SUPPLEMENTATION EFFECTS OF TANNIN AND SAPONIN EXTRACTS TO DIETS WITH DIFFERENT FORAGE TO CONCENTRATE RATIO ON In vitro RUMEN FERMENTATION AND METHANOGENESIS

Yogianto\textsuperscript{1}, A. Sudarman\textsuperscript{2}, E. Wina\textsuperscript{3} and A. Jayanegara\textsuperscript{2}

\textsuperscript{1}Graduate School of Nutrition and Feed Science, Faculty of Animal Science, Bogor Agricultural University, Jl. Agatis, Darmaga Campus, Bogor 16680 - Indonesia

\textsuperscript{2}Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Jl. Agatis, Darmaga Campus, Bogor 16680 - Indonesia

\textsuperscript{3}Indonesian Research Center for Animal Production, Ciawi - Bogor 16002 - Indonesia

Corresponding E-mail: anu\_jayanegara@yahoo.com

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ABSTRAK

Penelitian ini bertujuan untuk menginvestigasi pengaruh penambahan kombinasi ekstrak tanin dan saponin terhadap emisi gas metana dalam pakan dengan proporsi hijauan:konsentrat yang berbeda dalam fermentasi rumen secara \textit{in vitro}. Rancangan percobaan menggunakan rancangan acak kelompok (RAK) pola factorial. Faktor pertama adalah proporsi hijauan:konsentrat dalam pakan (70:30 and 30:70) dan faktor kedua adalah penambahan ekstrak tanin dan saponin (kontrol, tanin, saponin, tanin+saponin) pada dosis 2 mg/ml. Peubah yang diamati meliputi produksi gas, produksi gas metana, kecernaan bahan kering (KBK) dan bahan organik (KBO), dan konsentrasi amonia. Hasil menunjukkan bahwa penambahan tanin, saponin, dan kombinasinya secara umum menurunkan produksi gas dan metana pada kedua tipe pakan selama waktu inkubasi 24 dan 48 jam (P<0,05), namun kombinasi tanin dan saponin dibandingkan dengan penggunaan secara terpisah tidak menunjukkan perbedaan yang nyata. Penambahan kombinasi tanin dan saponin berpengaruh menurunkan KBK, KBO, dan ammonia secara nyata (P<0,05). Dapat disimpulkan bahwa penambahan ekstrak tanin, saponin dan kombinasi keduanya pada dosis 2mg/ml dapat menurunkan emisi gas metana tetapi diikuti dengan menurunnya KBK, KBO dan ammonia.

Kata kunci: tanin, saponin, hijauan, konsentrat, metana, in vitro

ABSTRACT

This experiment was aimed to investigate the effect of combining tannin and saponin extracts on ruminal methane emission of diets with different proportion of forage to concentrate in the \textit{in vitro} fermentation. The experiment was conducted in a factorial block design. The first factor was the proportion of forage:concentrate in diets (70:30 and 30:70) and the second was addition of tannin and saponin extracts (control, tannins, saponins, tannins + saponins) in the dose of 2 mg/ml. Variables observed were gas production kinetics, methane production, dry matter digestibility (DMD), organic matter digestibility (OMD) and ammonia concentration. Results revealed that addition of tannins, saponins and their combination generally lowered total gas and methane production during 24 and 48 h of incubation period in both types of diets (P<0.05), but combination of tannins and saponins compared with their separated forms did not show any significant differences. The addition of tannins, saponins and their combination reduced DMD, OMD and ammonia significantly (P<0.05). It can be concluded that the addition of tannin, saponin and their combination at a dose of 2 mg/ml could reduce methane emission but followed by a decline in the DMD, OMD and ammonia.

Keywords: tannin, saponin, forage, concentrate, methane, in vitro
INTRODUCTION

Global warming is a major environmental problem faced by mankind, especially in the last century. Intergovernmental Panel on Climate Change (IPCC) in 2007 reported that the average temperature of earth’s surface has increased by 0.74 ± 0.18°C in the 20th century and such fact is the largest temperature rise within the last few thousand years. Furthermore, modeling scenarios developed by IPCC also suggest that the earth’s surface temperature could increase by 2.4 to 6.4°C by the year of 2090 to 2099. If this is the case in the future, it will greatly impact on various aspects of human life. Main causes of global warming have been known to be associated with a very high rate of accumulation of greenhouse gases in the upper atmosphere such as carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O) and chlorofluorocarbon (CFC) as a result of the increasing intensity of various human activity (Thorpe, 2009). Methane is the second largest contributor to greenhouse gas (16% of total) after CO₂, but, its ability to retain heat (global warming potential) is 21 times higher than that of CO₂ (Iqbal, 2008).

