Advances in Biological Sciences Research, volume 21
Proceedings of the 6th International Seminar of Animal Nutrition and Feed Science (ISANFS 2021)

Pine Based Blend Essential Oil Did Not Reduce Methane Production But Increase Organic Matter Digestibility On Ruminal Feed Fermentation *In Vitro*

Asih Kurniawati[1,*], Muhlisin Muhlisin[1], Chusnul Hanim[1], and Muhsin Al Anas[1]

[1] *Department of Animal Nutrition and Feed Science Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281*
[*] *Corresponding author. Email:asihkurniawati@ugm.ac.id*

ABSTRACT
This research was done to investigate the effect of blend essential oil EOs1 with pine (*Pinus merkusii*; Jungh. and de Vriese) essential oil as the main component on feed nutrient digestibility and methane production. Blend essential oil consist of pine and eucalyptus (*Melaleuca leucadendra* (L.) essential oil in ratio 3:1 (EOs1). The EOs1 was added into the feed commonly used by the farmer, which consisted of king grass, rice bran, and pollard (60:20:20). The addition dosages of EOs1 was equal to 0, 100, and 200 µL/L of *in vitro* fermentation medium. *In vitro* gas production method using serum bottles was used to incubate feed material added EOs1 at 39°C for 24 h. At the end of incubation, gas produced was collected for methane analysis, and residual feeds were collected for further nutrients analysis, including dry matter, organic matter, crude protein, and crude fiber. Nutrient data then used to calculate the digestibility of dry matter, organic matter, crude protein, and crude fiber (DMD, OMD, CPD, and CFD), respectively. Data shows that all the treatments did not affect the methane production, DMD, CPD, and CFD, but increase OMD ($p<0.05$). It could be concluded that blend essential oil from pine and eucalyptus at ratio 3:1 potentially as a feed additive to improve nutrient metabolism but not for methane mitigation.

Keywords: *Pine, Eucalyptus, Essential Oil, Rumen Fermentation, Nutrient, Digestibility, Methane.*

1. INTRODUCTION

Essential oils are volatile and aromatic compounds that are composed of mixes fragrant and odorless substances. It is synthesized in the cytoplasm and located between cells in tiny droplets form. Essential oils in the naturally total mixture are more effective than active isolated ingredients or synthetic substitution. Every essential oil has an individual combination of its components that determined the interaction with target cell chemistry to take effect. Moreover, the effect of essential oil is usually supported and back up by combination with others [1]. The essentials oil showed antimicrobials activity [2–7] besides other activities for instant antioxidant and growth promoter for animals [8]. Due to antimicrobial activity, the essential oil was recently applied as an additive for ruminants to reduce methane and increase feed efficiency since rumen microbes carry out the feed fermentations. However, some studies showed essential oil application in the rumen, decreasing feed degradability accompanying the methane reduction, particularly in administering the high dose. In contrast, a lower dosage did not reduce nutrients degradability, likewise methane production. However, those effects varied depending on doses, and the types of essential oil used [9,10].

Since the application of essential oil in combination with others leads to interaction among active compounds, turning out synergistic, additive, or antagonistic effects [8,11], using essential oils in the mixture is expected to reduce individual doses of essential oils on methane mitigation without interfering with rumen fermentation. A lower dosage needed to gain the same effect in synergistic interaction compared to the sum of the individual essential oil effect. A combination of essential oils reduced the effective concentration of antimicrobial activity toward tested microbe. The synergistic and additive effects were shown when 1,8-cineole was combined with monoterpene hydrocarbons and sesquiterpene. The synergistic and additive effects were also demonstrated at the application of blending among monoterpene hydrocarbons components (α-pinene with linalool or limonene) [12].

Based on the laboratory analysis, the main component of pine essential oil is α pinene, a monoterpene hydrocarbon, and 1,8 cineole is the main component of eucalyptus essential oil.

