The Effect of Mixed Leaves Tannin Sources (*Acacia mangium* Willd, *Swietenia mahagoni*, and *Artocarpus heterophyllus*) in Pellets on *In Vitro* Methane Production

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ABSTRACT
Mitigation effort to reduce methane emissions using plant bioactive compounds has been extensively studied and applied as feed additives. The objective of this study was to investigate the effect of three leaves mixtures of tannin source (*Acacia mangium* Willd, *Swietenia mahagoni*, and *Artocarpus heterophyllus*) in pellet feed on *in vitro* methane and volatile fatty acids (VFA) production. Three experimental diets (grass: concentrate, 60:40) with substitution of pellet in concentrate equal to tannin level 0%, 1%, and 2% based on dry matter (DM) were used as substrate for *in vitro* fermentation by Menke and Steingass gas production method for 48 h of incubation. The addition of three leaves mixtures of tannin source did not affect (P>0.05) methane and VFAs production. It could be concluded that substitution of pellets containing three leaves mixtures of tannin source to concentrate up to 2% was not enough to reduce methane emissions.

Keywords: *Acacia, In vitro, Jackfruit, Mahogany, Methane mitigation, Tannin*

1. INTRODUCTION

Enteric fermentation had contributed greatly to emissions of greenhouse gases (GHG's) by 40% of the total emissions by the agricultural and livestock sector [1]. It is estimated to contribute to anthropogenic GHGs emissions as follows: 5% of CO_2, 53% of N_2O, and 33% of methane (CH_4) emissions which has the greatest potential to cause global warming [2]. The high production of methane also equal to the decrease in feed energy efficiency as 8% up to 14% [3].

Methane production is closely related to VFA production, when acetate is produced, re-oxidation of NADH occurs by production of hydrogen (H_2) that can be further used by methanogenic Archaea to reduce CO_2 into CH_4. Whereas in the formation of propionate, re-oxidation of NADH will produce succinate or lactate using H_2 [4]. One of the methane mitigation efforts can be carried out by utilizing plant bioactive compounds such as tannins as feed additives [6].

The potential of tannin compounds from acacia, mahogany, and jackfruit leaves as a single leaf has been shown in some studies to reduce methane production by *in vitro* respectively. The substitution of acacia leaf contains 4,5% of total tannin in the forage rations was able to reduce ammonia-N (NH_3-N) rate by *in vitro* up to 31,12% [5]. The addition of mahogany which contains 11,9% of total tannin was able to reduce methane production up to 39,1% [6], and the addition of jackfruit leaves flour which contain 7,8% of total tannin can reduce the production of methane by *in vitro* up to 7,1% [7].

The utilization of tannin as supplement or feed additive for ruminant should consider the level of tannin and the processing technology. The concentration of condensed tannin more than 50 g/kg of feed can reduce the feed digestibility, feed consumption levels, and the animal health [8]. Pellet feed contains three leaves mixtures of tannin source can be used as an alternative product to avoid the astringency effect of tannin on

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ruminant that can reduce feed digestibility and the consumption level [9].

2. MATERIALS AND METHOD

2.1. Experimental Treatments

Treatment was arranged in a one way design, the main factors being the levels of tannin from the leaves mixtures of *Acacia mangium* Willd, *Swietenia mahagoni*, and A*rtocarpus heterophyllus* in pellet feed. Three experimental diets shown as follows:
P0 = Elephant grass at 60% + Concentrate at 40% + Pellet feed at 0%,
P1 = Elephant grass at 60% + Concentrate 27,6% + Pellet feed at 12,4%, and
P2 = Elephant grass at 60% + Concentrate at 15,2% + Pellet feed at 24,8%.

Fermentation experiments were separately conducted for each treatment with three replicates each treatment.

2.2. Materials

Laboratory equipment used for *in vitro* gas production by Menke and Steingass method [10], extraction and determination of tannins by FAO [11], and determination of nutrient content of sample by AOAC [12]. The experimental substrate samples consisted of pellets, forage, and concentrates. The pellet feeds were composed of a mixture of acacia, mahogany and jackfruit leaves, soybean meal, molasses, and tapioca. The concentrate consisted of soybean meal, palm oil meal, polar, rice bran, molasses, premix, and corn cobs. The forage used in this experiment is elephant grass (*Pennisetum purpureum*).