Livestock, especially ruminants like cattle, goats, and sheep contribute to accumulation of methane emission in the atmosphere due to methanogenesis by archaean methanogen in the rumen (Cottle et al., 2011). Such emission does not only affect the global warming, but it also represents energy loss from the animals, in which the lost can be between, 8 to 14% from total digestible energy. Nutritional strategies to mitigate methane emission based on natural substances are preferred over the synthetic ones (Jayanegara et al., 2009a). Accordingly, secondary plant metabolites such as tannins and saponins are potential to be used in mitigating methane emissions from ruminants. Tannins can reduce methane emissions through a reduction in methanogen population (Bhatta et al., 2009) whereas saponins work through a reduction in protozoa population (Hess et al., 2003) in which part of the methanogen is living symbiotically (Finlay et al., 1994). If these two compounds are used simultaneously, is could be expected to decrease in methane emission further.

Therefore, the purpose of this study was to investigate the effect of combining tannin and saponin extracts when added into two types of rations with different forage to concentrate ratio on ruminal methane emission, gas production, digestibility of dry matter and organic matter, and ammonia concentration through an in vitro assay.

MATERIALS AND METHODS

Extraction of Tannins and Saponins

Tannin extract was taken from the leaves of mahogany (Swieteniamahagoni) while the saponin extract was taken from the lerak fruit (Sapindusrarak) in which both of them were collected from Bogor area. Mahogany leaves and lerak fruits were oven-dried at 60°C to obtain approximately 90% dry matter and then, ground immediately to obtain powdered forms. Mahogany leaves powder extracted with a combination of 70% methanol:30% water, while the lerak fruits powder was extracted with 100% methanol solvent by using an ultrasonic water bath for 30 min (Yuliana et al., 2014). Subsequently, the solid and liquid fractions were filtered using a Whatman paper. The liquid fraction was then evaporated in a rotary evaporator to evaporate the organic solvents, freeze dried and kept in air tight bags at freezer (-4°C). These procedures produced dried tannin and saponin extracts.

In Vitro Fermentation

The substrate used in the in vitro test was consisted of two types of diet with different forage to concentrate proportion, i.e.70:30 and 30:70, respectively. Forage used was elephant grass (Pennisetumpurpureum) with nutrient content (dry matter basis) of crude protein (CP): 8.96%, neutral detergent fibre (NDF): 65.61%, and acid detergent fibre (ADF): 44.72%. The concentrate was a commercial concentrate of dairy cows with trademark Lactofeed produced from CV. Tani Mulya, Bogor, Indonesia, contained of 11.45% CP. The grass was dried in an oven at 50°C until the moisture content was around 10%. The substrates were ground using a grinder to pass a 1 mm sieve size.

The in vitro fermentation technique was according to the method of Theodorou (1990). A total of 100 mg of substrate treatment was inserted into a 100 ml bottle size and buffered rumen fluid as the incubation medium. The incubation medium was consisted bicarbonate buffer solution: (24.1%), macro-mineral solution: (12.1%), micro-mineral solution: (0.00613%), resazurin: (0.0612%), distilled water: (36.2%), reducing solution: (2.3%) and rumen fluid: (25.3%). Rumen fluid was collected just before...
morning feeding from a rumen fistulated Friesian Holstein cow in Balai Penelitian Ternak, Ciawi, Bogor; the cow was fed with elephant grass and commercial concentrate at a ratio of 60:40, respectively. The rumen fluid was filtered through a nylon cloth and, then inserted into a container and immediately brought to the laboratory. Incubation was carried out in a water bath maintained at 39-42°C for 48 h. During the incubation, the bottles were shaken.