Mitigating methane from rumen fermentation without adverse effects on feed digestion or fermentation is an objective for future research. Thus, in this research combine pine (*Pinus merkusii*; Jungh. and de Vriese) and eucalyptus (*Melaleuca leucadendra* (L.)) essential oil as a feed additive to mitigate methane production.

2. MATERIALS AND METHOD

2.1. Materials

In vitro ruminal fermentation technique using serum bottle was used in this research [13]. As the source of rumen microbe, rumen fluid was collected from ruminally cannulated Bali crossbreed cow in the morning before feeding. Ten days before rumen fluid collection, cattle were fed *Pennisetum purpureum* 60% of total feed dry mater and concentrate commercial for beef cattle 40%. *Pennisetum purpureum*, rice bran, and pollard ratio 60:20:20 DM bases were used as feed substrates for *in vitro* fermentation. EOs1 was made by blending pine and eucalyptus essential oil in a ratio of 3:1. Pure pine and eucalyptus essential oil were bought from Lansida Company (Yogyakarta, Indonesia).

2.2. In Vitro Gas Production

Slightly modification of *in vitro* gas production from the original method of Theodorou [13] was done in this study. A smaller volume of serum bottles was used. Still, the fermentation medium and headspace ratio was approximately similar, while the proportion of feed sample, medium, and rumen microbe inoculant followed the method of Menke et al. [14]. 700 mg of feed substrate were incubated in a 70 mL buffered rumen fluid medium. Blend essential oil EOs1 was added into the weighed feed sample before medium addition to get the doses of 0, 100, and 200 µL/L of fermentation medium. The EOs1 was diluted in absolute alcohol for easy distribution into the bottle, and each bottle gets the same volume of alcohol, including control. Each treatment was examined in triplicate. Incubation was conducted at 39°C for 24 h. Before incubation, the gas pressure in the headspace was zeroing using a pressure transducer. At the end of incubation, the volume of gas produced during fermentation was measured, and the sample was collected for methane analysis using gas chromatography. At the same time, the residual feed was collected by filtration and further analyzed of nutrients content including dry matter, organic matter, crude protein, and crude fiber for calculation of digestibility of dry matter, organic matter, crude protein, and crude fibre.

2.3. Data Analysis

Collected data were analyzed as a complete randomized design using one way ANOVA procedure. Duncan's Multiple Range Test (DMRT) was used as a post hoc analysis. Statistical analysis was carried out using SPSS ver. 18 (IBM SPSS software, Chicago, USA).

3. RESULTS AND DISCUSSIONS

Data in Figure 1 showed that methane production during rumen fermentation *in vitro* was not reduced significantly by adding blend essential oil EOs1 containing pine eucalyptus essential oil with a ratio of 3:1. But, it can be seen from the graph there is a tendency decreasing of methane production with the increase of EOs1 doses. The previous study (data unpublished) indicating methane production was significantly reduced during *in vitro* rumen fermentation when both essential oils were added separately at doses 100 and 200 µL/L. While combining the two essential oils was given oppositely, pine: eucalyptus (1:3), methane reduced at dose 100 µL/L [15]. The properties and biological activity of essential oil are determined by two or three components present in high concentrations, even though the trace component can't be neglected. Besides the main component of essential oil, interaction among components, chemical structure, and doses of essential oil also determine the antimicrobial activity and others activities [16]. Combining two or more essential oil changes the composition and proposition of essential oil components, which will affect the activity.

Methane production was changed when the essential oil was applied to rumen fermentation. Those changes might be caused by the alteration of methanogens abundance or else by the changes of the methanogenesis pathway. The addition of essential oil into the rumen fermentation system affects the abundance of the rumen microbes, including methanogens, the archaea methane-producing. Adding eucalyptus and rosemary essential oil individually did not change the abundance of archaea, but both did reduce archaea abundance when combined with other essential oil. The magnitude of the reduction in archaea is not reflected accordingly in methane production. The reduction of methane in the system with individual essential oil was more remarkable than combining essential oils [17].

The essential oil in the rumen system not only affects methanogens but also affects other microbes that are responsible for feed fermentation and degradation. The addition of eucalyptus and rosemary essential oil with major component 1,8 cineol, did not affect total rumen bacteria abundance but reduced *Prevotella* spp.

