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AMMONIATION OF RICE STRAW AND SUPPLEMENTATION OF *Paraserianthes falcataria* AND *Sapindus rarak* ON *IN VITRO* RUMEN FERMENTATION AND METHANE PRODUCTION

AMONIASI JERAMI PADI DAN SUPLEMENTASI SENGON LAUT (Paraserianthes falcataria) DAN LERAK (Sapindus rarak) TERHADAP FERMENTASI RUMEN DAN PRODUKSI GAS METANA SECARA IN VITRO

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ABSTRACT

This study aimed to observe the effects of rice straw ammoniation and supplementation of *Paraserianthes falcataria* and *Sapindus rarak* on rumen fermentation and methane production *in vitro*. Rice straw was ammoniated by adding 2% urea. Rice straw, ammoniated rice straw, *P. falcataria* leaves and *S. rarak* fruits were oven-dried and finely ground. Experimental treatments were arranged as follow: rice straw (T1), ammoniated rice straw (T2), T2 80% + *P. falcataria* 20% (T3), T2 60% + *P. falcataria* 40% (T4), T2 80% + *P. falcataria* 10% + *S. rarak* 10% (T5), and T2 60% + *P. falcataria* 20% + *S. rarak* 20% (T6). An amount of 1 g sample from each treatment was added with 100 ml rumen fluid and buffer mixture (1:2 v/v), and incubated in a water bath at 39 ºC for 48 h. The incubation was performed in four replicates and each replicate was represented by four incubation bottles. Results showed that urea treatment increased gas production of rice straw at 24 and 48 h, higher ammonia production, higher IVDMD, and lower methane production as compared to the untreated rice straw (P<0.05). Addition of *P. falcataria* or *S. rarak* at lower level produced similar ammonia concentration as ammoniated rice straw whereas their addition at higher level decreased ammonia concentration (P<0.05). *Paraserianthes falcataria* addition to ammoniated rice straw decreased protozoa population (P<0.05) and *S. rarak* further decreased the fauna population (P<0.05) as well. Inclusion of *S. rarak* at 20% DM (T6) produced the lowest methane production both at 24 and 48 h after incubation and lowest methane production per unit of DM degraded. It was concluded that ammoniation of rice straw improved its nutritional quality with lower methane production, and its mixture with *P. falcataria* and *S. rarak* further increased the quality and lower the production of methane, respectively.

(Key words: Ammoniation, *In vitro* rumen, *Paraserianthes falcataria*, *Sapindus rarak*, Rice straw)

INTISARI

Penelitian ini bertujuan untuk mengamati pengaruh amoniasi jerami padi dan suplementasi sengon laut (Paraserianthes falcataria) dan lerak (Sapindus rarak) terhadap fermentasi rumen dan produksi gas metana secara in vitro. Jerami padi diamoniasi dengan menambahkan urea sebanyak 2%. Jerami padi, jerami padi amoniasi, daun P. falcataria dan buah S. rarak dikeringkan menggunakan oven dan digiling halus. Perlakuan yang dilakukan adalah sebagai berikut: jerami padi tanpa amoniasi (T1), jerami padi amoniasi (T2), perlakuan T2 sebanyak 80% ditambah P. falcataria 20% (T3), perlakuan T2 sebanyak 60% ditambah P. falcataria 40% (T4), perlakuan T2 sebanyak 80% ditambah P. falcataria sebanyak 10% dan S. rarak 10% (T5), dan perlakuan T2 sebanyak 60% ditambah P. falcataria 20% dan S. rarak 20% (T6). Sebanyak 1 g sampel dari masing-masing perlakuan ditambahkan 100 ml cairan rumen:buffer (1:2 v/v), dan diinkubasi di dalam penangas air pada suhu 39ºC selama 48 jam. Inkubasi dilakukan dalam empat ulangan dan masing-masing ulangan terdiri dari empat botol inkubasi. Hasil penelitian menunjukkan bahwa perlakuan urea meningkatkan produksi gas pada fermentasi rumen jerami padi pada jam ke 24 dan 48, menghasilan produksi amonia lebih tinggi, menunjukkan nilai degradasi bahan kering lebih tinggi, dan produksi metana lebih rendah dibandingkan dengan jerami padi yang tidak diamoniasi (P<0,05). Penambahan P. falcataria atau S. rarak pada level rendah menghasilkan konsentrasi amonia yang sama