Factorial (2 × 4) randomized complete block was used in this study. Factor A: Different forage to concentrate ratio:
- A1: 70% forage:30% concentrate
- A2: 30% forage:70% concentrate

Factor B : Addition of tannin and or saponin extracts at (a dose of 2mg/ml rumen fluid):
- B1: Control
- B2: B1 + 2 mg/ml tannin extract
- B3: B1 + 2 mg/ml saponin extract
- B4: B1 + 1 mg/ml tannin extract + 1 mg/mL saponin extract

Variable Measurements
Variables observed in this study were gas production kinetics, methane production, in vitro dry matter digestibility (DMD), in vitro organic matter digestibility (OMD) and ammonia concentration. Gas production was observed at 1, 3, 6, 10, 12, 14, 21, 24, 30, 36 and 48 h after incubation. Methane production was measured by using CO₂ trapping method with NaOH at the interval when te residue was filtered and dried in an oven at 105 °C for 24 h. Dry matter and organic matter residue were determined to calculate the DMD and OMD. Ammonia concentration was measured with the Conway micro-diffusion technique.

Statistical Analysis
Data obtained were analyzed by the factorial analysis of variance (ANOVA). When a particular variable showed significantly different at P<0.05 in the ANOVA result, a post-hoc test namely Duncan’s multiple range test was employed to compare among different treatment means. All statistical analyses were performed by using SPSS software version 17.

RESULTS AND DISCUSSION
Total Gas and Methane Production
Total gas production in vitro increased at higher incubation period but with a declining rate. This is due to the decreasing quantity of fermentable substrates (Jayanegara et al., 2006). At high forage ration (HFR), addition of tannin and/or saponin extracts did not affect total gas production up to 6 h of in vitro incubation as compared to control; the change was begun later (Table 1). After 24 h incubation, the addition of tannins, saponins and their combination at 2mg/ml in HFR significantly reduced the total gas production by 18.3, 16.9, and 11.2% from control, respectively (P<0.05). However, such additions to HCR did not decrease total gas production at 24 h. Different pattern was observed at 48 h of incubation; addition of tannin and saponin extracts in combination increased total gas production especially in HCR as compared to control (P<0.05).

Total gas in the in vitro rumen fermentation is produced from the fermentation of substrates, primarily composed of CO₂ and CH₄ (Getachew et al., 1998). The reduction of gas production at 24 h fermentation due to addition of tannin extract was in agreement with Jayanegara et al. (2009a) although the level tested was different. While the decline in gas production due to the addition of saponin extract was also observed by Makkar et al. (1995). Mechanism of tannins in reducing gas production is through their ability to interact with feed components mainly protein and fiber which have a major contribution in generating gas (Makkar, 2003; Makkar et al., 2007), whereas the mechanism of saponins is more ability to inhibit the activity of enzymes that degrade the fiber components (Hristov et al., 2003). Interestingly, when tannins and saponins were combined, the addition did not decrease gas production especially at longer incubation period. Apparently they interacts each other and alleviate the negative impact on the in vitro rumen fermentation activity. In relation to starting from 3 h of incubation until the end (48 h), HCR produced lower methane concentration than that of HFR (P<0.05; Table 2). Additions of tannins, saponins and the combination of tannins+saponins generally decreased methane concentration as compared to control both in HFR and HCR (P<0.05). The response was consistent until 48 h of incubation. No significant interaction was found between different forage to concentrate diet and tannin/saponin additions. Simultaneous addition of tannins+saponins lowered methane concentration than their individual addition especially during early incubation period and in HCR.
Effects of Tannin and Saponin Extracts on Rumen Fermentation (Yogianto et al.)

