

Evaluation of *Albazia chinensis* as Tannins Source for In Vitro Methane Production Inhibitor Agents Sheep Rumen Liquor

Anas, M. A., L. M. Yusiati, A. Kurniawati, C. Hanim

Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia

Corresponding email: muhsin_alanas@mail.ugm.ac.id

ABSTRACT: The objective of this study was to investigate the effect of *Albazia chinensis* as a source of tannin on in vitro methane production and rumen parameters. Two sheep were used as donor of rumen microbes. Four experimental diets (grass:concentrat, 60:40) with addition of *Albazia chinensis* 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. The addition of *Albazia chinensis* leaves decreased ($P < 0.01$) DM and OM digestibility with increasing of tannin level. Moreover, the treatment also decreased methane production. The significant decreasing occurred at tannin level of 6%. The addition of tannin did not affect ($P > 0.05$) number of protozoa, microbial proteins, ammonia, VFA and pH. It could be concluded that the addition of *Albazia chinensis* equal to tannin level of 6% is the optimal level to reduce methane production without any negative effects on rumen fermentation parameters.

Keywords: Methane, Tannin, *Albazia chinensis*, Sheep, In vitro

INTRODUCTION

Methane (CH₄) is gases which big potensi to cause of global warming (Reay *et al.*, 2010). Methane emissions from the agriculture sector represents 40% of total anthropogenic methane production (Key and Tallard, 2012), with the largest (25%) contibution comes from ruminants enteric fermentation (Thorpe, 2009). Small ruminants (sheep and goats) produce CH₄ emissions by 475 million tonnes of CO₂eq. Estimates of global CH₄ emissions by ruminants ranged between 65 to 85 million tonnes per year, while the total CH₄ emissions globally from 400 to 600 million tonnes per year (FAO, 2013). Methane emissions not only related to environmental issues, but also represents a loss of gross energy of feeds which can not be used for the production process. Approximately 2 to 12% of feed gross energy consumed by ruminant lost as CH₄ (Patra, 2012).

Several research using in vitro and in vivo method to mitigation of CH₄ production and improvement of ruminant performance have been done by supplementation of concentrate (Lovett *et al.*, 2005), fumarate (Ungerfeld *et al.*, 2007), sinamaldehyd (Macheboeuf *et al.*, 2008), antraquinone (Yusiati *et al.*, 2010), and essential oils (ME) (Calsamiglia *et al.*, 2007). Tannins or polyphenols is plant natural compound which could be use to reduce CH₄ emissions from rumen fermentation. Reduction methane supplementation of several legumes as tannin sources reduce CH₄ production from in vitro rumen fermentation are reported by Puchala *et al.*, (2005)

The mechanisms of tannins inhibition on CH₄ production are directly by inhibits the growth and activity of methanogens. (Tavendale *et al.*, 2005) and indirectly by form complex tannins protein binding which limiting methanogens acces to protein (McSweeney *et al.*, 2001). And also reducing fiber digestion that reduces the production of hydrogen (H₂) a precursor of CH₄ sythesis. Albazia chinensis is legume which has high polyphenol compound. Tannins content in Albazia chinensis is 7.21% DM based on Laboratory analysis. This study was conducted to determine the effect of Albazia chinensis as a source of tannins on CH₄ production of sheep in vitro.

MATERIALS AND METHODS

Albazia chinensis Preparation

Albazia chinensis was gain from Wonogiri, Central Java, Indonesia dried in an oven at temperature of 55°C for 3 days, then grounded using a Wiley mile and seave in size of 1 mm. Chemical composition of samples were analyzed using proximate analysis method (AOAC, 2005) and tannins content analyzed according Makkar (2003).

Inoculum preparation

Rumen fluid was obtained from two head of slaughtered sheeps before morning feeding. The sheeps had been adapted to diet consist of forages and concentrates in ratio 60:40 DM basic which offered in equal proportions twice a day.

In vitro gas production

In vitro gas production were used in this research according Menke and Steinngas, (1988). Substrate for fermentation consist of Pennisetum purpureum Schum (60%) and concentrates (40%) DM based. Albazia chinensis leaf were added to the fermentation diet equal to tannin content of 0, 2, 4, and 6% DM feed Fermentation was conducted for 48 h. At the end of incubation gas samples were collected for methane determination using gas chromatography. Residual feed were collected to calculate DM and OM digestibility, wherease pH, microbial protein synthesis, ammonia, and VFA were measured from the liquid media. Protein of microbe were determined using Lowry methode (Plummer, 1987), protozoa according to methode of Diaz *et al.*, (1993), ammonia followed Chaney and Marbach, (1962), and VFA acording to Filipek and Dvorak, (2009).