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dengan jerami padi amoniasi sedangkan penambahan P. falcataria atau S. rarak pada level yang lebih tinggi menurunkan konsentrasi amonia (P<0,05). Penambahan P. falcataria pada jerami padi amoniasi menurunkan populasi protozoa (P<0,05) dan penambahan S. rarak menurunkan populasi protozoa lebih rendah (P<0,05). Penambahan S. rarak pada 20% BK (T6) menghasilkan produksi metana paling rendah pada jam ke 24 dan 48 setelah inkubasi dan juga menghasilkan produksi metana per unit BK terdegradasi yang paling rendah dibandingkan perlakuan lainnya. Penelitian ini dapat disimpulkan bahwa amoniasi jerami padi meningkatkan kualitas nutrisinya sekaligus menurunkan produksi metana, serta penambahan campuran P. falcataria dan S. rarak dapat lebih meningkatkan kualitas jerami dan menurunkan produksi metana.

(Kata kunci: Amoniasi, In vitro rumen, Jerami padi, Lerak, Sengon laut)

Introduction

Rice straw is a common roughage consumed by ruminant in Indonesia particularly in traditional and small-scale farms. The by-product is abundantly available in the country due to the following reasons: (1) rice is a main carbohydrate-rich food consumed by Indonesians, (2) paddy rice cultivation dominates land use for agricultural crop production (Tsujino *et al.*, 2016), and (3) limited utilization of rice straw by farmers especially for those who have no livestock; burning of the straw is unfortunately widely practiced and thus contributes to zero economical value. Although it is produced in high quantity, nutritional quality of rice straw has been known to be low. Rice straw is characterized by high fiber content particularly lignocellulose and high in silica, in which these fractions are negatively correlated with digestibility and animal performance (Sarnklong *et al.*, 2010). Further, rice straw also contains low amount of protein. In the context of environmental protection concern, such fiber-rich feed like rice straw is not favourable due to the high methane emission when the material is entering the rumen and fermented by various anaerobic microorganisms including *Archaea methanogens* (Soder *et al.*, 2016).

Technology of ammoniation, either by using anhydrous ammonia or urea, has been widely applied to improve nutritional quality of rice straw. It had been shown that application of such technique decreased fiber content of rice straw (Fang *et al.*, 2012), increased its digestibility and improved animal performance (Gunun *et al.*, 2013). However, effect of rice straw ammoniation on methane production is limitedly investigated to date. Another strategy to improve nutritional quality of rice straw is to mix the material with certain feed that high in protein and low in fiber; such characteristics are generally found in legumes. Consuming only rice straw or other agricultural residues cannot meet protein requirement of ruminants and therefore supplementation with a high protein feedstuff is important. A number of forages rich in protein have been used as protein supplements in the diets for ruminant livestock in Indonesia such as *Moringa* leaves (Jayanegara *et al.*, 2010), *katu* leaves (Marwah *et al.*, 2010) and cassava leaves (Sudarman *et al.*, 2016). *Paraserianthes falcataria* (synonym: *Albizia falcataria*, *Falcataria moluccana*) or known as *sengon laut* is a legume tree species with considerable amount of protein content (Akkasaeng *et al.*, 1989; Merkel *et al.*, 1999). Therefore this forage legume is potential to be used for alleviating protein deficiency in rice straw.

High methane production of rice straw when being fermented in the rumen may be overcome by adding certain feed or plant material that rich in plant secondary compounds such as essential oil, tannin and saponin (Jayanegara *et al.*, 2013; 2015; Pirondini *et al.*, 2015). Such enteric methane emission contributes to global warming since the gas is a greenhouse gas. Methane is the second biggest greenhouse gas accumulated in the atmosphere but its capacity to retain heat is 28 times greater than that of carbon dioxide at 100 years period (Tian *et al.*, 2016). An Indonesian plant that rich in saponin content is *Sapindus rarak* or locally known as *lerak*, particularly in its fruit (Suharti *et al.*, 2009; Yuliana *et al.*, 2014). Thus *S. rarak* fruit may be combined with rice straw in order to reduce its effect on methane production. Together with urea treatment and *P. falcataria* leaf supplementation, it is expected that all these strategies may simultaneously improve nutritional quality and reduce methane production of rice straw. The present study aimed to observe the effects of rice straw ammoniation and supplementation of *P. falcataria* and *S. rarak* on rumen fermentation and methane production by *in vitro* analysis.