2.3. Methods

2.3.1. Extraction and Determination of Tannin Levels

Extraction and determination of tannin levels in acacia, mahogany, and jackfruit leaves were carried out using FAO and Porter method [11]. The results of each leaves tannin levels measurements shown in table 1.

Table 1. Tannin content of three types of leaves

Leaves	Tannin (%DM)		
	TT	CT	HT
Acacia	5,68	5,52	0,16
Mahogany	11,69	9,83	1,83
Jackfruit	7,80	5,34	2,49

TT: total tannin, CT: condensed tannin, HT: hydrolyzed tannin

2.3.2. Pelleting Process

The production of pellets is carried out by the cold method. Sequentially the processes of pellet production are grinding, mixing, pelleting and drying. Grinding process using a disc mill with 0,1 mm screen size. Pelleting using a die measuring 1,5 cm. Drying process using direct heat under the sun for 4 to 5 h till 88% up to 95% DM.

2.3.3. In vitro Gas Production

In vitro gas production was used in this study according to the method explained by [10]. Rumen fluid obtained from Bali cattle with fistula that has been adapted to standard feed for 5 days. Substrate of 300 mg was put into the syringe and incubated for 48 h under anaerobic condition at 39ºC. At the end of the incubation period, 10 mL of gas was taken in a vacutainer for analysis of methane gas production, while the fermented liquid was taken for VFA analysis.

2.4. Samples Collection and Analysis

2.4.1. Methane Production Measurement

Fermented gas samples were analyzed using the Gas Chromatography method (Balai Penelitian Lingkungan Pertanian, Kementrian Pertanian, Pati). Measurement of dry matter (DM) and organic matter (OM) and their digestibility using the method that was explained by [12]

2.4.2. Volatile Fatty Acids (VFA) Analysis

The filtrate of *in vitro* was prepared by centrifugation at 3000 rpm for 15 min, the VFA could be analyzed using gas chromatography (GC) based on the method [13] (Laboratory of Organic Chemistry, Faculty of Math and Science, Universitas Gadjah Mada).

2.5. Data Analysis

Obtained data were analyzed by one way analysis of variance (ANOVA) continued by Duncan's new multiple range test (DMRT).

3. RESULTS AND DISCUSSION

The addition of three leaves mixtures of tannin source (*Acacia mangium* Willd, *Swietenia mahagoni*, and *Artocarpus heterophyllus*) in pellet feed at tannin levels of 0%, 1%, and 2% DM feed have no effect (P>0.05) on methane and volatile fatty acids (VFA) production.

The results of this study showed that tannins in pellets which contains 5.8 mg/100 mg of total tannin up to level 2% based on DM rations did not give a significant effect on methane gas production in the percentage and digestibility parameters of dry matter and organic matter substrates (P>0.05). However, the treatment showed a decreasing trend in all methane production parameters compared to control. The experimental results also did not show a significant effect (P>0.05) on VFA levels but had a tendency to decrease the ratio of C2 and C3 at tannin levels of 2% compared to control (P<0.05).

Table 2. *In vitro* methane and VFAs production with different level of tannin from three leaves mixtures in pellet feed

Parameter	Tannin Concentration		
	0%	1%	2%
CH_4 (%) ^{ns}	8,33±0,42	7,72±0,37	7,32±0,60
CH_4/DM (mL/mg)^{ns}	0,033±0,004	0,029±0,004	0,025±0,002
CH_4/OM (mL/mg)^{ns}	0,043±0,002	0,040±0,006	0,034±0,001
VFA (*mMol*) ^{ns}	62,12±15,77	54,82±8,63	61,70±18,36
C2 ^{ns}	36,03±4,38	32,02±1,02	35,14±2,22
C3 ^{ns}	20,61±8,19	18,36±5,88	21,61±13,03
C4 ^{ns}	5,48±3,20	4,44±1,73	4,96±3,11
C2:C3 ^{ns}	1,73±0,02	1,72±0,06	1,60±0,04

CH_4: methane, DM: dry matter, OM: organic matter, VFA: volatile fatty acids, C2: acetate, C3: propionate, C4: butyrate, ^{ns} : not significant

In order to the results, CH_4 levels can increase through changes in the proportion of VFA towards an increase in the proportion of acetic acid that produces hydrogen gas as a substrate in methanogenic reactions [14]. This study shows that proportion of acetate with butyrate has decreased which indicates a decrease in acetate production and an increase in propionate due to hydrogen utilization. The decrease in the ratio of C2:C3 correlated with a tendency to decrease methane production.

