Parameter of ruminal feed fermentation in vitro with addition of clove essential oil (Syzygium aromaticum L.) as feed additive

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Abstract. Clove essential oils (CEO) effect as rumen modifier on rumen fermentation was studied using in vitro gas production technique. Feed consisted of king grass, wheat pollard and rice bran (60:20:20) were incubated in buffered rumen liquid at 39ºC for 24 hours with Ongole grade cattle rumen liquor as microbe donor. The CEO was added and mixed with feed to meet its levels of 0, 25, 50, 75, and 100 µL/L of fermentation medium. Volume of gas production was measured at the end of fermentation. Broth was collected for pH, protozoa number, ammonia, and microbial protein synthesis measurement. Medium pH ranges from 7.12 to 7.20, were not affected by CEO. Gas production reduced at CEO doses 75 and 100 µL/L (P<0.01). Ammonia concentration at all treatment was higher than control, whereas protozoa number were decreased by CEO (P<0.01). Protozoa ranged from 18.08 to 32.81 x 10^4 cell/ml reduced gradually by CEO doses 25 to 100 µL/L. Meanwhile, protein microbe at CEO 25 and 50 µL/L (141.54 and 141.11 mg/100 mL) were higher than control (P<0.01). CEO doses 75 and 100 µL/L did not change microbial protein. In conclusion, CEO addition up to 50 mg/l increase rumen feed efficiency.

1. Introduction

Feed fermentation in the rumen is crucial process that make ruminants can utilize feed nutrient to meet their nutrient requirement. Microbes inhabited in the rumen digest and ferment feed particle to produce a mixture compound which is required for animal growth and production. Primary products of ruminal fermentation are volatile fatty acids (VFA) such as acetate, propionate, and butyrate, as well as rumen microbial biomass and also CH₄, CO₂, H₂, and ammonia gases as additional product [1]. VFA is the major energy source for animal host, almost 70% of host energy requirement is fulfilled from VFA, whereas 90% of amino acids reaching the small intestine come from microbial protein [2]. Several study revealed the high correlation between feed efficiency and rumen microbe composition [2]. Addition of essential oils (EO) changed microbiota in the rumen and alter rumen fermentation [3]. Different EO have different effect on ruminal feed fermentation. Rosemary EO did not affect gas production up to dose 2000 mg/L but Oregano EO reduced gas production at dose 1500 mg/L. Both EO reduced ammonia and dry matter digestibility at doses 500 mg/l to 2000 mg/L [4]. Clove essential oil (CEO) with eugenol as main component has a chance as feed additive due to antimicrobial property of this essential oil [5].
2. Material and methods

2.1. Material

Feed material in this research consisted of *Pennisetum purpureum*, wheat pollard and rice bran in a ratio of 60:20:20 based on dry matter. Preparation of feed substrates for fermentation was done by drying feed material in the oven dryer at 55°C until air dried then they were ground to pass a 1 mm of sieve for further analysis. Essential oil was obtained from local EO shop Lansida, Kotagede, Yogyakarta, Indonesia.

2.2. Methods

In vitro methodology was performed according to Menke et al. [6] to observe the effect of CEO based rumen modifier in ruminal fermentation.

Rumen liquor collected from two Ongole grade cattle in the morning before feeding and filtered using 4-layer cheese cloth then quickly sent to the laboratory in vacuum flask bottle for further fermentation in vitro. Diet for the cattle as ruminen microbial resources consisted of King Grass and commercial concentrate for beef cattle (60:40).

Feed substrate as much as 200 mg were put into calibrated 100 ml syringe glass (Fortuna® Haberle Labortechnik, Germany) and pre-warmed overnight before 30 ml of buffered rumen liquor was added. CEO was added before pre-warmed to meet the doses in medium of 0, 25, 50, 75 and 100 µl/l. Ethanol was used to diluted CEO before added. Ethanol was added to control in equal volume with treatments (0.8% v/v). Ethanol addition at 10mg/mL did not affect ruminal fermentation [7]. Buffered rumen composition was made according to Menke et al. [6]. Incubation was done at 39°C for 24h. Total gas production were recorded at the end of incubation. All syringes contents were collected for pH measurement and analysis of ammonia, microbial protein synthesis and cell number of protozoa. One millilitre sample was taken for ammonia analysis according to Chaney and Marbach [8]. Sample for analysis of microbial protein synthesis was prepared by centrifugation and protein then determined by Lowry method [9]. Sample for analysis of protozoa number were preserved in 0.8 ml of formaldehyde saline solution in ratio of 1:9. One µl of prepared sample was put in to hemocytometer for direct counting under microscope.

Data in this research including total gas production, pH, ammonia, protozoa cell number and microbial protein synthesis were subjected to one-way analysis of variance, and Duncan Multiple Range Test was used to analysis the differences between mean due to the treatments.