Materials and Methods

Sample preparation

Rice straw was collected from paddy rice field in Purwokerto. The material was ammoniated by adding 2% of urea (dry matter basis) and kept for two weeks incubation. *Paraserianthes falcataria* leaves were freshly collected from Agrostology Experimental Field, Bogor Agricultural University. *Sapindus rarak* fruits were purchased from *Pasar Anyar* traditional market in Bogor. These materials were oven-dried at 60ºC for 24 h, ground with a hammer mill to pass a 1 mm sieve and used in further experimental steps.

Chemical composition analysis

Ground samples of rice straw (non-ammoniated and ammoniated), *Paraserianthes falcataria* leaves and *Sapindus rarak* fruits were analyzed for dry matter (DM), organic matter (OM), ash, crude protein (CP) and ether extract (EE) contents (AOAC, 2005). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin contents were determined by following the procedures of Van Soest *et al.* (1991). Hemicellulose and cellulose contents were obtained by difference between NDF and ADF and between ADF and lignin, respectively. Analyses of tannin and saponins contents were performed according to Makkar *et al.* (2007). All chemical composition analyses were conducted in duplicate.

In vitro analysis

Samples of unammoniated and ammoniated rice straw, *Paraserianthes falcataria* and *Sapindus rarak* were arranged and mixed according to the following experimental treatments (dry matter basis):

T1 : Rice straw
T2 : Ammoniated rice straw
T3 : T2 80% + *P. falcataria* 20%
T4 : T2 60% + *P. falcataria* 40%
T5 : T2 80% + *P. falcataria* 10% + *S. rarak* 10%
T6 : T2 60% + *P. falcataria* 20% + *S. rarak* 20%

These treatments were subjected to *in vitro* analysis according to Theodorou *et al.* (1994). An amount of 1 g sample from each treatment was inserted into a 125 ml serum bottle, followed by addition of 100 ml rumen fluid:buffer mixture (1:2 v/v). Rumen fluid was obtained from a fistulated Friesian Holstein crossbred cow before morning feeding. The cow was fed with napier grass and commercial concentrate (60:40 w/w). Buffer solution was prepared by mixing 9.8g $NaHCO_3$, 3.71g $Na_2HPO_4.7H_2O$, 0.57g KCl, 0.47 g NaCl, 0.12 g $MgSO_4.7H_2O$ and 0.04 g $CaCl_2$ in 1000 ml distilled water. Anaerobic condition of the *in vitro* system was maintained by continuous gassing of the incubation medium with CO_2 gas. Serum bottles were sealed with butyl rubber stoppers and aluminum crimp seals shortly before starting the incubation. The bottles were placed in a water bath at 39ºC for 48 h. At 3, 6, 9, 12, 24 and 48 h after the start of the *in vitro* incubation, gas production from each serum bottle was vented and recorded. The incubation was performed in four runs (replicates) and each treatment per run was represented by four incubation bottles.

Post-incubation measurement

Methane production was measured at 24 and 48 h after incubation by following the procedure of Yuliana *et al.* (2014). At the end of incubation, liquid samples were withdrawn for volatile fatty acid (VFA), ammonia (NH_3), total bacteria and protozoa determinations. Concentration of VFA was determined by using a gas chromatograph (GC 8A, Shimadzu Corp., Kyoto, Japan) with a column containing 10% SP-1200, 1% H_3PO_4 on 80/100 Cromosorb WAW. Chromatogram of VFA sample was compared with known concentration of VFA standard (acetate, propionate and butyrate). Ammonia concentration was measured by using Conway micro-diffusion technique. Total bacteria and protozoa population were enumerated by using roll tube and counting chamber methods, respectively. Solid residues after incubation were determined for dy matter contents and used to determine dry matter degradability (DMD).

