Effect of a mix essential oil of *Pinus merkusii* (Jungh. and de Vriese) and *Melaleuca leucadendra* (L.) on ruminal nutrient digestibility

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Abstract. This research was aimed to observe the effect of mix essential oil at different doses on nutrient digestibility in the in vitro rumen fermentation. Mix essential oil consisted of pine (*Pinus merkusii* (Jungh. and de Vriese)) and eucalyptus (*Melaleuca leucadendra* (L)) essential oil in ratio of 1:1. The doses of mix essential oils added were 0, 100 and 200 µL/L of fermentation media. Feed material included elephant grass, rice bran and pollard in ratio of 60:20:20 dry matter were used as fermentation substrate. Fermentation was conducted at 39ºC for 24h. Data collected included dry matter (DMD), organic matter (OMD), crude protein (CPD) and crude fibre digestibility (CFD). Results showed that the addition of mix essential oil at the level of 100 µL/L increased DMD (P<0.05), without an effect on OMD, CPD, and CFD. Furthermore, the addition of mix essential oil at the level of 200 µL/L reduced OMD and CPD (P<0.05) and did not affect DMD and CFD. In conclusion, combination of pine (*Pinus merkusii* (Jungh. and de Vriese)) and eucalyptus (*Melaleuca leucadendra* (L)) essential oil in ratio of 1:1 improve the effect of both essential oils particularly at doses 100 µL/L hence this mix essential oil potential to be used as feed additive.

1. Introduction

Essential oil is plant secondary metabolite that can be obtained by distillation, expression, or solvents. Essential oils usually contain bioactive constituents [1]. The chemical composition of essential oils depends on plant genetics, environmental conditions, conditions at harvest and the extraction process of active compounds [2]. Due to essential oil properties, essential oils can be used as a natural feed additive in livestock particularly to modified rumen fermentation [3]. Modification of rumen fermentation was done in intention to increase feed efficiency as generally acknowledgment that fermentations in rumin are played by rumin microbe.

Essential oils have been used by humans for a long time because they have properties as antibacterial, antiviral, anti-parasitic, anticancer and have the ability to suppress mycotoxin synthesis. Antimicrobial activity of essential oil is effective against various types of pathogenic bacteria such as *Listeria monocytogenes*, *L. innocua*, *Salmonella typhrium*, *S.enteritidis*, *Eschericia coli*, *Bacillus aureus* and others[2]. The mode of action of the essential oils antimicrobial activity are mostly disturb the activity which related to bacteria’s membrane cell including electron transport, protein translocation, phosphorylation, and other enzyme reactions [4]. The activity of essential oils is determined by the dosage and the active component of essential oil. In ruminants fermentation decreasing of methane
production was generated high EO doses of essential oil application, or about more than 300 mg/L [5]. Utilization of high dose of essential oil generally followed with reduction of nutrient digestibility. The use of high doses reduced digestibility [6].

Combination two or more essential oil enable interaction between its active compounds. The result of the interaction can be additive, antagonistic or synergistic [7]. In this research two essential oil, pine (Pinus merkusii) and eucalyptus (Melaleuca leucadendra) essential oil, were blend together in regard to get synergistic effect hence in high doses does not have negative effect on nutrient digestibility. Synergistic combination makes possible using lower doses of each essential oil compare to application in single essential oil to gain the same result [7].

2. Research method

2.1. Material

Feed material used as a fermentation substrate in this research consisting of Pennisetum purpureum, rice bran and pollard (60:20:20 DM bases). The rumen fluid for in vitro fermentation was collected from fistulated Bali cattle feed Pennisetum purpureum and commercial concentrate for beef cattle in the morning before feeding. Mix essential oil (MEO) were made by mix well of pine and eucalyptus essential oil in ratio 1:1. Base on laboratory analysis, main active compound of pine essential oil was α-pinene (24.49%) and 2-Methyl-3-ethylheptane (19.51%) whereas eucalyptus main component was 1,8-cineol (43.42%)

2.2. Method

Ruminal fermentation in this research was conducted using modified in vitro gas production technique according to Theodorou et al (1994) using serum bottle 150 mL. Modification were done in total volume, with the same ratio between media fermentation and head space volume. Seven hundred mg of feed sample were placed in the serum bottle than be added with MEO to gain the final concentration in in vitro medium of 0, 100 and 200 μL/L. To make easier on pipetting, MEOs were diluted in an amount of ethanol, and every bottles were design to get the equal amount of ethanol by added pure ethanol at 0 and 100 μL/L treatments. Buffered rumen fermentation as much as 63 mL were added a day before incubation with rumen microbes [8]. Bottles contain samples and fermentation medium were place at incubator 39°C overnight. In the next morning 7 mL of rumen fluid were added into each bottle and then incubated at 39°C for 24 h. At the end of incubation, residual feed material were filtered and for further analysis of dry matter, organic matter, crude protein and crude fibre for calculation of dry matter, organic matter, crude protein and crude fibre digestibility (DMD, OMD, CPD, and CFD) respectively.

