Improvement of ruminal feed fermentation by addition of eucalyptus based mix essential oil

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Abstract. In vitro fermentation methodology was used in this research to explore the effect of mix essential oil (MEO) of eucalyptus (Melaleuca leucadendra (L.) and pine (Pinus merkusii (Jungh. and de Vriese), in ratio 3:1, on feed digestibility and parameter of ruminal feed fermentation. Mix essential oil was added and mix with feed particle to meet the end concentration of 100 µL/L of fermentation media. Incubation was done at 39ºC for 24 h. Upon finished the incubation, gas produced were sampled for methane analysis. Residual feed was collected by filtration and then analysed the dry matter (DM) and organic matter (OM) content for calculation of DM and OM digestibility. Meanwhile filtrate also collected for determination of pH, ammonia, protozoa number and microbial protein synthesis. Data analysis showed MEO addition did not affect pH, ammonia concentration, DM and OM digestibility. Addition of MEO reduced methane production 24.62% (P<0.05), and increased microbial protein synthesis (P<0.05) from 133.89 mg/L to 189.66. Otherwise, protozoa number in MEO treatment was lower compared to control (P<0.05). In conclusion, mix essential oil of eucalyptus and pine essential oil up to level 100µL/L could be used as feed additive to improve the fermentation of feed in the rumen.

1. Introduction

Enteric feed fermentation is the primary feed digestion process in ruminant. Rumen microbe including bacteria, fungi and protozoa digest and ferment feed nutrient to produce volatile fatty acid (VFA: acetate, propionate and butyrate), microbial cell protein, and addition product that consider as waste are ammonia, CO2, and H2 [1]. To maintain favorable system for normal growth of rumen microbe and feed fermentation, VFA, CO2, and H2 should be clearance from rumen cavities. VFAs are absorbed through rumen walls and metabolism by animal as main source of energy, whereas CO2 was discarded through eructation transported into the lung via circulation. Clearance of metabolic H2 is either through VFA production or, predominantly, conversion to CH4 [1]. Besides contribute to atmosphere pollutant as greenhouse gas, methane produced during rumen fermentation indicated inefficiency energy metabolism in rumen. The lost feed energy in the form of CH4 is around 2 to 12% of feed gross energy [2]. Increasing feed efficiency in ruminant can be done by minimizing loss of energy through manipulation of rumen fermentation, improving diet digestibility and decreasing methane production [3]. Monensin an antibiotic from ionophore group improves feed efficiency, by suppress methane production and successful in reducing energy and protein loss [4] and also increase energy supply for animal host [5]. However, the use of antibiotics for nonmedical treatment in animal production nowadays has been banned including in Indonesia.
Essential oils (EO) are plants secondary metabolite, which can be extracted by simple hydrodistillation. It has antibacterial, antifungal and antiprotozoal properties, like antibiotic [6]. Several research show EO have potency as antibiotic alternative to modify rumen fermentation, increase feed efficiency and reduce methane production [4,7–9]. Activity of EO was determined by the doses administered. Mostly methane reduction occur at a high doses, over than 300 µL/L and addition EO at high doses typically be associated with reduction of nutrient digestibility [10]. Beside doses of administration, EO activities also affected by its components. Interaction of two or more EO component may lead an additive, antagonistic or synergistic effect [11]. In synergistic effect, of EO component combination, doses needed will lower than sum of doses pure component to gain the same effect on rumen fermentation [11]. Based on previous study application of EO of eucalyptus (Melaleuca leucadendra (L.) and pine (Pinus merkusii (Jungh. and de Vriese) in in vitro rumen fermentation reduced methane at doses more than 100 µL/L (un published data). Data of GCMS analysis showed that main component of eucalyptus EO was 1,8-cineole (eucalyptol) (43.42%) whereas for pine EO did not dominated by one component only but there were fourth the hights main component i.e. α-pinene (24.49%), 2-Methyl-3-ethylheptane (19.44), 2,6-Dimethylundecane (19.51) and odecane (15.89). Hence in this research was aimed to study the combination of eucalyptus and pine essential oil on nutrient digestibility and rumen fermentation.

2. Material and Methods

2.1. Material

Pennisetum purpureum, rice bran and wheat pollard, in dry meals form, were used as fermentation substrate in this research. Ratio of thus three material was 60:20:20 based on DM. Nutrients composition of mix feed were dry matter (DM) 88.94, organic matter (OM) 84.53, crude protein (CP) 13.40 and crude fiber (CF) 21.44 and non-nitrogen extract (NNE) 46.5%.

Mix essential oil MEO consist of Eucalyptus (Melaleuca leucadendra (L.) and pine (Pinus merkusii (Jungh. and de Vriese) EO in ratio 3:1. Commercial EO were purchased from local EO shop Lansida in Yogyakarta, Indonesia.