Calculation and data analysis

Chemical compositions of mixtures between ammoniated rice straw, *P. falcataria* and *S. rarak* at various proportions were calculated from their individual compositions. Data on *in vitro* incubation, i.e. gas production, rumen fermentation and methane production were analyzed by analysis of variance (ANOVA) by following a randomixed complete block design. Different batch of rumen fluid sampling at different experimental run served as the block in ANOVA. Outliers were detected through standardized residuals in which their values were lower than –3 or higher than 3, and subsequently were removed from the dataset. Comparison among different treatments was performed by applying Duncan's multiple range test. All the statistical analyses were performed by using SPSS software version 20.0.

Results and Discussion

Chemical composition

Paraserianthes falcataria leaves contained a relatively high CP content, i.e. above 15% DM, whereas CP content of *S. rarak* fruits was approximately half of *P. falcataria* (Table 1). Both plants had low EE contents. Much higher NDF and ADF were observed in *P. falcataria* than those of *S. rarak*; both contained negligible amounts of lignin. *Paraserianthes falcataria* and *S. rarak* contained considerable amounts of plant secondary metabolites, i.e. tannin and saponin, respectively. Content of CP was low in rice straw (T1), and urea treatment (T2) increased substantially the CP content (Table 2). In comparison to *P. falcataria*, NDF and ADF contents of rice straw were higher by 46.5 and 20.6%, respectively. Urea treatment decreased NDF, ADF and lignin contents of rice straw by 15.1, 11.8 and 5.0%, respectively. Addition of *P. falcataria* to ammoniated rice straw (T3 and T4) increased its CP and tannin contents, and decreased its NDF, ADF and lignin contents. Addition of *S. rarak* to ammoniated rice straw and *P. falcataria* mixture (T5 and T6) further decreased its NDF and ADF contents, and added a certain amount of saponin.

The CP content of *P. falcataria* in this study was slightly lower than that of reported by Akkasaeng *et al.* (1989); the authors reported that the species contained 18.6 and 19.5% CP (DM basis), obtained at two different collection periods. Merkel *et al.* (1999) even reported a higher value of CP in *P. falcataria*, i.e. 21.3% DM, and used the plant as a protein supplement (fed up to 50% of dietary CP) for growing lambs. The NDF values of *P. falcataria* reported in literatures ranged from 40.7 to 51.1% DM (Akkasaeng *et al.*, 1989; Merkel *et al.*, 1999); these were lower than what was observed in the present study. It seems that *P. falcataria* used in this study is of lower quality than the literatures as shown by the lower CP and higher NDF values, most probably due to older age of the plant sampled. As a forage gets more mature, cell wall proportion increases, and thus elevate its fiber proportion on one side and decrease its protein proportion on the other side. Moderate level of tannin present in *P. falcataria* was also reported in other studies. D'Mello (1992), for instance, reported that *P. falcataria* contained 4.7% condensed tannin. Further, Jayanegara *et al.* (2011) reported that the plant contained 6.4% tannin, in which 4.6% was in the form of condensed tannin and 1.8% was hydrolysable tannin.

Content of CP in *S. rarak* was relatively similar to that of reported by Suharti *et al.* (2009), i.e. around 7% DM. The fruit has been well-known to contain considerable amount of saponin (Yogianto *et al.*, 2014; Yuliana *et al.*, 2014). Suharti *et al.* (2009)

Table 1. Chemical composition of *Paraserianthes falcataria* and *Sapindus rarak* (dry matter basis)

Component	*P. falcataria*	*S. rarak*
Organic matter	94.7	97.9
Ash	5.3	2.1
Crude protein	16.5	7.8
Ether extract	0.8	0.7
NDF	59.6	24.0
ADF	50.1	19.4
Lignin	0.8	nd
Hemicellulose	9.5	4.6
Cellulose	49.3	19.4
Tannin	3.1	nd
Saponin	nd	6.9

ADF, acid detergent fiber; nd, not detected; NDF, neutral detergent fiber.

