin and saponin sources are Acacia (*Acacia* sp.) and Lerak (*Sapindus rarak*). These plants are reported to contain high levels of tannin (22-48%) and saponin (81.47%), respectively (Astuti et al. 2008; Fathoni 2010). However, there is limited information on the effects of tannin and saponin on reducing methane production from agricultural and plantation byproduct based diet fermented in the rumen. Therefore, the study was aimed to investigate the effect of tannin and saponin extracted from local sources, namely Acacia and Lerak, on reducing methane produced from agricultural and plantation byproduct based diet fermented in the rumen.

## MATERIAL AND METHODS

### Experimental feeds and design

#### *Feed samples*

Eight different feeds were used in this study, namely palm oil leaves (POL), palm oil leaves silage (POLS), sugarcane top (SCT), sugarcane top silage (SCTS), maize straw (MS), maize straw silage (MSS), rice straw (RS), and rice straw silage (RSS). Fresh material of the feeds were oven-dried at 60°C for three days, grinded and kept in refrigerator before used. The nutrient contents of each feed were analyzed by the procedure of Association of Official Analytical Chemists (AOAC 1990).

#### *Saponin and tannin extraction*

Tannin was extracted from dry leaves of Acacia (*Acacia* sp.) using methanol 50% (1:4 w/v). Tannin resulted from this method was total condensed tannin. Saponin from Lerak fruit (*Sapindus rarak*) was extracted using methanol 50% (1:4 w/v) (Makkar et al. 1995b). Acacia leaves and lerak fruit were dried using oven 60°C for three days then were grinded. Five gram of Acacia leaves meal was mixed with 20 ml of methanol (50%) and kept for 24 h. The solution then was evaporated using rotary-evaporator at temperature of 40°C, then was dried using freeze drying and kept in refrigerator before used. By using similar method, 5 gram of lerak fruit meal was added by 20 ml methanol (50%) then kept for 24 h at room temperature.

### Experimental design

The feed samples were individually evaluated for their potency to produce methane using *in vitro* rumen fermentation technique (Makkar et al. 1995b). Tannin and saponin were individually added at the level of 2.5% of DM to each feed sample. Purified tannin at the minimal level of 0.25 mg/ml buffer solution or equivalent with 25 mg/g of DM feed sample, can reduced methane produced from mixed diet of hay and concentrate (Yugianto et al. 2014). The total samples evaluated were 24 as presented in Table 1. Each feed sample was subjected to *in vitro* fermentation to measure methane production after 48 h of incubation.

**Table 1.** Feed samples and addition of tannin and saponin for each feed sample evaluated in the experiment

No.	Sample evaluated	Code	No.	Sample evaluated	Code
1.	Palm oil leaves	POL	13.	Palm oil leaves-silage	POLS
2.	POL + tannin	POL-T	14.	POLS + Tannin	POLS-T
3.	POL + saponin	POL-S	15.	POLS + Saponin	POLS-S
4.	Sugarcane top	SCT	16.	Sugarcane top-silage	SCTS
5.	SCT + tannin	SCT-T	17.	SCTS + Tannin	SCTS-T
6.	SCT + saponin	SCT-S	18.	SCTS + Saponin	SCTS-S
7.	Maize straw	MS	19.	Maize straw-silage	MSS
8.	MS + tannin	MS-T	20.	MSS + Tannin	MSS-T
9.	MS + saponin	MS-S	21.	MSS + Saponin	MSS-S
10.	Rice straw	RS	22.	Rice straw-silage	RSS
11.	RS + tannin	RS-T	23.	RSS + Tannin	RSS-T
12.	RS + saponin	RS-S	24.	RSS + Saponin	RSS-S

### Rumen fluid collection and buffer preparation

Rumen fluid was collected from rumen cannulated Frisien Holstein cows fed twice a day with king grass and concentrate at the ratio 2:1. Five hundred milligrams of each feed sample inclusion of 2.5% of tannin or saponin was included in the *in vitro* fermentation. The *in vitro* solution used in the study was composed of ruminal fluid 22.6%; buffer solution 25.1%, mineral solution 12.5%, reducing agent solution 2.1%, and aquadest (Makkar et al. 1995a). Rumen fluid and buffer were mixed at the ratio of 1:3 and flushed with carbondioxide (CO<sub>2</sub>) gas. Fifty ml of buffered rumen fluid was anaerobically dispensed into serum bottle then sealed with butyl-rubber stopper. Each sample was replicated three times and maintained in water bath at 39°C for 48 h. During incubation, gas production (total and methane) were monitored at 0, 6, 12, 18, 24, 30, 36, 42, and 48 h.

