

In Vitro Gas Production of Lemongrass Leaves as Essential Oil Source and its Effect on The Kinetics of Gas Production

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ABSTRACT

The research was aimed to evaluate the effect of lemongrass leaves (*Cymbopogon citratus*) supplementation as essential oil (LL) in the ration toward total gas production and kinetics of gas production. The ration used in the study contained 9.94% crude protein and 88.5% total digestible nutrient. Essential oil (EO) was added in the following levels: 0 (control), 25, 50, 75, and 100 mg/L and each treatment are replicated 3 times. Two cannulated Ongole crossbreed cows were used as inoculum donors. In vitro gas production was analyzed using Menke and Steingass (1988) method and the gas produced was recorded every hour for 48 h. The kinetics of gas production was analyzed using Fit Curve. The collected data were analyzed using one way variance analysis and the difference between mean was tested by Duncan's New Multiple Range Test (DMRT). Total gas production at 48 h decreased ($P < 0.01$) 25.14; 21.19; 16.06% with LL supplementation 50, 75 and 100 mg/L compared to control, whereas LL supplementation 25 mg/L did not affect total gas production. LL supplementation did not affect initial gas production (a). Lower potential gas production ($P < 0.01$) was observed with LL supplementation 50, 75 and 100 mg/L compared to control and greater LL supplementation showed lower potential gas production. Increasing LL supplementation did not affect the fractional rate of gas production (c). In general, based on this study, increasing EO supplementation from lemongrass leaves affected total gas production at 48 h and the kinetics of gas production.

Keywords: Essential oil, Lemongrass leaves, Beef cattle, *In vitro* gas production

INTRODUCTION

Methane is one of prominent gas which significantly contribute to global warming. Strategies to mitigate methane emission from livestock has been continuously conducted using an antibiotic (Tamminga, 1996; Sirohi *et al.*, 2012). However, the use of antibiotics in animal feeds to alleviate methane and improve nutrient utilization has been prohibited due to the risk of transmitting residue in animal products. Therefore, scientists are interested in using natural antimicrobial from plants as a safe modulator in animal feeds (Sirohi *et al.*, 2012). Essential oil (EO) have been known to enclose antimicrobial properties and the use of EO for ruminal fermentation modification have been used in prior studies to alleviate methane emission and improving nutrient efficacy (Patra and Saxena, 2010; Klevenhusen *et al.*, 2011).

Studies regarding different EO have been investigated to look for the effectiveness of EO to modify ruminal fermentation and metabolism to enhance nutrient efficiency and improve animal health and production (McIntosh *et al.*, 2003; Wallace *et al.*, 2003; Newbold *et al.*, 2004; Castillejos *et al.*, 2005; Kamalak *et al.*, 2011). Thymol, bioactive compound from oregano (*Origanum vulgare*), decreased methane emission, however, greater supplementation up to 300 mg/L also decreased nutrient utilization in *in vitro* technique (Wallace *et al.*, 2004; Benchaar and Greathead, 2011). Eugenol, bioactive compound present

in clove (*Syzigium aromaticum*) have been tested for the effects of ruminal fermentatin with the same as thymol level used, but showed no effect in decreasing methane emission or nutrient utilization (Calsamiglia *et al.*, 2007; Castillejos *et al.*, 2006). The effectiveness of EO as rumen fermentation modulators varies depending on the source, type and level of EO used in the studies due to high variations on bioactive compound of EO and each bioactive compounds had different limiting levels to manipulate rumen fermentation (Bakkali *et al.*, 2008; Jimenez-Peralta *et al.*, 2011; Kamalak *et al.*, 2011).

Lemongrass leaves (*Cymbopogon citratus*) (LL), is an herb and spice plant that has been mostly used by humans for cooking and contain 2.1 to 2.34% EO. The EO from LL consisted exclusively in monoterpenoids which three main constituents have been accounted for, in which 48.1% geranial, 34.6% neral, and 11.0% β -myrcene (Bassolé *et al.*, 2011) The findings on the constituents of LL were identified to hold antimicrobial and antifungal activity which are likely benefit to modifying ruminal fermentation therefore enhance the efficiency of nutrient utilization. Generally, only a few studies have investigated the use of EO from LL to comprehend the effect on ruminal fermentation. For this reason, there had been increasing interest in justifying the use of LL as feed additives in cattle feeds using in vitro gas production technique. The objetive of this in vitro study was to study the effect of lemongrass leaves as essential oil source on reducing total gas production and identify the effect of lemongrass leaves on gas production kinetics in cattle rumen fluid using in vitro gas production technique.