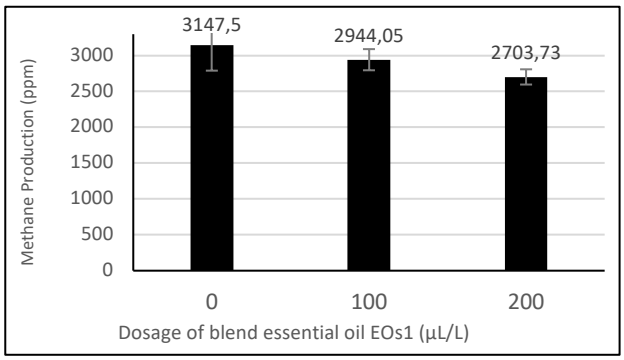

Figure 1. Methane production in rumen fermentation *in vitro* with the addition of Blend essential EOs1

Hence in combination with other essential oil also did not affect total bacteria but changed the abundance of rumen bacteria, including bacteria members of *Prevotella* spp. in the different pattern when combined with different essential oil [17]. *Prevotella* spp. members are bacteria that produce extracellular hydrolytic enzymes, including amylolytic, hemicellulolytic, and proteolytic [18], crucial enzymes for nutrient degradation.

The addition of blend essential oil EOs1 at 100 and 200 µL/L did not affect crude protein digestibility as well as crude fiber and dry matter digestibility, but organic matter digestibility increased significantly in line with the increased doses of blend essential oil EOs1 (Table 1). A previous study showed that addition of pine essential oil individually did not affect crude protein and organic matter digestibility up to doses 800 µL/L, but reduced dry matter digestibility at 200 µL/L and up also reduced crude fiber digestibility start from doses 100 to 800µl/L on administered. Meanwhile, eucalyptus addition individualy did not affect crude protein digestibility up to dose 800 µL/L. Moreover, dry matter and crude fiber digestibility decreased by eucalyptus essential oil at dose 100 till 800 µL/L, and organic matter digestibility was reduced by eucalyptus essential oil at dose 200 µL/L and above [19]. The addition of pine and eucalyptus essential oil in combination 3:1 in this research reduced the negative effect toward nutrient digestibility compared application in separately. The different effect of individual and blend essential oils on nutrient digestibility might be due to the different doses of each active compound and the varieties of the active compound when added in separate or combined applications. Several studies also reported the different effects of essential oil on nutrients digestibility due to the differences of the active compound and its doses. Blend essential oil based on eugenol and cinnamaldehyde showed no effect on most nutrient digestibility except neutral detergent fiber (NDF) digestibility was increased. Addition blend essential oil in higher level reduced all nutrient digestibility [20]. Addition of 125, 250 and 375 thyme essential oil with major active compounds thymol and p-cymene, did not affect DM OM CP and NDF digestibility [21]. Eucalyptus at 50, 100, 200 400 linearly

Table 1. Nutrient digestibility in rumen fermentation in vitro with the addition of Blend essential BEO1.

Parameters	Level Of Blend Essential Oil (BEO1) (µL/L)		
	0	100	200
CPD (%)ns	44.00±2.93	40.08±2.63	47.77±3.56
CFD (%)ns	54.14±2.43	53.11±1.65	52.04±3.87
OMD (%)*	61.16±2.22^{a}	63.15±0.33^{ab}	66.62±2.89^{b}
DMD (%)ns	42.72±1.01	44.72±4.23	44.72±2.06

Description: DMD: dry matter digestibility, OMD: organic matter digestibility; CPD: crude protein digestibility, CFD: crude fiber digestibility

reduced DMD and methane production[22]. Eucalyptus essential oil with major component 1,8 cineole and rosemary essential oil with α-pinene as major component showed different effect toward dry matter and neutral detergent fiber when combined with different others essential oil [17].