2.2. Methods

In vitro gas production technique according to Menke [12] were applied in this research. Around 300 mg of mix of feed sample and 30 µL MEO were put into calibrated glass syringes of 100 ml (Fortuna®, Häberle Labortechnik, Germany), and then pre-warmed at 39°C for a night. At the next morning 30 ml of buffered rumen fluid anaerobically was added in to the syringe and then further incubated at 39°C for 24 h in triplicate and fermentation was repeated twice. A correspond fermentation were done in serum bottles for feed digestibility calculation. Rumen fluid as source of rumen microbe, was collected before morning fed from two ruminally fistulated Ongole grade. Cattles were fed a ration consisting of Pennisetum purpureum and beef cattle concentrate 60:40 DM bases.

After 24 h of incubation volume of produced gas were measured and 5 ml was sampled in plain vacuum tube (Becton Dickinson Vacutainer System) for methane analysis using gas chromatography. Syringe gas content were collected for further analysosn of protozoa according to [13], ammonia according [14] and rumen microbial protein according to [15]. Residual feed in serume bottles were filtered and measured the DM and OM content for calculation DM and OM digestibility (DMD and OMD). Obtained data were subjected independent sample T test analysis.

3. Results and Discussion

Addition of MEO at dose 100 µL/L did not affect pH of medium (Table 1). For normal rumen microbe activity pH in the rumen are maintain in physiological range between 5.5 to 6.9 [16].

Methane production reduced by addition of MEO 100 µL/L (P<0.05). Based on data in Table 1 methane production reduce 24.62% from the control. Several result showed that addition EO with active compound similar with MEO, effective reduce methane at high doses. Addition rosemary with α-pinene (23.02%), camphor (21.86%) reduced methane at doses around 1500 µL/L but DMD and
fibre degradability reduced at lower dose 1000 µL [17]. Reduction of methane mostly need high doses of EO, 1125 µL [18], 250 and 500 µL [19] and companying by reduction of nutrient digestibility. Combination of different component of EO affect their antimicrobial activity. A potency to reduce methane with little or no negative effect on feed digestibility in the rumen shows at moderate dose of EO combination [20]. Effect of EO in reducing methane production could be directly inhibit methanogens growth and activity or indirectly by reducing protozoa number [19]. In this research the reducing methane production in rumen fermentation may be as a consequence of the decreasing of protozoa number. Since 9 to 25% of methanogens in the rumen are associated with protozoa. Associated methanogen contribute around 37% of methane produced during feed fermentation in the rumen [21]. As shown in Table 1 MEO reduce protozoa number as much 75% from the control treatment (P<0.05). Actually, methanogenesis is a mechanism of hydrogen sink produced during nutrient metabolism by rumen microbe. Inhibition of methane production may lead increasing of hydrogen accumulation in rumen and further disturb the rumen metabolism. Microbial protein synthesis were significantly increased by addition of MEO (P<0.01) as shown in Table1. Microbial growth may as alternative of hydrogen sink. Inhibition of methanogenesis decreased potential redox in the system through increasing reducing equivalent. Some anabolism possess utilized reducing equivalent, for instant nitrogen fixation into carbon backbone to form amino acid and also synthesis of fatty acid to promote rumen microbe synthesis [22]. Ammonia is the nitrogen source for synthesis of rumen microbe. Ammonia concentration did not affected by the treatment. Microbial protein synthesis increase 27% in sheep when methane decreased [23]. Increasing of microbial protein is beneficial for animal host since microbe cell is the main protein source.

Digestibility of DM and OM did not affected by addition of MEO at level 100 µl/L. Addition of EO in rumen fermentation more than 100 µl/L reduced nutrient digestibility. Some reduced nutrient digestibility at higher doses [18]. Reduction nutrient digestibility is undesirable in animal production as indicator inefficiency of feed utilization.

### Table 1. Parameters of rumen fermentation with addition of mix of eucalyptus and pine essential oil (MEO)

| Level of mix essential oil (µl/L) | 0             | 100            |
|----------------------------------|---------------|----------------|
| pH                               | 6.85±0.07     | 6.91±0.05      |
| Methane (ppm)*                   | 106.21±5.33   | 80.06±4.78     |
| Protozoa (cell x10³)*            | 3.21±0.58     | 0.80±0.01      |
| DMD (%)*                         | 38.03±1.26    | 35.57±1.11     |
| OMD (%)*                         | 61.16±1.29    | 64.92±0.53     |
| Ammonia (mg/100 ml)*             | 29.94±2.69    | 30.29±2.46     |
| N microbe (mg/100 ml)**          | 133.89±6.39   | 189.66±2.25    |

* significantly different (P<0.05), ** (P<0.01)

**4. Conclusion**

Mix essential oil consist of mix essential oil of eucalyptus and pine essential oil up to level 100µl/L reduced methane production and increase microbial protein synthesis. It could be concluded that MEO potential to be used as feed additive for improvement the fermentation of feed in the rumen.

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