MATERIALS AND METHODS

Two ruminally cannulated Ongole crossbreed cows were used to obtain rumen inoculum which then used in in vitro technique. In vitro gas production techniques was used to refine the use of experimental animal. Animals involved in this study were nursed according to the guiding principle of Faculty of Animal Science, Universitas Gadjah Mada. Five treatments were used in the study: a control (without EO addition) and four different level EO from lemongrass leaves (LL) addition 25, 50, 75, and 100 mg/L. LL addition was mixed with diet to produce a homogeny between the EO and diet.

Diet and Treatments. Diet used in this study was classically a beef cattle diet, a mixture feed contain 40% rice bran and 60% king grass (9.94% CP and 88.5% TDN on a dry matter basis). Samples of feedstuff and LL were ground and milled pass a 1 mm sieve in a Wiley mill and used for chemical analysis and in vitro gas production study. Proximate analysis: dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF) and extract ether (EE) of each feedstuff and LL were analysed using AOAC method (2005). The EO concentration of LEM was determined using steam distillation method as described by Bassole *et al.* (2011). The distillation was conducted in Clevenger apparatus containing 200 and 500 mL distilled water and boiled for 6 h. The apparatus was also streamed down with the cold water until it reached 80°F. The steam and the EO evaporated and detained in the Clevenger apparatus. The steam was cold down to become water and separated from the EO. Collected EO from apparatus was then calculated using this calculation.

$$\text{Essential oil concentration \% (v/m)} = \frac{\text{EO volume (mL)}}{\text{sample weight (g)}} \times 100\%$$

In vitro gas production. In vitro gas production was measured using Menke and Steinngas method (1988). Rumen fluid was obtained from two fistulated Ongole crossbreed cows, fed a 60:40 forage:concentrate ratio diet. The collected rumen fluid were transferred into two pre-warmed thermos flasks at 39°C, combined, filtered through four layers of cheesecloth and flushed with CO₂ and mixed with buffered-mineral solution with ratio 1:2 (v/v). Buffered-mineral solution was prepared by mixing solutions as described by Menke

and Steinngas (1988). Equilibrated buffered-rumen solution as much as 30 ml was mixed with the 0.3 g feed sample in 100 mL syringe, then flushed with CO₂ and incubated at waterbath 39°C for 48 h. The gas production was recorded at interval 0, 2, 4, 6, 8, 12, 24, and 48 h. Cumulative gas production was evaluated using Fit Curve program to obtain the kinetics of gas production which were: intercept value at initial gas production (**a**), gas production from the insoluble fraction(**b**), potential extent of gas production (**a+b**) and fractional rate constant of gas production for the insoluble fraction (**c**).

Statistic. Each treatment was examined in triplicate. Results of the total gas production at 48 h and the kinetics of gas production were analysed as a randomized complete design using one-way ANOVA procedure of SPSS (2014). Duncan's Multiple Range Test (DMRT) was used as a post-hoc analysis as described by Astuti (2007). Comparison test and significance level were declared at P<0.05 or P<0.001.

RESULTS AND DISCUSSION

Cumulative gas production for each of treatments was presented as gas production curves (Figure 1) and values for kinetics of gas production parameters are given in Table 1. Cumulative gas production at 48 h was highly significant different among treatments (P<0.001) and was significant (P<0.05) when compared between control and other groups of treatments. It was found that cumulative gas was linearly decreasing when the level of LL supplementation was increased.