### Analysis *in vitro* fermentation parameters

Total gas production was measured in each of serum bottle using a scaled glass syringe connected to three waytap attached to another scaled glass syringe. Measurement of methane gas produced followed the methods described by Tjandraatmadja (1981). The DM content of the feed was determined by drying to a constant weight in an oven at 105°C overnight. The ash in the feed sample and residue was determined by combusting in a

muffle furnace at 550°C for 6 h. The N content of the feed was determined by the micro Kjeldahl method (AOAC 1990), NDF was analyzed according to Van Soest et al. (1991), gross energy (GE) was determined by using an auto bomb calorimeter. Chemical composition of the feed samples evaluated in this study are presented in Table 2.

**Table 2.** Chemical composition of feed samples used in the experiment.

Sample	Ash	Crude fibre	Crude protein	Crude fat	GE	NDF	Ca	P
	----- % -----				(kcal/kg)	----- % -----		
Palm oil leaves	7.64	53.48	4.70	1.58	4,138	72.56	0.46	0.80
Palm oil leaves-silage	5.70	57.99	4.21	1.76	4,291	77.44	0.49	0.06
Sugarcane top	10.18	39.78	5.47	1.83	3,868	72.62	0.25	0.11
Sugarcane top-silage	10.42	46.69	5.14	2.12	3,993	70.42	0.57	0.08
Maize straw	10.90	40.13	7.16	1.0	3,768	68.41	0.27	0.11
Maize straw-silage	10.45	38.09	6.87	1.81	3,908	62.62	0.41	0.02
Rice straw	20.40	32.45	5.32	1.01	3,212	69.03	0.17	0.10
Rice straw-silage	19.65	38.80	4.99	1.56	3,446	70.45	0.26	0.08

### Statistical analysis

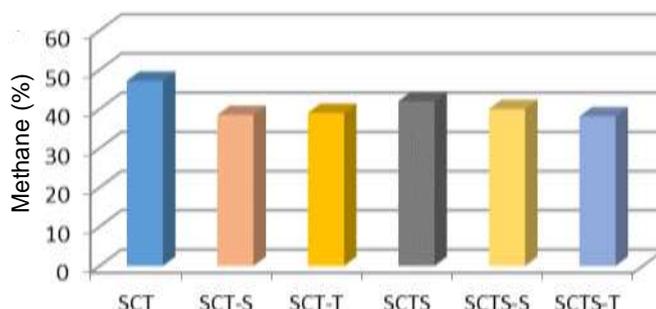
All treatments were conducted in triplicates. The means of each variable measured in this study were analysed by Analysis of Variance (ANOVA) using the procedures of the IBM IPSS Statistics version 20. The differences between means were compared by a least significant difference method (LSD).

## RESULTS AND DISCUSSION

Among the byproducts investigated in this experiment, palm oil leaves have the lowest protein but the highest NDF contents. While maize straw contains more protein but less NDF compared to other feedstuffs. These data indicated the quality of feedstuffs. The NDF content of feed materials would have an effect on the rate of rumen digestion and the amount of methane produced. Many studies showed that there was a positive correlation between NDF content of the feed materials and enteric methane production (Tavendale et al. 2005; Kulivand & Kafilzadeh 2015). Proximate analyses showed that the ensiling process did not change the chemical compositions in particular the protein contents of the feed materials (Table 2).