The data analysis used was a unidirectional ANOVA, the average difference was tested by Duncan's Multiple Range Test (DMRT).

3. Results and discussion

The results (table 1) showed that the addition of blend essential oils affected the digestibility of dry matter, organic matter, and crude protein (P<0.05). The addition of MEO at the level of 100 μL/L increased in DMD by 8.42% compared control, while at dose of 200 μL/L did not changed DMD. Single essential oil addition of pine at level of 100 did not change DMD but at 200 μL/L reduce DMD whereas eucalyptus reduce DMD at both doses [6]. Combination of pine and eucalyptus essential oil (ratio 1:1) at dose up to 200 μL/L did not changed much on DMD. Dry matter digestibility is an indicator to measure forage quality using in vitro and in vivo methods [9]. Furthermore Faquay et al (1972) reported that DMD is influenced by the physical form of the feed and individual factors [10].

Organic matter digestibility were reduced by addition of MEO at the level of 200 μL/L (P<0.05) but at dose 100 μL/L OMD did not significantly affected (table 1). Previous research with single essential oil pine at 100 and 200 μL/L showed have no effect on OMD but eucalyptus essential oil reduced OMD at both level [6]. Combination of pine and eucalyptus at ratio 1:1 can compensate of negative effect eucalyptus essential oil particularly at level 100 μL/L. Another research showed extract contain α-pinene i.e., alcoholic and organic extract Rosemary both decrease OMD [11]. Digestibility of organic matter
can be used to measure the energy available to ruminants and to evaluate feed protein [12]. The level of organic matter digestibility was determined by microbial activity during the fermentation process. Complex organic matter would be degraded into less complex particle for further utilized by microbes for their growth. Organic matter digestibility is related to dry matter digestibility, so that if dry matter increases, organic matter will also increase and vice versa [13].

### Table 1. Nutrient digestibility (%) of in vitro rumen fermentation with addition of mix essential oil of pine (*Pinus merkusii* (Jung and de Vriese)) and eucalyptus (*Melaleuca leucadendra* (L.)) essential oil.

| Parameters                        | Doses of mix essential oil (µL/L) |
|-----------------------------------|-----------------------------------|
|                                   | 0       | 100     | 200     |
| Dry matter digestibility          | 42.15±0.00<sup>a</sup> | 45.69±1.50<sup>b</sup> | 41.77±1.75<sup>a</sup> |
| Organic matter digestibility      | 42.18±0.00<sup>b</sup> | 43.22±0.30<sup>b</sup> | 39.88±0.97<sup>a</sup> |
| Crude protein digestibility       | 49.70±0.00<sup>b</sup> | 50.96±3.68<sup>b</sup> | 40.68±3.22<sup>a</sup> |
| Crude fiber digestibility         | 51.36±0.00 | 52.06±4.44 | 51.65±2.74 |

<sup>a</sup>Different superscript in the same raw showed significant differences (P<0.05).

As shown on table 1, the addition of MEO at the dose of 100 µL/L did not effect on CPD but at dose 200 µL/L reduced CFD by 18.14% from control (P<0.05). Addition of single pine and eucalyptus essential oil at up to dose 800 µL/L did not affect CPD [6]. From those data can be seen that mixing of both essential oils alter the effect on CPD. Protein degradation in the rumen depends on three catabolic processes, namely proteolysis, peptidolysis and deamination. The most important factors that affect microbes in degrading protein in a food is the type of protein, interactions with other nutrients, especially the energy complex on the ration, travel rates and rumen phases [14].

Crude fibre digestibility did not affected by addition on both doses of MEO (table 1). Those effect was different with previous study with addition of single essential oil of pine and eucalyptus which reduce CFD at dose 100 µL/L and above [6]. Combination of both essential oil improve the effect of both essential oil on CFD.

Another study also reported that addition essential oil in the form combination of two or more essential oil add the benefit on rumen fermentation. Addition of blend essential oil (BEO, Crina® for ruminants) have not detrimental effect on nutrients digestibility and increase VFA production and reduce ratio of acetate and propionic acid [15].

### 4. Conclusion

Combination of pine (*Pinus merkusii* (Jung. and de Vriese)) and eucalyptus (*Melaleuca leucadendra* (L.)) essential oil in ratio of 1:1 improve the effect of both essential oil particularly at doses 100 µL/L. It can be concluded that this mix essential oil is potential to be used as feed additive.

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