3. Results and discussion

The CEO addition at doses 75 and 100 µl/l reduced total volume of gas production (P<0.01) by 9.05% and 14.93%, respectively (Table 1). Previous study showed that CEO reduced gas production at higher doses i.e. 250 mg/L [10]. Gas volume which produced during in vitro ruminal fermentation can be used to predict feed digestion rate [11]. Feed digestibility is one of parameter to determine feed quality that support the animal performance and productivity. The greater gas volume production indicates more feed nutrients digested and the higher feed utilized by animal.

Rumen is a complex ecosystem [12], in which microbiota inhabited is the important part on the ruminant’s digestion system, metabolism and health [13]. To improve feed efficiency, control and manipulation of rumen fermentation can be done by altering rumen microbe activity [14]. Several feed additives – for instance antimicrobial agent that can be antibiotic or natural plant secondary metabolite – can be used to manipulate rumen fermentation [15]. The gas chromatography analysis, showed the main component of CEO in this research were eugenol (71.40%) and caryophyllene (22.48%). Eugenol is an EO component that contain phenol group which displaying a high antibacterial activity, high reactivity and has ability to form hydrogen bond with enzymes and other protein [16], and cause lysis of cell [17].
Addition of CEO did not affect the pH of fermentation which range from 7.12 to 7.20 (Table 1). This pH range is in physiological pH for optimum activity for rumen microbes. In the rumen environment, pH is the most variable factor. Maintenance a constant pH at physiological level stimulates cellulolytic bacteria that lead the improvement of feed digestibility [12].

**Table 1.** Parameters of in vitro feed ruminal fermentation with addition of rumen modifier based on Clove (Syzygium aromaticum. L.) essential oil

| Parameters | Doses of Clove essential oil (µl/l) |
|------------|-----------------------------------|
|            | 0       | 25       | 50       | 75       | 100      |
| Total gas production (ml/g feed DM)** | 119.89 ±2.95b | 119.33 ±4.18b | 121.887 ±2.56b | 109.03 ±6.28a | 101.99±3.55a |
| pH         | 7.12 ±0.03 | 7.16 ±0.02 | 7.16 ±0.04 | 7.19 ±0.05 | 7.20±0.03 |
| Microbial protein synthesis (mg/100 ml)* | 127.23 ±3.47a | 141.54 ±9.64b | 141.11 ±4.19b | 120.25 ±5.56a | 118.65±10.77a |
| Ammonia (mg/100 ml)** | 20.48 ±5.33a | 33.04 ±2.01b | 29.14 ±3.89b | 27.44 ±1.55b | 29.31±0.73b |
| Protozoa (x 10⁵ cell)** | 32.81 ±0.11c | 29.62 ±2.86c | 24.84 ±0.47b | 21.32 ±0.27ab | 18.08±0.09a |

*a,b,c* different superscript in the same row shows significant differences *: (P<0.05) ** (P<0.01)

As shown in Table 1, addition CEO at doses 25 and 50 µl/l enhanced microbial protein synthesis by 11.25 and 10.91%, respectively (P<0.05). However, the rate of protein microbial synthesis at doses CEO 75 and 100 µl/l did not differ from the control. This tendency is in accordance with Calsamiglia et al. [18] that EO has ability to improve feed efficiency in rumen and promote microbial growth.

All fermentations with CEO addition have a greater ammonia concentration (P<0.01, Table 1). Previous research reported that the addition of eugenol at doses of 25 to 75 mg/kg [19] and the higher doses 400 to 1000 mg/kg in diet did not affect to ammonia [20]. The increasing ammonia concentration in this research was probably caused by the lower of eugenol doses applied. Addition of CEO in this research was between 25 to 100 µL/L equal to 3.75 to 15 mg/kg feed, and eugenol content in CEO was 71.40%. Several data indicated that eugenol at low level (0.3 and 3 mg/L) tended to increased ammonia concentration, medium level (30 mg/l) had no impact, however high doses 300 to 5000 mg/L significantly decreased ammonia [21].

Addition of CEO at level 50 to 100 µL/L decreased the cell number of protozoa (P<0.01) by 24.29% to 44.89% (Table 1). Likewise, three other doses, addition CEO at 25 µL/L also reduced protozoa number even not significant. Reduction of protozoa is beneficial for increasing the ruminant production and growth [21].

**4. Conclusion**

Result of this research show that utilization of clove essential oil as feed additive at 50 µL/L is the best doses to increase feed efficiency since this does has no effect on gas production as representatives of nutrient digestibility. Addition of clove essential oil at 50 µL/L yielded a higher microbial protein, low number of protozoa and did not alter rumen pH.

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