In vitro gas production kinetics as influenced by combination of Acacia magium, Swietenia mahagoni and Artocarpus heterophyllus as tannin source

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Abstract. Acacia mangium, Swietenia mahagoni, and Artocarpus heterophyllus commonly used in ruminant feeding practices and which contains CT with potential to reduce CH₄ emissions and improve feed protein utilization. This study was aimed to determine the effect of combination of Acacia mangium, Swietenia mahagoni, and Artocarpus heterophyllus as a tannin source on in vitro ruminal fermentation kinetics. The combination leaves consists of Swietenia mahagoni (40%), Acacia mangium (30%), and Artocarpus heterophyllus (30%). The levels of combination leaves which used were 0%, 50%, and 100% as forage sources (60%) and concentrate 40%. Feed fermentation was conducted using Menke and Steingass gas production technique for 48 hours. Kinetic of gas production was analyzed using the Fit Curve program. Data obtained were analyzed using one way ANOVA, and continued by DMRT. The results showed that the gas production from soluble fractions (a) was affected by the treatment. Gas production from the potentially degraded fraction (b) did not different between control and treatment (P>0.05). The potential extent of gas production (a+b) decreased significantly (P<0.05) at 50% combination leaves. The gas production rate (c) did not affected (P<0.05) by combination leaves. Inclusion of 50% of combination leaves in the diet could reduce rumen gas production, the soluble fraction (a) and the potentially degraded fraction (b).

1. Introduction

Ruminant livestock is one sector that contributes Green House Gasses (GHGs) emissions that can cause global warming. Total amount of GHGs emissions produced by the livestock sector in 2000 were 2.45 gigatonnes CO₂ eq. The largest source of GHGs emissions is methane gas emissions produced by ruminant livestock enteric fermentation, which is 1.6 gigatonnes CO₂ eq [1].

Tannin is a plant secondary metabolite compound that can reduce methane gas production and manipulate the fermentation process in the rumen by inhibiting the growth of several microbes that play a role in feed degradation [2]. Tannins can be toxic to methanogenic bacteria and other bacteria in the rumen in three ways: 1) inhibiting the activity of enzymes and binding the substrate needed for bacterial growth, 2) forming complexes with bacterial membranes so that it blocks the substrate and
nutrients to entering the cell for bacterial growth, 3) forming complexes with metal ions in bacteria that has a function as a coenzyme, so that it will inhibit bacterial growth [3]. Giving chestnut tannins in hay could reduce methane gas production and total gas production in rumen fermentation [4]. Tannins can also interact with proteins and form strong complexes in the rumen. The tannin and protein complexes are stable in the rumen (pH 3.5-7.5) and will be released when in the abomasum (pH < 3.5) [3]. Giving tannins with levels of 2% to 4% of DM in feed could inhibit protein degradation in the rumen [5].

*Acacia mangium*, *Swietenia mahagoni*, and *Artocarpus heterophyllus* are legumes that have high tannin content. Total tannin contents in *Acacia mangium*, *Swietenia mahagoni*, and *Artocarpus heterophyllus* leaves are 9.5%, 11.93%, and 3.32% of DM respectively [6–8]. *Acacia mangium*, *Swietenia mahagoni*, and *Artocarpus heterophyllus* has been spread in various regions in Indonesia so that it is easily obtained and used as animal feed. Based on this description, it is necessary to examine the effect of combination of *Acacia mangium*, *Swietenia mahagoni*, and *Artocarpus heterophyllus* leaves as a tannin source on in vitro ruminal fermentation kinetics.

2. Materials and methods

2.1. Samples collection and preparation

Forages were collected from Special Region of Yogyakarta, Indonesia. Samples were dried at 55°C for 48 hours and ground to pass a 0.5 mm screen for chemical composition and tannin essay. Samples were proximate analyzed. Forage samples were analyzed for the total tannins and their condensed tannins content.

2.2. In vitro gas production kinetics

Two cattle were fed with elephant grass and concentrate (forages: concentrate, 60:40) for feed adaptation. Rumen fluid is taken by fistula hole. In vitro fermentation were conducted by Menke and Steingass (1988) method for 48 hours. Syringe is filled with substrate, which is feed material (forage and concentrate; 60:40) with different levels (0%, 50%, and 100%) of combination leaves as forage and tannin source. Fermented gas production was measured at 1, 2, 4, 6, 8, 12, 24, 36, and 48 hours. Gas production kinetics was measured using fit curve program by Chen (1994).