The treatment showed an insignificant effect that could occur because the tannin level in the ration had a relatively in small level. Non-significant effect of small level tanin also reported by Bauchemin et al. [15] the addition of condensed tannin extract from *Schinopsis quebracho* up to a level of 2% had no effect on the decrease of methane production. The addition of *Albizia chinensis* as a source of tannins at the level of 0%, 2%, and 4% as an *in vitro* substrate did not have a significant effect on methane production while at the level of 6% it could have a significant effect on decreasing methane production without giving a negative effect on rumen fermentation [16]. In addition ruminants are able to tolerate alkaloid compounds such as tannins at low levels [17].

Low tannin levels will not affect the production of VFA and methane. Thus, the formation of methane requires hydrogen that results from the formation of acetate so that the VFA level is related to the level of methane production [18]. The decrease of acetate to propionate ratio can occur due to the influence of tannins which can suppress the methanogenesis process in the rumen. Fermentation of glucose into acetate can produce hydrogen which is used as the main substrate in the formation of methane while the formation of propionate requires hydrogen as a precursor [19].

The differences results of the studies on the effect of adding tannins on methane production and fermentation parameters, caused by several factors. Different doses of tannins, the type of tannin, and the basic substrate of fermentation can cause different effects on *in vitro* methane production and fermentation parameters results. Types of condensed and hydrolyzed tannins have different levels of toxicity, hydrolyzed tannins have a higher toxicity effect than condensed tannins, the addition of higher hydrolyzed tannins can reduce methane emissions more significant when compared to condensed tannins [15]. This is related to the protein precipitation capacity of tannins which indicates that hydrolyzed tannins can precipitate more Bovine Serum Albumine (BSA) protein than condensed tannins [20].

The type of substrate for fermentation would also affect the ability of tannins to reduce methane production. This is related to the rate of feed degradation to the gas formation process, as Widiawati et al. [21] stated that substrates with high fiber content will increase methane production when compared to substrates with higher protein content. Substrates with higher ADF (cellulose) content can increase the production of acetate and hydrogen. The affiliation of hydrogen with CO_2 will form methane. The high production and concentration of methane gas formed can reduce the efficiency of feed use and indicate a large amount of wasted feed energy.

The pelleting process also affects the tannin content even though the pelleting process is carried out by the cold method. The pelleting process can reduce tannin levels, both of total tannins, condensed tannins, and hydrolyzed tannins. The pelleting process showed a decrease on total tannin by 1,46%, meanwhile, the levels of hydrolyzed and condensed tannins decreased by 0,6% and 0,8%, respectively. The decrease in water content and mechanical processes are indicated to reduce tannin levels in the pelleting process [22].

4. CONCLUSION

In conclusion addition of three leaves mixtures of tannin source (*Acacia mangium* Willd, *Swietenia mahagoni*, and *Artocarpus heterophyllus*) up to 2% in pellet feed on *in vitro* was not enough to reduce the methane production and it has no significant effect on volatile fatty acids production.

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REFERENCES

[1] P.J Gerber, H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falcucci, Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities, Rome, 2013.

[2] IPCC, Climate change mitigation, Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change Cambridge, United Kingdom and New York, USA, 2007.

[3] D. J. Cottle, J. V. Nolan, S. G. Wiedemann, Ruminant enteric methane mitigation : a review, Anim. Prod. Sci., 51(6), 2011, pp. 491-514.

[4] A.R Moss, J.P Jouany, J. Newbold, Methane production by ruminants: its contribution to global warming, In Annales de Zootechnie, 49, 2000, pp. 231-253.