Various *Ferula* sp. essential oils have α-pinene as a major component, showed selective antimicrobial effects. Supplementation at dosage 50mg/L did not affect methane production but tended to increase *in vitro* dry matter and organic matter disappearance [23]. Each essential oils have their minimal inhibitory concentration related to their antimicrobial activity, and every microbe has their sensitiveness toward various essential oils. There were many numbers and varieties of microbes in the rumen. Hence, looking for effective essentials oil to modulate the efficiency of rumen nutrients utilization is still needed. Combination of pine and eucalyptus essential oil in ratio 3:1, even though it did not significantly reduce methane, but it did not negatively affect nutrient digestibility, instead of increasing organic matter digestibility.

4. CONCLUSIONS

Blend essential oil from pine and eucalyptus in ratio 3:1 (EOs1) potentially as a feed additive to improve nutrient metabolism but not for methane mitigation.

REFERENCES

[1] J.L. Ríos, 2016 Essential oils: What they are and how the terms are used and defined Essential Oils in Food Preservation, Flavor and Safety (Elsevier Inc.), 2016, pp 3–10.

[2] M. Ghavam, M.L. Manca, M. Manconi G. Bacchetta, Chemical composition and antimicrobial activity of essential oils obtained from leaves and

flowers of *Salvia hydrangea* DC. ex Benth,Nature Scientific Report, 10: 15647, 2020, pp. 1–10.

[3] G. Dogan, N. Kara,E. Bagci, S. Gur, Chemical composition and biological activities of leaf and fruit essential oils from *Eucalyptus camaldulensis*, Zeitschrift fur Naturforsch. - Section C Journal of Biosciences, vol. 72, 2017, pp. 483–489

[4] Q. Chen, X. Zhao, T. Lu, Y. Yang, Y Hong, M., Tian, Y. Zhou, Chemical composition, antibacterial, and anti-inflammatory activities of essential oils from flower, leaf, and stem of *Rhynchanthus beesianus,* Biomed Research International*,* 2021, pp. 1–11.

[5] A. Guetat, A. Faraj, Al-Ghamdi, A. K. Osman, 1 , 8-cineole , α -pinene and verbenone chemotype of essential oil of species *Rosmarinus officinalis* L . from Saudi Arabia, International Journal of Herbal Medicine,. Vol. 2 (2), 2014, pp. 137–41.

[6] K. Sebei, F. Sakouhi, W. Herchi, M.L. Khouja, S. Boukhchina, Chemical composition and antibacterial activities of seven *Eucalyptus* species essential oils leaves Biological. Research, vol. 48:7, 2015, pp. 1–5.

[7] T. Ujilestari, R. Martien, B. Ariyadi, N.D. Dono, Zuprizal, Antibacterial effects of essential oils of *Cymbopogon citratus* and *Amomum compactum* under self-nanoemulsifying drug delivery system (SNEDDS), IOP Confence Series: Earth and Environmental Science, 387, 2019, pp. 012071.

[8] M. Hyldgaard, T. Mygind, R.L. Meyer, Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components, Frontrier in Microbiology.vol. 3, 2012, pp. 1–24.

[9] G. Cobellis, M. Trabalza-Marinucci, Z. Yu, Critical evaluation of essential oils as rumen modifiers in ruminant nutrition: A review, Science of the Total Environment, vol. 545–546, 2016, pp. 556–68.

[10] M. Günal,B, Pinski,A.A. AbuGhazaleh, Evaluating the effects of essential oils on methane production and fermentation under in vitro conditions, Italian Journal Of Animal Science vol. 16, 2017, pp. 16 500–6.

[11] R.L. Van Zyl, S.T. Seatlholo, A.M. Viljoen, Pharmacological interactions of essential oil constituents on the in vitro growth of *Plasmodium falciparum* South African Journal of Botany, vol. 76, 2010, pp. 662–7.

[12] I.H.N. Bassolé, H.R. Juliani, Essential oils in combination and their antimicrobial properties, Molecules vol. 17, 2012, pp. 3989–4006.