### Table 1. Gas Production Kinetics (in ml) of High Forage Ration (HFR) and High Concentrate Ration (HCR) on Addition of Tannin and Saponin Extracts

| Time (h) | HFR (70%F:30%C) | HCR (30%F:70%C) | Significance |
|----------|-----------------|-----------------|--------------|
|          | Ctl T S T+S     | Ctl T S T+S     | FC TS INT    |
| 1        | 15.7a 16.2ab 16.2ab 20.8abc | 17.8ab 19.8ab 20.9bc 24.8c | ** ** ns    |
| 3        | 30.6ab 28.6a 31.7ab 33.8ab | 34.6ab 35.3bc 40.5cd 41.3d | ** ** ns    |
| 6        | 46.3a 42.7a 47.8ab 51.4abc | 59.7cde 56.8bcd 62.8de 68.9e | ** * ns     |
| 10       | 77.9bc 58.2a 69ab 72.1b  | 99.1de 87.7cd 96.7de 104e | ** * ns     |
| 12       | 94.9bc 67.7a 81.5b 83.2b  | 116.9de 104.7cd 119.9c 123.5e | ** ** ns    |
| 14       | 109.6c 77.1a 91.6b 93.1b  | 131.9de 119.4cd 136.6e 139.5e | ** ** ns    |
| 21       | 140.2b 110.4a 113.6a 121a  | 158.9c 154.1e 160.5c 164.3c | ** * *     |
| 24       | 151b 123.4a 125.5a 134.1a  | 167.5c 165.8c 169.3c 174c  | ** * *     |
| 30       | 164.9b 147.7a 141.7b 152.2ab | 178c 180.4c 180.9c 185.8c | ** ns *     |
| 36       | 173.2bc 162.1ab 156.4a 168.9a | 183.7cd 188.6d 190.3d 195.5d | ** ns ns    |
| 48       | 184.8bc 174.3ab 169.5a 187.4c | 190.1cd 199.2d 203.2e 207.5e | ** * **    |

Different superscripts within the same row are significantly different at P<0.05
F= forage; C = concentrate; Ctl= control; T = tannin; S = saponin; T+S = tannin + saponin; FC= factor forage to concentrate ratio; TS = factor addition of tannin and saponin; INT = interaction between FC and TS; ** = highly significant (P <0.01); * = Significant (P<0.05); ns = non-significant

### Table 2. Methane Production (in % Total Gas) of High Forage Ration (HFR) and High Concentrate Ration (HCR) on Addition of Tannin and Saponin Extracts

| Time (h) | HFR (70%F:30%C) | HCR (30%F:70%C) | Significance |
|----------|-----------------|-----------------|--------------|
|          | Ctl T S T+S     | Ctl T S T+S     | FC TS INT    |
| 1        | 34.14c 26.002ab 27.55ab 23.099ab | 29.03bc 26.21ab 25.02ab 22.06a | ns ** ns    |
| 3        | 36.20d 26.83bc 25.34bc 23.094ab | 28.33c 27.58c 22.95ab 20.91a | * ** ns     |
| 6        | 34.48d 28.65c 26.61abc 24.38ab | 28.11bc 27.42bc 24.11ab 22.85a | ** ** ns    |
| 10       | 33.81d 28.82c 28.11bc 24.51ab | 27.90bc 26.45bc 24.45ab 22.19a | ** ** ns    |
| 12       | 32.87d 27.77c 27.30bc 24.73bc | 27.35bc 25.59abc 24.12ab 22.27a | ** ** ns    |
| 14       | 32.08d 27.67c 26.63bc 24.68ab | 26.53bc 25.29bc 23.88ab 22.14a | ** ** ns    |
| 21       | 31.21d 26.85bc 26.50bc 24.68abc | 26.99c 22.66abc 24.03ab 22.66a | ** ** ns    |
| 24       | 31.23d 26.60c 26.22bc 24.78abc | 27.07c 25.12abc 23.91ab 22.75a | ** ** ns    |
| 30       | 30.99d 24.70abc 26.30bc 24.56bc | 27.12c 25.14abc 24.05ab 22.47a | ** ** ns    |
| 36       | 30.95d 24.32ab 25.96bc 24.21bc | 27.22c 25.08abc 24.22ab 22.54a | * ** ns     |
| 48       | 31.13c 23.90a 25.37ab 24.46a  | 27.31b 24.96ab 24.24a 22.68a | * ** ns     |