Table 2. Chemical composition of experimental treatments (dry matter basis)

Component	T1	T2	T3	T4	T5	T6
Organic matter	79.8	80.7	83.5	86.3	83.8	86.9
Ash	20.2	19.3	16.5	13.7	16.2	13.1
Crude protein	6.5	11.7	12.7	13.6	11.8	11.9
Ether extract	0.3	1.1	1.0	1.0	1.0	1.0
NDF	87.3	74.1	71.2	68.3	67.6	61.2
ADF	60.4	53.3	52.7	52.0	49.6	45.9
Lignin	15.9	15.1	12.2	9.4	12.2	9.2
Hemicellulose	26.9	20.8	18.5	16.3	18.1	15.3
Cellulose	44.5	38.2	40.4	42.6	37.4	36.7
Tannin	nd	nd	0.6	1.2	0.3	0.6
Saponin	nd	nd	nd	nd	0.7	1.4

T1: rice straw; T2: ammoniated rice straw; T3: T2 80% + *P. falcataria* 20%; T4: T2 60% + *P. falcataria* 40%; T5: T2 80% + *P. falcataria* 10% + *S. rarak* 10%; T6: T2 60% + *P. falcataria* 20% + *S. rarak* 20%.
ADF: acid detergent fiber; nd: not detected; NDF: neutral detergent fiber.

observed that saponin content of S. Rarak meal was 3.87% DM; this was lower in comparison to our result. Other species of sapindus such as *Sapindus saponaria* and *Sapindus mukorossi* also contain significant concentration of saponin (Kamra *et al.*, 2008; Soliva *et al.*, 2008; Delgado *et al.*, 2012). Due to its low CP but high saponin contents, *S. rarak* is often used as an extracted form rather than its intact form.

Lower NDF and ADF in urea-treated rice straw in comparison to that of the untreated was expected. Urea may be used for ammoniation technique of high-fiber materials since it is converted to ammonia after dissolved in water and by the action of urease enzyme. Ammonia released is absorbed into cell wall, cleaves lignocellulose component, and converts it into lignin, cellulose and some other smaller molecules (Sarnklong *et al.*, 2010). Other authors also reported a decrease in fiber content of rice straw after applying urea treatment (Vadiveloo and Fadel, 2009; Fang *et al.*, 2012; Gunun *et al.*, 2013). Fiber-decreasing effect of urea treatment does not only apply to rice straw but also to other materials or by-products rich in fiber. Laconi and Jayanegara (2015), for instance, reported that urea treatment of cocoa pod (1.5% addition level) decreased crude fiber and ADF contents by 8.6 and 11.8%, respectively. Another advantage of using urea, apart from its ability to break down lignocellulose, is that the compound supplies nitrogen and can further be converted into microbial protein (Gunun *et al.*, 2016). Increasing concentration of CP observed in ammoniated rice straw in this study was clearly a contribution from urea as a source of non-protein nitrogen.

Rumen fermentation

All treatments produced similar gas production up to 12 h after incubation (Table 3). Urea treatment increased gas production of rice straw by 18.9 and 20.2% at 24 and 48 h of *in vitro* analysis, respectively (P<0.05). Addition of *P. falcataria* to ammoniated rice straw at 20 and 40% DM did not further improved gas production. Addition of *S. rarak* to ammoniated rice straw and *P. falcataria* mixture at different levels resulted in a different response; addition at 10% DM produced similar gas production whereas at 20% DM decreased gas production (P<0.05) as compared to T3 and T4, respectively. Urea treatment and addition of *P. falcataria* and/or *S. rarak* did not change total VFA production and molar proportion of individual VFA of rice straw during 24 h *in vitro* incubation (Table 4). Rice straw treated with urea had higher ammonia concentration by 72% than that of the untreated rice straw (P<0.05). Addition of *P. falcataria* or *S. rarak* at lower level produced similar ammonia concentration as ammoniated rice straw whereas their addition at higher level decreased ammonia concentration (P<0.05). Ammoniation of rice straw increased its degradability by 37.1% (P<0.05; Table 5). Addition of both *P. falcataria* and/or *S. rarak* did not further increase the degradability of ammoniated rice straw. Dietary treatments showed indifferent total bacteria population but, on the contrary, revealed different protozoa population. *Paraserianthes falcataria* addition to ammoniated rice straw decreased the protozoa population (P<0.05) and *S. rarak* further decreased the fauna population (P<0.05).

