Calliandra calothyrsus as Tannins Source for *In Vitro* Methane Production Inhibitor Agents

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ABSTRACT

An *in vitro* study was conducted to investigate the effect of *Calliandra calothyrsus* as a source of tannin on *in vitro* methane production and rumen parameters. Two sheeps were used as donor of rumen microbes. Four experimental diets (grass:concentrat, 60:40) supplemented with *Calliandra calothyrsus* leaves equal to tannin level of 0%, 2%, 4% and 6% based on dry matter (DM) were used as substrate for *in vitro* fermentation by Menke and Steingass gas production method for 48 hours of incubation. Methane production, DM and organic matter (OM) digestibility, and parameters of rumen fermentation were measured at the end of incubation. Data obtained were analyzed by one way analysis of variance (ANOVA) continued by DMRT. Degradation of DM and OM decreased (P<0,01) when supplemented by *Calliandra calothyrsus* leaves with increasing of tannin level. The significant decreasing occured at tannin level of 4%. The supplementation of tannin did not affect (P>0,05) number of microbial proteins, ammonia, VFA and pH, but increasing protozoa population. Moreover, the treatment also decreased methane production. These result suggest that supplementation of *Calliandra calothyrsus* can be useful to reduce methane production without any negative effects on rumen fermentation parameters.

Keywords: Methane, Tannin, *Calliandra calothyrsus*, Sheep, *In vitro*

INTRODUCTION

Global warming is considered as one of the most important issue in the world recently, ruminant fermentation is one of the most important source of Methane (CH4) emission which is responsible for approximately 25 % of CH4 global emission (EPA, 2010). According to Makkar et al. (2009), plant extracts and plant secondary metabolites (i.e., essential oils, tannins, saponins, flavonoids) as natural alternatives to improve livestock productivity and reduce their impact on the environment by reducing environment pollutants such as CH4 in fermentation gases, as well as P and N in manure. Tannis are water-soluble polyphenolic compunds with high molecular weight, which have a potentially wide range of effects on rumen fermentation, suc as reducing protein degradation in the rumen, decreasing methane production, preventing bloat and increasing conjugated linoleic acid in the ruminant derived foods (Patra Saxena, 2011).

Calliandra calothyrsus is legumes contain relatively high amounts of tannins (Jackson et al., 1996), which is important for the feed combination in small ruminants (Hess et al., 2007). However, the beneficial effects of tannis depends on the source and level of tannins applied. Negative effects of tannins have been associated with their potential toxicity to some rumen micro-organisms (Goel et al., 2005) and in the metabolism of the ruminant, particularly when HT are involved (Reed, 1995). Therefore, the exact concentration of tannins supplemented to animal is the key factor in the success of reducing methane production in ruminant. This research was conducted to evaluate the effect of different level of tannins from *Calliandra calothyrsus* on in vitro methane production and rumen parameters.

MATERIALS AND METHODS

Sample preparation. The effects of *Calliandra calothyrsus* in an *in vitro* batch fermentation were conducted according to Theodorou et al. (2000) with modification. The diet consists of *Pennisetum purpureum* (Schumach; was cut before flowering age) (60%) and concentrate (40%; consists of 20% of rice brand and 20% of wheat pollard), supplemented with *Calliandra calothyrsus* leaves equal to tannin level of 0%, 2%, 4% and 6% based on DM. Incubations were conducted using rumen fluid from ruminal canulated sheep, fed with *P. purpureum* and concentrate (60:40). Rumen fluid was collected before morning feeding and squeezed through polyester cloth into a vacuum flask thermos, and immediately sent to the laboratory. The incubation was conducted in a 125 ml bottle containing 70 ml of diluted rumen fluid and 700 mg of the diet per bottle.

The bottles were set into three triplicate bottles, for dry matter digestibility (DMD), organic matter digestibility (OMD) and gas production determination. Each tube was gassed with CO_2 gas before sealing with butyl rubber stopper plus aluminium crimp cap and pre-warmed overnight at 39 ˚C. In the next morning, 7 mL collected rumen fluid was added into each bottle using 10 mL plastic syringe. Bottles then incubated for 24 h at 39 ˚C.

After 24 h, the pH was measured immediately after opening the bottles and then residual feed were collected by filtration using filter paper for further residual nutrients analysis, including DM, OM according to AOAC (2010). Five ml gas were collected using syringe and transferred into 5 ml plain vacutainer for methane analysis using gas chromatography.

Statistic. The differences of pH, protozoa, microbial protein, methane (CH_4), ammonia (NH_3), dry matter and organic matter digestibilit, and Volatile Fatty Acids (VFA) were analisis using ANOVA followed by Duncan's multiple range test (SPSS, 2010).

RESULTS AND DISCUSSION

Results. Results of *In vitro* rumen fermentation with different level of tannin from *Calliandra calothyrsus* is presented in Table 1. There were no differences on pH, microbial protein, ammonia gas production and volatile fatty acids as the effect of different level of *Calliandra calothyrsus*. However, the pH and VFA were tended to increase as increased level of *Calliandra calothyrsus*. The using of *Calliandra calothyrsus* significantly increased the protozoa counts, but the different level of tannin gave no effects on protozoa counts. Different level of tannin from *Calliandra calothyrsus* significantly reduced dry matter and organic matter digestibility. Tannin from *Calliandra calothyrsus* did not affect the methane gas production, however tannin significantly reduced methane production based on dry matter and organic matter content, regardless level of tannin.

Table 1. *In vitro* rumen fermentation parameter with different level of tannin from *Calliandra calothyrsus*

Parameter	Tannin concentration (%)			
	0	2	4	6
pH	6.91	6.91	6.89	6.87
Protozoa ($x10^3$)	32.66^{a}	37.81^{ab}	38.12^{ab}	40.47^{b}
Microbial protein (mg/ml)	0.65	0.65	0.73	0.67
NH_3 (mg/100ml)	60.21	63.16	62.35	55.30
DM digesbility (%)	67.00^{c}	52.41^{b}	46.27^{a}	51.07^{b}
OM digesbility (%)	75.56^{c}	56.51^{b}	53.81^{a}	58.07^{b}
VFA (mol/100mol)	56.84	59.13	66.92	71.28
C2	41.64	43.40	48.01	51.50
C3	9.85	10.27	12.89	13.46
C4	5.35	5.45	6.03	6.31
CH_4 (ml)	5.11	5.16	5.14	4.83
CH_4/DM	40.33^{b}	39.90^{b}	34.53^{ab}	28.93^{a}
CH_4/OM	36.40^{b}	31.99^{a}	30.78^{a}	28.60^{a}

CH_4: methane, NH_3: ammonia, DM: dry matter, OM: organic matter, VFA: Volatil Fatty Acids, C2: Acetate, C3: Propionate, C4: Butirate.
[a,b,c] *different superscripts at the row showed significant differences*

CONCLUSIONS

Tannin from *Calliandra calothyrsus* did not affect pH, microbial protein, ammonia gas production and volatile fatty acids. The using of *Calliandra calothyrsus* significantly increased the protozoa counts regardless the level of tannin. Different level of tannin from *Calliandra calothyrsus* significantly reduced dry matter and organic matter digestibility. Tannin from *Calliandra calothyrsus* did not affect the methane gas production, however tannin significantly reduced methane production based on dry matter and organic matter content, regardless level of tannin.

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