2.3. Data analysis

Data of fermentation were analyzed using one-way analysis of variance with IBM SPSS 16 for Microsoft Windows and statistically significant differences between means were determined by Duncan’s Multiple Range Test (DMRT) when the effects of treatment (P<0.05) were detected. Microbial diversity data were analyzed descriptively

3. Results and discussion

3.1. Effect of combination leaves on rumen gas production kinetics

The effect of combination leaves on rumen gas production kinetics can be observed in Table 1 and Figure 1.

| Fraction | Feed treatments | Sig. |
|----------|-----------------|------|
|          | 0% CA | 50% CA | 100% CA |
| a (ml)   | 20.44 ± 2.092^c | 5.32 ± 2.850^a | 13.72 ± 0.972^b | <0.001 |
| b (ml)   | 70.78 ± 10.870 | 65.70 ± 2.723 | 89.88 ± 16.679 | 0.093 |
| a+b (ml) | 91.22 ± 11.824^ab | 71.02 ± 5.508^a | 103.60 ± 16.981^b | 0.047 |
| c (ml/jam) | 0.041 ± 0.004 | 0.047 ± 0.006 | 0.036 ± 0.013 | 0.365 |

CA= Combination leaves, TT = Total tannin, CT = Condensed Tannin

[^a,b]: Means within the same row with different superscript letters differ significantly (P<0.05)
Figure 1. Graphic of fermented gas production with 0%, 50%, and 100% combination leaves.

3.1.1. Effect of combination leaves on gas production from feed soluble fraction (a)
The result (Table 1.) showed that the gas production from soluble fractions (a) was reduced by giving 50% of combination leaves. Previous study also reported that giving tannins from quebracho at the level of 3% of DM reduced gas production from soluble fractions (a) of alfalfa hay by 22.07% [9]. The difference in gas production from soluble fractions (a) is probably caused by changes in the microbial population. Tannins contained in combination leaves might affect the activity of microbes which are responsible for degrading soluble fractions in feed. Previous study reported that giving tannins from *Samanea saman* pod extract at the level of 6% of DM reduced amylolytic activity in the rumen by 18.52% [2]. Other study also reported that condensed tannins from *Lotus corniculatus* leaves extracts at the level of 0.15% of DM decreased proteolysis activity of *Prevotella ruminicola* 23, *Prevotella ruminicola* C21a, and *Clostridium proteoclasticum* B316 by 81.63%, 45.19%, and 85.27% [10].

Changes in microbial activity caused changes in the digestive value of feed soluble fraction in the rumen. Tannins extracted from *leucaena leucocephala* leaves at the level of 1.23% of DM reduced methane production, dry matter digestibility, and organic matter digestibility of Tifton hay in the rumen by 14.13%, 2.63% and 1.53% respectively [11]. Other study stated that giving condensed tannins from rain tree pod meal at the level of 0.5% of DM reduced protein digestibility of concentrate (consist of cassava chip, rice bran, brewery grain, palm kernel, coconut meal, urea, molasses, mineral premix, salt, and sulfur) by 9.3% [12].

3.1.2. Effect of combination leaves gas production from fraction (b)
The result (Table 1.) showed that the gas production from feed potentially degraded fractions (b) did not affected by the treatments. This shows that tannins contained in combination leaves did not influence the activity of microbes which are responsible for degrading potentially degraded fraction (b) in the rumen. Previous study reported that giving quebracho tannin at the level of 1% of DM did not affect the value of b of alfalfa hay, but giving quebracho tannin at the level of 3% of DM significantly reduced the value of b by 8.94% [9].

3.1.3. Effect of combination leaves on gas production from soluble and potentially degraded fraction (a+b)
The result (Table 1.) showed that although the production of gas produced from the potentially degraded fraction (b) did not significantly different, the total gas production (a+b) reduced significantly at 50% of combination leaves. Previous study also reported that tannin from chestnut
extract at the level of 4% of DM reduced the value of a and of a+b significantly, but did not reduce the value of b of alfalfa silage [13].

The difference in total gas production (a+b) is probably caused by changes in the microbial population. The result showed that tannins contained in combination leaves had more influence on the microbes which are responsible for the degradation of soluble fraction (a) than the microbes which are responsible for the degradation of potentially degraded fractions (b). Previous study reported that condensed tannins from rain tree pod meal at the level of 0.5% of DM reduced proteolytic population by 17.95%, but did not affect the cellulytic population in rumen [12]. Other study also reported that giving tannin source forages like Leucaena leucocephala reduced population of proteolytic bacteria by 16.36% [14].

### 3.1.4. Effect of combination leaves on feed degradation rate (c)

The result (Table 1.) showed that the feed degradation (c) did not affected by the treatments. This showed that although the addition of combination leaves reduced total gas production, the tannins contained in combination leaves did not have sufficient capacity to reduce the degradation rate in the rumen. Same result also reported in previous study which stated that giving quebracho tannin at the level of 2% of DM did not affect the degradation rate (c), but significantly reduced total gas production (a+b) of soya bean meals by 5.93% [9].

### 4. Conclusion

The conclusion of this study is the use of 50% combination leaves as forage source reduced rumen gas production kinetics, the soluble fraction (a) and the potentially degraded fraction (a+b).

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