Table 3. Gas production kinetics of experimental treatments

Treatment	Gas production (ml/g)					
	3 h	6 h	9 h	12 h	24 h	48 h
T1	5.3	10.5	16.0	20.5	45.6[a]	77.4[a]
T2	5.8	9.7	14.8	22.3	54.2[b]	93.0[b]
T3	5.8	10.1	14.9	22.6	57.8[b]	92.3[b]
T4	7.5	12.4	17.3	23.4	53.7[b]	86.9[b]
T5	6.3	11.4	17.3	23.6	54.1[b]	91.1[b]
T6	4.7	9.1	14.4	18.9	44.2[a]	75.8[a]
SEM	0.42	0.53	0.64	0.81	1.21	1.64
P-value	0.109	0.093	0.214	0.075	<0.001	<0.001

[a,b] Different superscripts within the same column are significantly different at P<0.05.
T1: rice straw; T2: ammoniated rice straw; T3: T2 80% + *P. falcataria* 20%; T4: T2 60% + *P. falcataria* 40%; T5: T2 80% + *P. falcataria* 10% + *S. rarak* 10%; T6: T2 60% + *P. falcataria* 20% + *S. rarak* 20%.
SEM: standard error of mean.

Table 4. Rumen fermentation of experimental treatments

Treatment	Total VFA (mmol/l)	C_2 (%)	C_3 (%)	C_4 (%)	NH_3 (mmol/l)
T1	63.9	62.1	25.6	12.3	15.4[a]
T2	57.1	65.2	23.8	11.0	26.5[c]
T3	74.3	60.0	27.4	12.6	26.5[c]
T4	67.8	54.8	26.3	18.9	20.3[b]
T5	59.1	64.3	24.6	11.1	24.6[c]
T6	62.5	60.0	25.1	15.0	20.7[b]
SEM	3.98	1.58	1.02	0.96	0.87
P-value	0.597	0.220	0.920	0.071	<0.001

[a,b,c] Different superscripts within the same column are significantly different at P<0.05.
T1: rice straw; T2: ammoniated rice straw; T3: T2 80% + *P. falcataria* 20%; T4: T2 60% + *P. falcataria* 40%; T5: T2 80% + *P. falcataria* 10% + *S. rarak* 10%; T6: T2 60% + *P. falcataria* 20% + *S. rarak* 20%.
C_2, acetate; C_3, propionate; C_4, butyrate; NH_3, ammonia; SEM, standard error of mean; VFA, volatile fatty acid.

Table 5. Degradability and microbial population of experimental treatments

Treatment	IVDMD (%)	Bacteria (10^9/ml)	Protozoa (10^5/ml)
T1	34.5[a]	4.10	12.38[c]
T2	47.3[c]	6.29	10.13[c]
T3	47.3[c]	5.17	8.25[b]
T4	42.5[b]	4.35	8.63[b]
T5	42.0[b]	4.27	5.63[a]
T6	41.1[b]	4.82	4.25[a]
SEM	0.86	0.46	0.06
P-value	<0.001	0.182	<0.001

[a,b,c] Different superscripts within the same column are significantly different at P<0.05.
T1: rice straw; T2: ammoniated rice straw; T3: T2 80% + *P. falcataria* 20%; T4: T2 60% + *P. falcataria* 40%; T5: T2 80% + *P. falcataria* 10% + *S. rarak* 10%; T6: T2 60% + *P. falcataria* 20% + *S. rarak* 20%.
IVDMD: *in vitro* dry matter degradability; SEM: standard error of mean.

Cell wall expansion and degradation of rice straw due to urea treatment enables rumen microbes to attack, colonize and degrade the fiber component more easily. It was confirmed by the increase of gas production and degradability of ammoniated rice straw in comparison to the untreated rice straw. Gas produced in an *in vitro* rumen fermentation system is originated from two sources; it is an end product of microbial metabolism during feed degradation and fermentation, and as a result of VFA buffering process by bicarbonate buffer present in the incubation medium (Jayanegara *et al.*, 2017). Therefore gas production is considered as an indicator of feed degradability or digestibility. A number of studies have shown a positive correlation between gas production and feed digestibility (Sebata *et al.*, 2011; Plaizier and Li, 2013). An increase of rumen ammonia concentration in the incubation of urea-treated rice straw indicates that the urea is converted to ammonia and further solubilized in the rumen. With regard to VFA produced, in contrast to our expectation, total VFA and its individual components did not change by urea treatment. Generally an increase of gas production is accompanied by an increase of total VFA concentration since both parameters are end products of metabolism of rumen microbes, particularly from carbohydrate metabolism (Morvay *et al.*, 2011; Jayanegara *et al.*, 2017). Apparently the insignificancy of total and individual VFA in the present study is due to the high variation among replicates.

