INTISARI

KAJIAN MOLEKULER METANOGEN RUMEN DAN EVALUASI PRODUKSI METAN PADA RUMINANSIA PASCA PENAMBAHAN SUMBER *ESSENTIAL OIL* ASAL TANAMAN

Penelitian ini bertujuan untuk memperoleh kandidat aditif pakan penurun metan berbasis *essential oil* (EO). Seleksi kandidat aditif pakan dari *raw material* sumber EO dan destilat EO komersial di lakukan dengan melihat pengaruhnya terhadap produksi metan, parameter fermentasi, kecernaan nutrien pakan dan aktifitas enzim hidrolitik pada fermentasi rumen serta keragaman dan kelimpahan metanogen secara metagenomik.

Empat *raw material* sumber EO yaitu kapulogo (*Amomum compactum)*, kencur(*Kaempferia galanga* L.), jahe merah (*Zingiber officinale Var.Rubrum*) dan jinten (*Cuminum cyminum*) serta empat destilat EO komersial yaitu adas (*Foeniculum vulgare* (Mill)), pinus (*Pinus merkusii* (Jungh. and de Vriese)), sereh wangi (*Cymbopogon nardus* (L.) Rendle) dan kayu putih ambon (*Melaleuca leucadendra* (L.)) digunakan dalam seleksi aditif pakan. Kandungan nutrisi *raw material* sumber EO dianalisis dengan metode proksimat sedangkan kandungan EO dianalisis dengan menggunakan metode *Stahl distilation*. Komponen aktif EO baik dari *raw material* sumber EO maupun destilat EO dianalisis dengan menggunakan *gas chromatography–mass spectrometry* (GCMS*)*. Metode *in vitro* produksi gas digunakan dalam penelitian ini sebagai simulasi fermentasi pakan di dalam rumen. Empat *raw material* sumber EO dan empat destilat EO komersial masing-masing ditambahkan ke dalam substrat pakan pada fermentasi rumen *in vitro*. Dosis masing-masing sumber EO adalah dosis rendah yang setara dengan konsentrasi EO di dalam media fermentasi setingkat 0, 25, 50, 100 mg/l dan dosis tinggi pada konsentrasi 0, 100, 200, 400 dan 800 mg/l. Pakan yang diberikan sebagai substrat fermentasi berupa rumput raja, bekatul dan *wheat pollard* dengan perbandingan berdasar bahan kering 60:20:20. Kandungan nutrien bahan pakan adalah PK 13,4%, SK 21,44% dan BETN 46,50%. Inkubasi dilakukan pada 39ºC selama 24 jam. Data yang diambil setelah fermentasi adalah produksi metan dan parameter fermentasi yang meliputi pH, produksi gas, konsentrasi *volatile fatty acid* (VFA), asetat, propionat, butirat, konsentrasi amonia, jumlah sel protozoa, sintesis protein mikrobia, kecernaan nutrien yang meliputi kecernaan bahan kering (KcBK), kecernaan bahan organik (KcBO), kecernaan protein kasar (KcPK) dan kecernaan serat kasar (KcSK) serta aktifitas enzim hidrolitik yang meliputi enzim amilase, endoglukanase dan proteoase. Data dianalisis dengan analisis variansi rancangan acak lengkap pola faktorial (4x5) dilanjutkan dengan uji *Duncan's Multiple Range test* (*DMRT*) untuk melihat perbedaan antar rata-rata bila terjadi pengaruh perlakuan yang signifikan. Data keragaman dan kelimpahan metanogen diambil pada fermentasi dengan macam EO terpilih dengan dosis optimum dalam menurunkan metan yang tidak mengganggu fermentasi pakan. Keragaman metanogen dianalisis dengan dua metode yaitu *terminal restriction fragment length polymorphism* (TRFLP) berdasar gen *mcrA* yang mengkode enzim metil-ko M reductase, serta dengan metode metagenomik hasil sekuensing *next-*

generation sequencing (NGS) dengan *illumina platform* berdasar *hypervariable regions* V4, gen yang mengkode 16S rRNA arhkaea. Enzim restriksi yang digunakan untuk memotong amplikon pada metode TRFLP adalah MspI, BsrI dan TaqI. Data kelimpahan metanogen aktif di peroleh dengan metode *quantitative polymerase chain reaction* (qPCR) berdasar ekpresi gen *mcrA* (total metanogen), *mer* (hidrogenotrop), *mtaA1* (metilotrop), *acs2* (asetiklastik) dengan gen *reference unimet.* Data kelimpahan hasil analisis qPCR dianalisis dengan analisis variansi *independent sample T-Test.*