Statistic Analysis

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

RESULTS AND DISCUSSION

Addition of Albazia chinensis at tannin levels of 0, 2, 4, and 6% DM feed have no effect on pH, protozoa, microbial protein, and NH₃. pH media range from 6.92 to 6.97. Normal pH for microbial activity range between 5.5 to 7.6 and optimal at 6.7 to 7.0 (Owens and Zinn, 1988) This result is in accordance to Chaudhary *et al.*, (2011).that reported giving tannins from Ficus infectoria leaves in ruminant did not influence significantly on the pH value For protozoa number, Aghamohamadi *et al.*, (2014) reported that tannin from Quercus persica have no effect on protozoa population in rumen fermentation of sheep. Microbial protein were reported did not affected by tannin extract (Wischer *et al.*, 2012). Utilization of Quercus libani Oliv. in the diet equal to tannin content 16.57 to 22.43 mg/100 ml did not showed the influence on the NH₃ content (Abarghuei, 2011). The concentration of NH₃ in the rumen depends on feed protein content. The higher the protein content of the feed the higher the concentration of NH₃ (McDonald, 2002).

Table 1. In vitro rumen fermentation parameter and microbial activities with different level of tannin from *Albazia chinensis*

Parameter	Tannin concentration (%)			
	0	2	4	6
pH	6.95	6.92	6.97	6.92
Protozoa (x103)	31.09	35.63	34.38	38.28
Microbial protein (mg/ml)	0.60	0.67	0.63	0.70
NH ₃ (mg/100ml)	64.85	61.52	59.47	64.37
DM digesbilty (%)	67.73 ^c	53.83 ^a	57.27 ^b	55.87 ^{ab}
OM digesbilty (%)	75.67 ^b	61.37 ^a	65.27 ^a	63.50 ^a
VFA (mol/100mol)	68.89	70.30	66.70	68.06
C2	50.47	51.50	48.73	50.52
C3	11.72	11.89	11.85	10.41
C4	6.70	6.91	6.42	7.13
CH ₄ (ml)	4.37	4.40	4.85	4.37
CH ₄ /DM	34.09 ^b	38.88 ^b	34.09 ^b	29.96 ^a
CH ₄ /OM	34.47 ^b	37.35 ^b	34.70 ^b	28.87 ^a

CH₄: methane, NH₃: 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

Albazia chinensis as a source of tannin decreased ($P < 0.01$) digestibility of DM and OM. Digestibility of DM and OM with the addition of 2% tannin level significantly lower than the level 4 and 6%. Tannins from various sources able to reduce DM and OM on feed fermentation in the rumen (Attia *et al.*, 2013). Digestibility of DM and OM associated with protein digestibility and other organic materials such as carbohydrates and fats (Kurniawati *et al.*, 2013). Tannin in moderate level could be used to protect feed protein from microbial rumen degradability.

The results showed that the addition *Albazia chinensis* as a source of tannin did not affect significantly ($P > 0.05$) the production of total VFA and VFA components (acetate, propionate, and butyrate). Same result was reported by Dentinho *et al.*, (2014) that feeding containing tannins, both in high and low levels did not affect the VFA production or component. Different tannin source contain different nutrients, particularly carbohydrates content such as cellulose and hemicellulose which will affect the total production of VFA. The proportion of VFA in rumen fluid varies depending on the kind of feed consumed (McDonald *et al.*, 2002).

Albazia chinensis as tannin source at level 0, 2, 4 and 6% did not affect significantly on total volume CH₄ production. But CH₄ production based on DM and OM digestibility were reduced significantly by addition of *Albazia chinensis* at tannin level of 6%. CH₄ production decreased as much as 12.11%/DM digestibility and 16.24%/OM digestibility compared to control. Declining of CH₄ production in ruminants by tannins also reported by Aghamohamadi *et al.*, (2014). Tannins affinities to bind to protein and other nutrient are varies depend on tannins sources. Sasongko, (2010) stated that the tannins have optimal holding capacity to bovine serum albumin at a certain concentration. Utilization of jackfruit leaves as a source of tannins with varying concentrations have different affinity on binding to protein. At certain tannin concentrations the binding affinity will decreased due to saturation point.

CONCLUSIONS

In conclusion addition of *Albazia chinensis* leaves at tannin level of 6%, reduce CH₄ production with no negative effect on rumen fermentation. The decreasing of DM and OM digestibility due to reduction of protein digestibility.

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