Supplementation of *P. falcataria* at 40% DM decreased *in vitro* DM degradability and ammonia concentration apparently was due to tannin present in the plant. Tannin has the ability to form complexes with macromolecules such as protein and carbohydrate particularly via its multiple hydroxyl groups, making the nutrients less available for rumen microbial degradation (Piluzza *et al.*, 2014) and hence less degradability. Through such complex formation, stepwise protein degradation to peptides and amino acids, and subsequent deamination to form ammonia is hampered; this was confirmed by the lower rumen ammonia concentration at 40% DM *P. falcataria* supplementation, in which the mixed diet contained 1.2% tannin. In agreement with the current finding, in a meta-analysis study, it was shown that higher concentration of condensed tannin in diet led to a lower rumen ammonia and *iso*VFA concentrations both in *in vitro* and *in vivo* experiments (Jayanegara and Palupi, 2010). Jolazadeh *et al.* (2015) also observed that tannin (extracted from pistachio hulls) treatment of soybean meal lowered its proportion of rumen degradable protein and increased proportion of rumen undegradable protein. Lower protozoa population due to *P. falcataria* supplementation was apparently related to defaunation activity of tannin as also observed by other authors (Bhatta *et al.*, 2009).

The decrease of protozoa population by addition of *S. rarak* indicates that saponin has a defaunation activity against the fauna. Saponin forms a complex with cholesterol in the cell membrane of protozoa, changes its permeability and induces cell lysis (Jayanegara *et al.*, 2014). Supporting the present finding, studies performed by other authors also demonstrated a decrease of protozoa population by addition of saponin (Zhou *et al.*, 2011; Ramos-Morales *et al.*, 2017). A meta-analysis study of Jayanegara *et al.* (2014) revealed that all saponin sources evaluated, i.e. quillaja, tea and yucca saponins had the ability to reduce protozoa population in the rumen with relatively similar magnitude of reduction among the sources. With regard to ammonia decreasing effect of saponin in *S. rarak*, it is apparently related to the following mechanisms: (1) sugar component of saponin interacts with ammonia, making the ammonia less available, (2) reduction of predation intensity of protozoa on rumen bacteria as a result of anti-protozoal effect of saponin in which such predation leads to microbial protein degradation to form ammonia; when the predation intensity is reduced then formation of ammonia is reduced as well, and (3) saponin inhibits the growth of some rumen microbes that degrade protein to amino acids and ammonia such as *Butyrivibrio fibrisolvens*, *Streptococcus bovis* and *Prevotella bryantii* (Patra and Saxena, 2009; Jayanegara *et al.*, 2014).

Methane production

Inclusion of *S. rarak* at 20% DM (T6) produced the lowest methane production both at 24 and 48 h after incubation (Table 6). When methane was presented as percentage to total gas, ammoniation of rice straw (T2) resulted in a lower methane concentration than that of untreated rice straw (T1; P<0.05); the decrease was 19.7 and 18.5% at 24 and 48 h, respectively. Such response was also similar when methane was expressed as a proportion to degradable DM with a higher magnitude of decrease, i.e. 29.0%. Methane production per unit of DM degraded was lowest at the highest *S. rarak* inclusion level (T6). Addition of *P. falcataria* lowered methane production at 48 h incubation in comparison to ammoniated rice straw (P<0.05), but it was non-significant for methane concentration and methane per unit of DM degraded.

The decrease of methane percentage to total gas and methane per unit of degradable DM in ammoniated rice straw incubation was apparently related to the change of carbohydrate fractions after urea treatment. Urea treatment decreased NDF and ADF contents of rice straw and converted them to more readily available carbohydrate as discussed above. Such cell wall fractions are positively correlated with methane production since fiber degradation by rumen microbes produce large amount of hydrogen that acts as a main substrate for methanogenesis (Jayanegara *et al.*, 2011). The present result indicates that urea