[13] M.K. Theodorou, B.A. Williams, M.S. Dhanoa, A.B. McAllan, J. France, A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds, Animimal Feed Science and Technology, vol. 48, 1994, pp. 185–97.

[14] K.H. Menke, L. Raab, ,A. Salewski, H. Steingass, D. Fritz, W. Schneider, The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor in vitro The Journal of Agricultural Science, vo. 93, 1979, pp. 217–22.

[15] A. Kurniawati, D.N. Wigati, C. Hasanah, L.M. Yusiati, Improvement of ruminal feed fermentation by addition of eucalyptus based mix essential oil, IOP Conference Series: Earth and Environmental Science vol.. 425, 2020 pp. 012086

[16] G. Cobellis, M. Trabalza-marinucci, Z. Yu, Critical evaluation of essential oils as rumen modifiers in ruminant nutrition : A review Science of the Total Environmen, vol. 546, 2016, pp. 556–68.

[17] G. Cobellis, T. Massimo, M. Marcotullioc, Z. Yu, Evaluation of different essential oils in modulating methane and ammonia production, rumen fermentation, and rumen bacteria in vitro, Animal Feed Science and Technology, vol. 215, 2016, pp. 25–36.

[18] R. Danielsson, J. Dicksved, L. Sun, H. Gonda, B. Müller, A. Schnürer, J. Bertilsson, Methane production in dairy cows correlates with rumen methanogenic and bacterial community structure, Frontiers in Microbiology, vol. 8, 2017, pp. 1–15.

[19] A. Kurniawati, Widodo, W.T. Artama, L.M.Yusiati, Effects of four essential oils on nutrients digestibility of in vitro ruminal fermentation, Buletin Peternakan, vol. 42 2018, pp. 122–6.

[20] J.A. Tekippe, R. Tacoma, A.N. Hristov, C. Lee, J. Oh, K.S. Heyler, T.W. Cassidy, G.A. Varga, D. Bravo, Effect of essential oils on ruminal fermentation and lactation performance of dairy cows, Journal of Dairy Science, vol. 96, 2013, pp. 7892–903.[21] A.D.B. Ribeiro, M.V.C.F. Junior, D.M. Polizel, A.A. Miszura L.G.M. Gobato, J.P.R. BarrosoI. Susin, A.V. Pires, Thyme essential oil for sheep: effect on rumen fermentation, nutrient digestibility, nitrogen metabolism, and growth, Arquivo Brasileiro Medocine Veterinaria. e Zootecnia, vol. 71, 2019, pp. 2065–74. [22] B. Wang, M. Jia, L. Fang, L. Jiang, Y. Li, Effects of eucalyptus oil and anise oil supplementation on rumen fermentation characteristics, methane

emission, and digestibility in sheep Journal of Animal Science, vol. 96, 2018, pp. 3460–70.

[23] F. Klevenhusen, K. Deckardt, O. Sizmaz, S. Wimmer, A. Muro-Reyes, R. Khiaosa-Ard, R. Chizzola, Q. Zebeli, Effects of black seed oil and *Ferula elaeochytris* supplementation on ruminal fermentation as tested in vitro with the rumen simulation technique (Rusitec). Animal Productio Science, vol. 55 2015, pp. 736–44.

[24] M. Joch, L. Cermak, J. Hakl, B. Hucko, D. Duskova, M. Marounek, In vitro screening of essential oil active compounds for manipulation of rumen fermentation and methane mitigation Asian-Australasian Journal of Animal Science, vol. 29, 2016, pp. 952–9.

[25] F. Mcintosh, P. Williams, R. Losa, R.J. Wallace, C.J. Newbold, D.A. Beever, Effects of essential oils on ruminal microorganisms and their protein metabolism Applied and Environmental Microbiology vol. 69, 2003, pp 5011–5014.

[26] C.J. Newbold, F.M. Mcintosh, P. Williams, R. Losa, R.J. Wallace, Effects of a specific blend of essential oil compounds on rumen fermentation Animal Feed Science and Technologi vol. 114. 2004, pp. 105–12.