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Effect of Nutmeg (*Myristica fragrans Houtt*) Leaves Tannin on *in Vitro* Fermentation Parameters and Methane Mitigation

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ABSTRACT
This research aimed to find out the effect of addition nutmeg (*Myristica fragrans Houtt*) leaves tannin on in vitro microbial protein, total protozoa, ammonia concentration (NH_3), and methane (CH_4) production. Materials used in this research were nutmeg (*Myristica fragrans Houtt*) leaves as a source of tannin and rumen fluid from fistulated Bali cow. Fermentation substrates consisted of Pennisetum purpureum and soybean meal with ratio 60:40 DM based. Treatments in this research were Myristica fragrans leaves addition equals to tannin contents of 0, 2 and 4%. In vitro fermentation was done in 48 hours. Variables measured in this research were microbial protein, total protozoa count, NH_3, and CH_4, then analyzed with One-Way Analysis design. The result showed that adding up to 4% of nutmeg leaves tannin in the feed ration did not affect microbial protein, total protozoa, NH_3, CH_4. Therefore, the addition of tannin from nutmeg leaves up to 4% did not give an adverse effect on rumen fermentation and methane production.

Keywords: *In vitro, Methane, Nutmeg leaves, Rumen fermentation, Tannin.*

1. INTRODUCTION

Ruminants are one of the largest sources of methane emissions that contribute to greenhouse gases (GHG) in the atmosphere. Ruminants contribute 77% of emissions from the livestock sector [1]. These gas emissions from ruminants were mainly from rumen fermentation. Rumen microbes play an essential role in the feed fermentation in ruminants by producing enzymes that can degrade feed. Degradation of feed ingredients in the rumen will produce fermentation products such as volatile fatty acid (VFA), ammonia, methane and CO_2. Methanogenic archaea uses molecular hydrogen (H_2) from carbohydrate fermentation to produce CH_4 [2]. Methane from ruminants represent about 6-10% gross energy loss to the animal [3]. Therefore, it is necessary to reduce ruminants enteric CH_4 without altering animal production. Various methods can do methane mitigation, one of them is using plant's secondary metabolites in feed, include tannins [4].

Tannins are phenolic compounds that exhibit potential to modify rumen fermentation including reducing enteric CH_4 emissions [5].Tannins from different plants have different biological activities depend on their chemical structures. Tannins classified into two major groups, hydrolysable (HT) and condensed tannins (CT) [6]. Tannins may form complex substances with other macromolecules like proteins, starch, cellulose, vitamins and minerals [7]. The ability of tannins to precipitate protein can cause positive and adverse effects. Tannins can protect high-quality protein feed ingredients where this protected protein can escape from rumen degradation and become a direct source of amino acids for livestock. However, tannins may also reduce rumen microbial activity due to its ability to bind with enzymes or microbial cell walls. Then, it may inhibit microbial growth and decrease rumen fermentation [8]. Considering that tannins may perform adverse effect when given at the inappropriate level,

thus further study needs to be conducted to minimize these effects.. Nutmeg is one of the tropical plants contained tannins quite high in its leaves. It has become a potential source to protect feed proteins. The use of tannin from various sources can have different effects on rumen fermentation. There is some research using tannins from a different source on *in vitro* feed fermentation to reduce rumen fermentation and methane production. Gambier leaf residue, *Mangifera indica*, *Cassia fistula*, *Acacia nilotica* leaves on in vitro feed fermentation has proven to decrease the ruminal fermentation and methane production [9,10].

2. MATERIAL AND METHODS

2.1. Samples Collection and Preparation

Myristica fragrans leaves were collected from Kebun Pala Ngobo Afdeling Gebugan (PT Perkebunan Nusantara IX), Bergas, Semarang, Central Java Province. Samples were dried at 55ºC for 72 h and ground to pass 1 mm sieve for chemical compositions and tannins assay. Rumen fluids were collected from fistulated Bali cattle. The following treatments were tested: 60% *Pennisetum purpureum* + 40% soybean meal without tannins as control, 60% *Pennisetum purpureum* + 40% soybean meal+ 2% tannins and 60% *Pennisetum purpureum* + 40% soybean meal+ 4% tannins.

2.2. In Vitro Incubation

The rumen fermentation characteristic parameters were tested by using gas production *in vitro* according to Menke and Steinngas [11]. Samples with amounts 300 mg DM were incubated together with buffered rumen fluid at 39ºC for 48 h in 3 replications. After 48 h incubation, the collected gas was analyzed for methane and the fermentation fluid was analyzed for ammonia concentrations, microbial protein, and total protozoa [12, 13, 14].

2.3. Statistical Analysis

The data were examined by One-way analysis of variance (ANOVA).