Table 6. Methane (CH_4) production of experimental treatments

Treatment	CH_4 (ml/g)		CH_4 (%gas)		CH_4/IVDMD (ml/g)
	24 h	48 h	24 h	48 h	
T1	12.0^{b}	19.1^{c}	25.4^{b}	23.3^{c}	54.1^{c}
T2	11.6^{b}	18.6^{c}	20.4^{a}	19.0^{a}	38.4^{ab}
T3	11.7^{b}	17.9^{bc}	19.5^{a}	18.8^{a}	37.6^{a}
T4	11.0^{b}	16.4^{b}	20.2^{a}	18.6^{a}	38.5^{ab}
T5	11.7^{b}	19.6^{c}	21.2^{a}	20.9^{b}	43.8^{b}
T6	8.4^{a}	14.5^{a}	19.4^{a}	19.5^{ab}	34.1^{a}
SEM	0.54	0.79	0.90	0.80	2.00
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

^{a,b,c} Different superscripts within the same column are significantly different at P<0.05.
T1: rice straw; T2: ammoniated rice straw; T3: T2 80% + *P. falcataria* 20%; T4: T2 60% + *P. falcataria* 40%; T5: T2 80% + *P. falcataria* 10% + *S. rarak* 10%; T6: T2 60% + *P. falcataria* 20% + *S. rarak* 20%.
IVDMD: *in vitro* dry matter degradability; SEM: standard error of mean.

treatment of rice straw does not only improve its nutritive value but also beneficial with regard to decreasing environmental emission. Supporting this result, increasing forage to concentrate ratio from 47:53 (lower fiber diet) to 68:32 (higher fiber diet) increased methane emission of dairy cows from 538 to 648 g/day (Aguerre *et al.*, 2011). More recently, Adejoro and Hassen (2017) supplemented or treated *Eragrostis curvula* hay with urea and observed the effects of such experimental treatments on digestibility and *in vitro* fermentation. The authors reported that hay treated with urea resulted in lower methane production, methane to total gas and methane per unit OM digested in comparison to the control hay. Urea treatment also increased total gas production, *in vitro* OM digestibility, and total VFA production of the hay, but no change of individual VFA proportion (acetate, propionate, butyrate and acetate-to-propionate molar ratio) was observed.

Methane reduction effect of *S. rarak* supplementation at 20% DM (T6) was evident. Supplementation of the fruit at 10% DM (T5) was not significant apparently because of the lower level of saponin present in the mixed diet, i.e. 0.7% DM, whereas saponin content in T6 diet was 1.4% DM. Such methane decrease by saponin seems to be related with its defaunation activity against rumen protozoa. Certain population of archaea methanogen live in symbiosis with protozoa in which they take advantage from the fauna by consuming the hydrogen released and use it as a primary substrate for methanogenesis (Morgavi *et al.*, 2010). Therefore in the presence of saponin, it is expected that methanogen population is reduced as well. Accordingly, Goel *et al.* (2008) demonstrated that addition of saponin-rich fractions from carduus (*Carduus pycnocephalus*), sesbania (*Sesbania sesban*), knautia (*Knautia arvensis*) or fenugreek (*Trigonella foenum-graecum*) into hay:concentrate diet (1:1 w/w) decreased both protozoal numbers and methanogen relative abundance. A study of Narvaez *et al.* (2013) showed that supplementation of *Yucca schidigera* extract (containing 153 mg/g DM steroidal sapogenin) at a level of 650 µg/ml decreased methanogenic archaea population from 247.8 × 10^8 to 118.3 × 10^8 gene copies. In addition to such population decrease, saponin might also reduce the activity of methanogenic archaea. Another plausible explanation of the lower methane production due to saponin is that the compound had been demonstrated to inhibit cellulolytic bacteria and anaerobic fungi in which both microbial groups are responsible for fiber degradation in the rumen (Guo *et al.*, 2008). Such inhibition may lead to further reduction of hydrogen provision for methane formation.

Conclusion

Urea treatment of rice straw effectively improves its nutritive value as indicated by the lower NDF and ADF contents, higher gas production, rumen ammonia concentration and *in vitro* DM degradability. Further, such treatment provide an additional advantage by lowering enteric methane production, a major greenhouse gas in the atmosphere contributing to global warming. Supplementation of *P. falcataria* to ammoniated rice straw may provide further benefit with regard to higher protein content and a shift toward more proportion of rumen undegradable protein due to its tannin content. Although supplementation of *S. rarak* does not improve nutritional quality of ammoniated and *P. falcataria* mixture, it may strategically be used to further lower the methane production since the plant is rich in saponin.

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