[5] B. Santoso, B. T. Hariadi. Effect of *Acacia mangium* Willd supplementation on *Pennisetum purpureum* to fermentation characteristic and *in vitro* methane production, Med. Anim. Sci., 30, 2007, pp. 106-113.

[6] C. Hasanah, A. Kurniawati, L. M. Yusiati, Muhlisin, Z. Bachruddin, Methane production and methanogens diversity in *in vitro* ruminal fermentation with *Swietenia mahagoni* leaves meal as tannin source, IOP Conference Series: Earth Environmental Science, 478, 2019, pp. 1-5.

[7] T. Wahyono, W. T. Sasongko, M. Sholihah, M. R. Pikoli, Effect the addition of jackfruit leaves tannin (*Artocarpus heterophyllus*) to biological value of *Moringa oleifera* and *Vigna radiata* in *in vitro*. Anim. Sci. Bul., 41, 2017, pp. 15-25.

[8] I. Mueller-Harvey, Characterisation of phenolic compounds including flavonoids and rannins of ten ethiopian browse species by high performance liquid chromatography. J. Sci. Food Agric., 86, 2017, pp. 2010-2037.

[9] S. Suharti, D. A. Astuti, E. Wina, T. Toharmat. Rumen microbial population in the *in vitro* fermentation of different ratios of forage and concentrate in the presence of *Sapindus rarak* fruit extract. J. Anim. Sci., 24, 2011, pp. 1086-1091.

[10] K. H. Menke, H. Steinngas, Estimation of energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid, Anim. Res. Develop., 28, 1988, pp. 7-55.

[11] FAO, Quantification of Tannins in Tree Foliage, Animal Production and Health Sub-Programme, Vienna, 2000.

[12] AOAC, Official method of analysis of the association of official analytical chemists, 18th ed, Maryland: AOAC International, William Harwitz (ed), United States of America, 2005.

[13] J. Filípek, R. Dvořák. Determination of the volatile fatty acid content in the rumen liquid : comparison of gas chromatography and capillary isotachophoresis, Act. Vet. Brn., 2009.

[14] A. Jayanegara. Reducing methane emissions from livestock: nutritional approaches, Proceedings of ISSM, ISTECS European, 2008, pp. 18-21.

[15] K. A. Beauchemin, S. M. McGinn, T. S. Martinez, T. A. Mc.Allister, Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle, J. Anim. Sci., 85, 2007, pp. 1990-1996.

[16] M. A. Anas, L. M. Yusiati, A. Kurniawati, C. Hanim, Evaluation of Albizia chinensis as tannin source for in vitro methane production inhibitor agents sheep rumen liquor, Int. Semin. Trop. Anim. Prod., 2015, pp. 261-265.

[17] M. Gonzales., L. Pabon, J. Carulla, Effect of tanin s on in vitro amonia release and dry matter degradation of soybean meal, Prod. Anim., 10, 2002, pp. 97-101.

[18] C. Martin, M. Doreau, D. P. Morgavi, Methane mitigation in ruminants: from rumen microbes to the animal, Livestock and Global Climate Change Conference, Hammamet, Tunisia, 2008.

[19] R. W. S. Ningrat, M. Zain, Erpomen, H. Suryani, Effects of doses and different sources of tanins to *in vitro* fermentation characteristics, Asian J. Anim. Sci., 11, 2017, pp. 47-53.

[20] A. Jayanegara, A. Sofyan, H. P. S. Makkar, K. Becker, The kinetic of gas production, digestion of organic matter and methane production by in vitro of tannin-suplemented hay and straw, J. Med. Anim. Sci., 32, 2009, pp. 185-19.

[21] Y. Widiawati, M. Winugroho, P. Mahyuddin. Estimasi produksi gas metana dari rumput dan tanaman leguminosa yang diukur secara in vitro. Prosiding seminar nasional teknologi peternakan

dan veteriner 3-4 Agustus 2010, Bogor, 2010, pp. 131-136.

[22] Puspitasari, Y. E. Effect of three leaves mixtures of tannin source to nutritional digestion of thin tailed sheep, Thesis, Faculty of Animal Science Gadjah Mada University, (not published).