The total cumulative gas after 48 hours of fermentation of sugarcane-top (116.17 ml) increased when saponin (163.17 ml) ( $P < 0.01$ ) or tannin (132.50 ml) was added ( $P < 0.05$ ). However the proportion of methane to total gas was reduced by 16.41% and 17.36% when saponin or tannin was added, respectively (Figure 1). These results indicated that tannin gave more effect in reducing methane gas production than saponin. Silage of sugarcane-top did not change the total gas produced during rumen fermentation (116.17 vs 118.50 ml). However, the processing reduced the proportion of methane to total gas from sugarcane-top by 11%. Similar effect of tannin and saponin was also indicated in sugarcane-top silage. The proportion of methane to total gas was reduced by 19.18% when

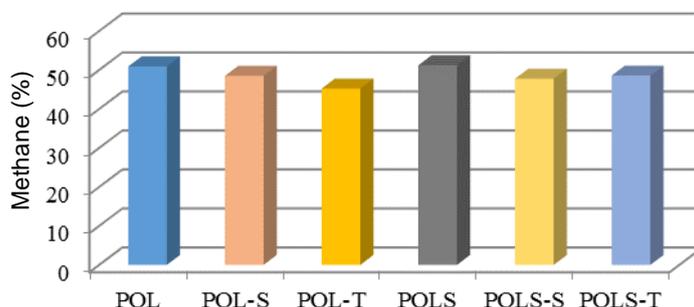
tannin was added, while addition of saponin only reduced the proportion of methane to total gas by 15.24%.



SCT: Sugarcane top; SCT-S: Sugarcane top + saponin; SCT-T: Sugarcane top + tannin; SCTS: Sugarcane top silage; SCTS-S: Sugarcane top silage + saponin; SCTS-T: Sugarcane top silage + tannin

**Figure 1.**Proportion of methane from total gas cumulative (%) when sugarcane top (SCT) and sugarcane top silage (SCTS) were fermented with addition of tannin or saponin

Addition of saponin and tannin increased total gas cumulative when palm oil leaves was fermented for 48 hours, from 60.83 ml to become 75 and 69 ml, respectively ( $P < 0.05$ ). However, the proportion of methane to total gas was reduced by 4.75% and 11.20% when saponin or tannin was added, respectively (Figure 2). Ensilage of palm oil leaves did not change the total gas produced during rumen fermentation (60.83 vs 55.33 ml). Ensilage of palm oil leaves also had no effect on methane production (50.9% of total gas vs 50.09% of total gas). The effect of saponin and tannin was different based on the substrate/feed being fermented. When fresh palm oil leaves was fermented, tannin had more effect in reducing methane compared to saponin ( $P < 0.05$ ), while when palm oil leaves was ensilaged, the saponin and tannin had similar effect in reducing methane gas produced (4.64 and 4.19%).

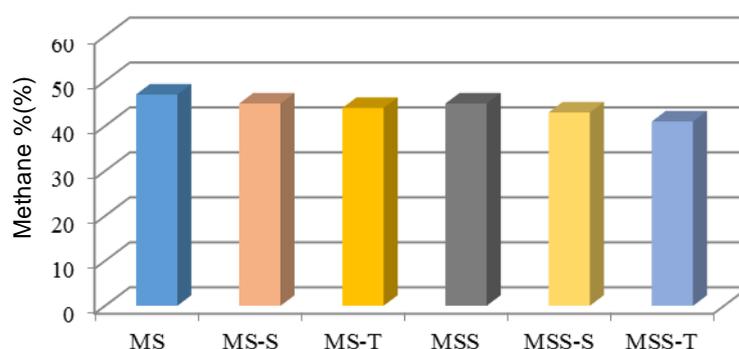


POL: Palm oil leaves; POL-S: Palm oil leaves + saponin; POL-T: Palm oil leaves + tannin; POLS: Palm oil leaves silage; POLS-S: Palm oil leaves silage +saponin; POLS-T: Palm oil leaves silage + tannin

**Figure 2.**Proportion of methane from total gas cumulative (%) when palm oil leaves (POL) and palm oil leaves silage (POLS) were fermented with addition of tannin or saponin

There were no effect of additional saponin and tannin on total gas cumulative when maize straw was fermented (98.83 vs 99.33 vs 94.00 ml). The effect of saponin and tannin was very small as shown by the low reduction of methane produced (4.26 and 6.38%) (Figure 3). Ensilage of maize straw increased total gas produced up to 106.17 ml compared to non-ensilage (98.83 ml) ( $P < 0.05$ ), but has no effect on the proportion of methane gas from the total gas cumulative. Additional saponin and tannin on maize straw silage