Explanation of Ctl, T, S, TS, INT, * and **: see Table 1
Effect of tannins in reducing methane emission on high forage ration was in line with that reported by Jayanegara et al. (2010); addition of purified tannins from chestnut and Sumach at 1 mg/ml into hay:concentrate (70:30) diet decreased methane concentration by 6.5 and 7.2%, respectively. With regard to saponins, in contrast to the present study, saponins from lerak fruits did not decrease rumen methanogens which can be correlated with the methane emissions. Such difference may occur because of the differences in the dose of saponin extracts, duration of incubation, and the incubation medium. Other reports have shown the methane mitigation effect of saponins from various sources, such as from Camellia sinensis (Guo et al., 2008) and Knautia arvensis (Goel et al., 2008). The ability of tannins and saponins to reduce ruminal methane emissions has different mechanisms. Tannins, including hydrolysable and condensed tannins reduce methane through a direct inhibition on archa metanogen population in the rumen (Bhatta et al., 2009). On the other hand, saponins decrease methane through a reduction in ruminal protozoal population (Hess et al., 2003) in which part of the methanogens are symbiotically living together with the fauna and contribute up to 37% of the total methane emissions from the rumen (Finlay et al., 1994).

Feed Digestibility and Fermentation

Data on feed dry matter digestibility (DMD) and organic matter digestibility (OMD) are presented in Figure 1 and Figure 2, respectively. The addition of tannins, saponins and their combination at 2 mg/ml during the 48 h incubation period significantly decreased DMD and OMD (P<0.05). The DMD decrease on the addition of tannins, saponins and the combination in HFR 20.7%, 20.5% and 24.7%, respectively, in HCR were 19.0%, 12.2% and 13.3%, respectively. Similarly, OMD decrease due to addition of tannins, saponins and tannins+saponins were 35.0%, 27.2% and 30.7%, respectively in HFR and 19.9%, 16.2% and 16.1% in HCR. The decline of ruminal digestibility due to addition of tannins and/or saponins has also been reported by some other authors (Makkar et al., 1995; Wina et al., 2005; Jayanegara et al., 2009b). The mechanisms of tannins and saponins in reducing ruminal digestibility of dry matter and organic matter are similar as in the reduction of gas production; tannins inhibit feed degradation process through their interactions with protein and fiber components (Makkar, 2003; Makkar et al., 2007) while saponins inhibit the activity of enzymes that degrade fiber components (Hristov et al., 2003).

Addition of saponins and the combination resulted in the decrease of rumen ammonia concentration both in HFR and HCR (P<0.05; Figure 3). No difference was observed between HFR and HCR with regard to ammonia concentration. Combination of tannins+saponins decreased ammonia further than those of their individuals (P<0.05). This may indicate the

![Figure 1. Dry Matter Digestibility (DMD) of High Forage Ration (HFR) and High Concentrate Ration (HCR) on Addition of Tannin and Saponin Extracts. Ctl = control; T = tannin; S = saponin; T+S = tannin + saponin; HFR= high forage ration; HCR= high concentrate ration; F= forage; C= concentrate.](image-url)
presence of associative effect between tannins and saponins in decreasing rumen ammonia concentration. A number of studies have reported that tannins and saponins reduced ammonia concentration in the rumen, both in vitro and in vivo (Makkar et al., 1998; Wina et al., 2005).

Concentration of ammonia in the rumen is derived from the lysis of microbes and degradation of feed protein. Most of ammonia is absorbed through the rumen wall and the rest is used directly by rumen microbes to meet the needs of nitrogen; about 50-80% requirements for microbial nitrogen is derived from ammonia (Leng, 1984). Tannins decrease ammonia concentrations of ammonia by binding with feed protein and, hence, prevent its degradation by proteolytic microbes (Tanner et al., 1994). The decrease in ammonia due to the addition of saponins occurred by an indirect mechanism through a reduced protozoal population (VanSoest, 1994). When both tannins and saponins were added simultaneously, apparently both mechanisms occur and lead a synergistic effect for further reduction of rumen ammonia.

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CONCLUSION

Addition of tannins, saponins and their combination were able to reduce ruminal methane emissions \textit{in vitro} when added both in high fiber and high concentrate rations at 2 mg/ml. Although the additions also decreased DMD, OMD and ammonia concentration in the rumen, it does not always mean that a negative effect on animal performance will occur. It has to be noted that the depression of digestibility is taken place in the rumen, not in the total digestive tract. Further \textit{in vivo} study is therefore needed to confirm the present \textit{in vitro} results and to investigate their effects on animal performance.

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