3. RESULT AND DISCUSSION

3.1. Rumen fermentation parameters

Rumen fermentation parameters were analyzed to find out the influence of protein protection by tannin from *M. fragrans* leaves contained 13.4% total tannins. Feed fermentation with the addition of *M. fragrans* leaves tannins up to 4% were not significantly different for microbial protein, total protozoa and NH_3 (Table 1).

Table 1. Rumen fermentation parameters from feed fermentation with addition of *Myristica fragrans* leaves tannins for 48 h

Parameters	Addition of *Myristica fragrans* leaves tannins		
	0%	2%	4%
Microbial protein (mg/ml)	0.58 ± 0.05	0.52 ± 0.04	0.51 ± 0.08
Total protozoa ($x10^3$)	100.00 ± 10.87	94.37 ± 7.04	106.25 ± 12.23
NH_3 (mg/100 ml)	41.15 ± 3.18	40.93 ± 7.54	39.91 ± 7.25

Microbial protein measured in this study showed a non-significant difference between control and increasing the level of *M. fragrans* leaves tannin. The supplementation of *Calliandra calothyrsus* as tannin source up to 6% on *in vitro* feed fermentation did not affect number of microbial proteins [15]. The results showed that the tannins addition did not interfere with microbial protein synthesis, and the need for N precursors and energy for microbes to develop remained fulfilled. The availability of N precursors and energy strongly influences microbial protein synthesis [16]. The result also showed microbes which were tolerant or resistant to tannins in the rumen fluid used. Groups of tolerant-tannins bacteria are from genus Streptococcus described as *S. gallolyticus*, and family Enterobacteria in the gamma subdivision of the Proteobacteria class [17].

The result of total protozoa did not show a significant difference between treatment and control. Tannin from *M. fragrans* did not cause defaunation in protozoa. Tannin from each of the different plant species has a different ability to rumen microbes. The addition of *Autocarpus integrifolis* up to 30% and utilization of *Calliandra calothyrsus* in the feed mixture did not contribute a significant difference in the total population of protozoa, fungi and proteolytic bacteria [18, 19].

The result showed that addition *M. fragrans* leaves tannins up to 4% did not affect NH_3 level. The solubility and protein content of the feed affects the concentration of NH_3 formed in the rumen fermentation. Protein degradability from feedstuff and the ability of the protein to survive from rumen fermentation can also affect NH_3 concentration [20]. The addition of *Albazia chinensis*, *Gliricidia sephium*, *Leucaena leucocephala* and *Manihot esculenta* leaves on *in vitro* feed fermentation also showed no significant different on NH_3 concentration [21, 22].

3.2. Methane production

The effect of the addition of *M. fragrans* leaves as source of tannins to the methane production did not show significant difference (Figure 1).

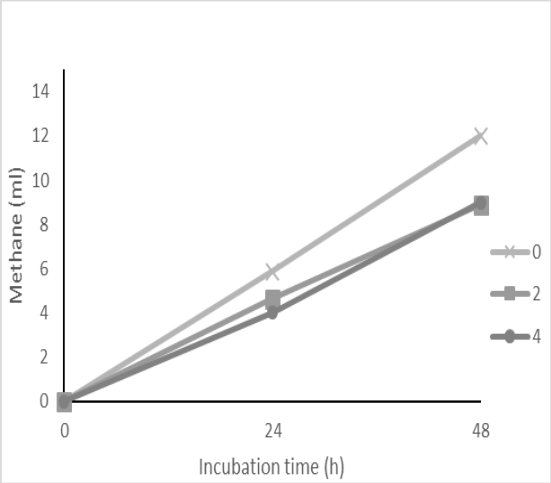

Figure 1. In vitro methane production (ml) of feed with addition of M. fragrans leaves tannins

Methane is one of the gases produced from feed fermentation by rumen microbes. The reduction of CO_2 by H_2 is the primary pathway for methane production and some of which derived from formic acid [23]. Methane productions in ruminants are influenced by many factors, including type and quality of feed, level intake and environmental temperature [24].

Addition of *M. fragrans* leaves tannin up to 4% slightly decreased (P>0.05) CH_4 level at 24 and 48 h incubation as compared to control. Utilization of *M. fragrans* fruit as herbal feed additives on in vitro gas production did not show a significant difference in methane production [25]. The result was related to the binding ability of tannins from *M. fragrans* leaves, which contained higher condensed tannins than hydrolysable tannins. Hydrolysable tannins are more effective in suppressing methanogenesis than condensed tannin [26].

4. CONSLUSION

Based on the results, the addition of *M. fragrans* leaves contained tannins up to 4% did not give a negative effect on *in vitro* microbial protein, total protozoa, ammonia concentration, and methane production.

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