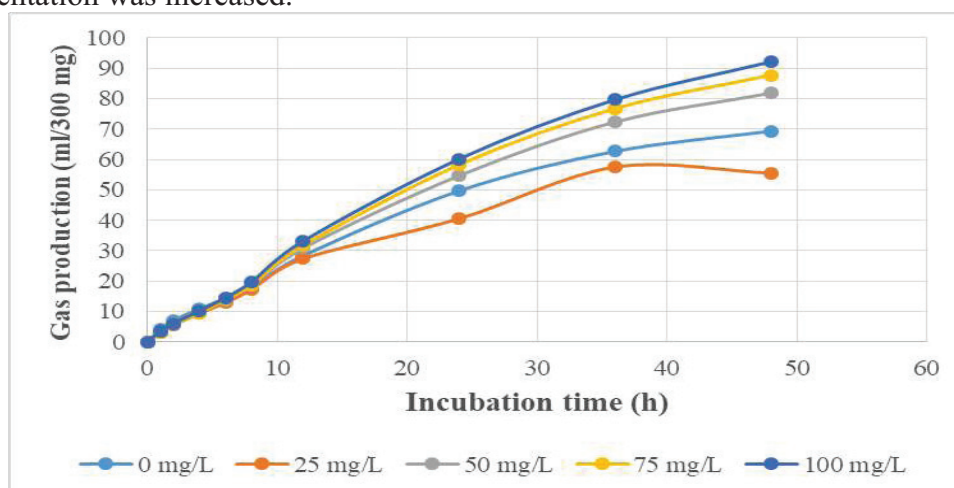


Figure 1. The effect of level of lemongrass leaves (LL) on cumulative gas production at different times of incubation

Table 1. Effect level supplementation of LL on total gas production at 48 h and the kinetics of gas production

Parameter	LL supplementation (mg/L medium)					SEM	P
	0	25	50	75	100		
Total gas production at 48 h	46.70 ^c	45.30 ^c	39.20 ^b	36.80 ^{ab}	34.59 ^a	1.29	0.000
Fermentation kinetics value ¹							
A	-1.54	-1.37	-2.74	-2.32	-2.33	0.29	0.574
b	58.73 ^{bc}	62.11 ^c	54.35 ^{ab}	50.06 ^a	49.89 ^a	1.52	0.008
a+b	57.19 ^{bc}	60.73 ^c	51.60 ^{ab}	47.73 ^a	47.53 ^a	1.63	0.005
C	0.037	0.033	0.030	0.033	0.030	0.01	0.382

LL, essential oil from lemongrass leaves

¹ a= intercept value at initial gas production, b= gas production from the insoluble fraction, a+b= potential extent of gas production, c= fractional rate constant of gas production for the insoluble fraction

(a,b,c) Means with different superscript in the same row differ (P<0.05)

Increasing level of LL decreased total gas production at 48 h ($P < 0.001$). Supplementation of LL 50 and 75 mg/L decreased total gas production at 48 h as much as 16.05% and 21.19% compared to control ($P < 0.05$). Greater LL inclusion up to 100 mg/L decreased 25.93% compared to control ($P < 0.05$). The result from this study is in agreement with the findings of Spoby and Samir (2010) and Kamalak *et al.* (2011) that reported addition of EO decreased the gas production. Gas production is related to volatile fatty acid (VFA) production following substrate fermentation, therefore greater fermentation lead to greater gas production (Blummel and Orskov, 1993). The decreasing gas production might be because of the VFA production also decreased could be explained along with the increasing EO supplementation (Benchaar *et al.*, 2007).

The intercept value (a) for the different treatments representing gas production from soluble fraction range from -2.74 to -1.37 and was not significantly different among treatments ($P > 0.05$). Whereas, gas production from the insoluble fraction (b) and potential extent of gas production were significantly different ($P < 0.01$). Meanwhile, gas production rate constant for the insoluble fraction (c) was not significantly different among treatments group. Increasing level of LL supplementation from 50 to 100 mg/L decreased potential gas production significantly ($P < 0.05$). This result from this study is in agreement with the finding of Kamalak *et al.* (2011) that reported EO supplementation reduced the potential gas production. In the experiment, the decrease in gas production from the insoluble fraction and potential extent of gas production with increasing level of EO supplementation from LL could be explained. This result consistent with that a decrease in total gas production and decreasing VFA production in rumen may yield adverse nutrient consequences leading into a decreased gas production and the values of the gas production kinetics itself.

CONCLUSIONS

In general, increasing level of essential oil supplementation from lemongrass leaves decreased total gas production at 48 h, gas production from insoluble fraction (b) and the potential extent of gas production significantly ($P < 0.01$).

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