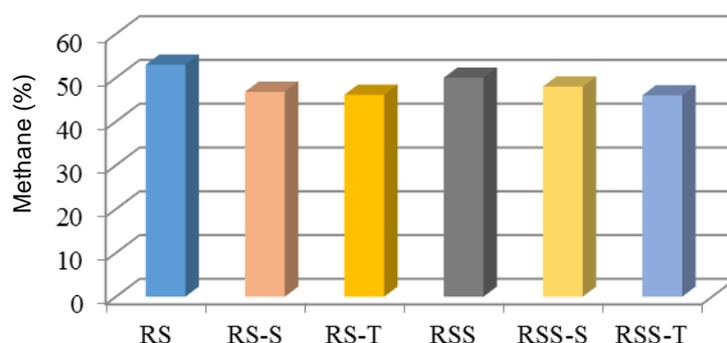
reduced the proportion of methane gas produced by 8.51 and 12.77% respectively. The results showed that saponin dan tannin have larger effects in reducing methane production when the maize straw was ensilaged than when as fresh maize straw.



MS: Maize straw; MS-S: Maize straw + saponin; MS-TL: Maize straw + tannin; MSS: Maize straw silage; MSS-S: Maize straw silage + saponin; MSS-T: Maize straw silage + tannin

**Figure 3.** Proportion of methane from total gas cumulative (%) when maize straw (MS) and maize straw silage (MSS) were fermented with addition of tannin or saponin

There were no effect of additional saponin and tannin on total gas cumulative of rice straw (73.67 vs 83.83 vs 79.17 ml). The proportion of methane to total gas cumulative was also similar for RS (48.68%), RS-S (46.81%), and RS-T (46.12%). The reduction of proportion methane gas per total gas cumulative was accounted for 11.68 and 12.98% when saponin and tannin was added. Ensiling process reduced total gas cumulative up to 66.33 ml, as well as the proportion of methane to total gas by 5.66%. The reduction of proportion methane to total gas was increased up to 9.43% and 13.21% when rice straw silage was added by saponin and tannin, respectively (Figure 4).



RS: Rice straw; RS-S: Rice straw + saponin; RS-T: Rice straw + tannin; RSS: Rice straw silage; RSS-S: Rice straw silage + saponin; RSS-T: Rice straw silage + tannin

**Figure 4.** Proportion of methane from total gas cumulative (%) when rice straw (RS) and rice straw silage (RSS) were fermented with addition of tannin or saponin

Among the four feedstuffs investigated, total gas produced during the 48 hours of incubation was different ( $P < 0.05$ ). The gas produced from sugarcane-top fermentation was the highest, while total gas produced from palm oil leaves was the lowest. The gas produced during the rumen digestion of feeds can indicate the amount of substrate being digested. The amount of gas produced linearly with the amount of dry matter digested (Hart et al. 2009). The two feeds contain similar amount of NDF but sugarcane-top

contains more protein than palm oil leaves (Table 2). The ratio of NDF:CP for palm oil leaves is 15.44, while sugarcane-top is only 13.28, indicated the quality of the feed.

Findings of the study indicated that the effect of tannin more stronger than saponin in reducing the proportion of methane to total gas. The results were coherent with the report of Jayanegara et al. (2012) indicated that tannin has an antimethanogenic effect. Differences in the effect of tannin and saponin for each feed may be due to differences in the chemical composition, in particularly NDF and energy content of the feed. The NDF content has negative corelation with digestibility.

## CONCLUSION

It is concluded that the two agents (condensed tannin and saponin) can reduce the proportion of methane to total gas during the rumen fermentation of feedstuffs. However, the effect of tannin and saponin was different among the feedstuff investigated. Tannin has larger effect on reducing the proportion of methane to total gas than saponin. The largest effect of tannin and saponin was on sugarcane top. Ensiling did not constantly reduce methane production among the feedstuffs investigated.

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