Data hasil penelitian menunjukan kandungan EO *raw material* kapulogo kencur, jahe merah dan jinten berturut-turut sebesar 3,88%; 3,09%; 2,89% dan 2,20% dengan komponen aktif utama untuk masing-masing *raw material* berturut-turut adalah *eucalyptol* (68,75%), *hexadecane* (64,98), *zingiberene* (31,46%) *carvone* (86,04%). Komponen aktif utama empat destilat EO komersial adalah adas *trans-anethole* (68,76%), pinus *α-pinene* (24,49%), *2,6,-dimethylundecane* (19,51%) dan *2-methyl-3-ethylheptane* (19,44), sereh wangi *geraniol* (22,70%) dan *citronella* (15,57%), dan kayu putih ambon *eucalyptole* (43,42%). Macam *raw material* sumber EO pada pemberian dosis 0-100 mg/l tidak berpengaruh terhadap pH, amonia, sintesis protein mikorbia, butirat, rasio asetat:propionat, KcBK dan KcBO, serta aktivitas enzim protease. Dosis pemberian *raw material* sumber EO tidak berpengaruh terhadap pH, produksi metan/BK pakan, amonia, sintesis protein mikrobia, total VFA, asetat, propionat, butirat, rasio asetat:propionat, dan aktivitas enzim endoglukanase. Macam *raw material* sumber EO berpengaruh terhadap total produksi metan, produksi metan/BK pakan, volume produksi gas, jumlah protozoa kcPK dan KcSK ($P<0,01$). Peningkatan dosis *raw material* sumber EO meningkatkan produksi metan, produksi gas, protozoa, KcSK ($P<0,01$) dan KCBO ($P<0,05$), serta menurunkan KcPK ($P<0,01$). Pemberian destilat EO pada dosis rendah (0-100 mg/l) belum mampu menurunkan produksi metan. Pengaruh destilat EO yang berbeda menunjukan pola yang berbeda antara masing-masing destilat EO terhadap parameter fermentasi, VFA, kecernaan nutrien dan aktivitas enzim hidrolitik. Pengaruh yang signifikan terlihat pada pemberian destilat EO pada dosis tinggi (0-800 mg/l). Data yang diperoleh menunjukan bahwa pemberian destilat EO pada dosis tinggi meningkatkan pH ($P<0,01$) meskipun masih dalam kisaran pH normal rumen. Produksi metan, volume produksi gas, protozoa, sintesis protein mikrobia, KcBK, KcBO, dan KcSK mengalami penurunan ($P<0,01$) sejalan dengan peningkatan dosis destilat EO pada pemberian dosis tinggi. Pola hubunga antara penurunan dengan peningkatan dosis berbeda pada masing-masing destilat EO. Pemberian destilat EO dengan dosis di atas 200 mg/l selain menurunkan metan juga menyebabkan penurunan produksi gas, sintesis protein mikrobia, KcBK, KcBO dan KcSK, amun tidak berpengaruh terhadap total VFA, asetat, propionat, butirat serta rasio asetat:propionat. Penurunan metan pada pemberian EO pinus 100 dan 200 mg/l lebih tinggi dibanding perlakuan yang lain, dengan penurunan metan sebesar 31,55 dan 40,02% dari kontrol. Hasil analysis sekuen TRFLP menunjukan keragaman genetik metanogen dipengaruhi oleh penambahan EO pinus pada dosis 100 dan 200 mg/l. *Methanomicrobiales* merupakan metanogen yang dominan pada semua media fermentasi meskipun proporsinya

berbeda. Berdasar hasil metagenomik data sekuensing dengan NGS menunjukan *Metanobrevibacter* mendominasi pada kelompok kontrol dan perlakuan EO pinus 200 mg/l sedangkan *Methanomassiliicoccaceae* dominan pada perlakuan EO pinus 100 mg/l. Berdasar ekpresi gen yang berperan dalam metanogenesis, total metanogen, metanogen asetoklastik dan metilotropik mengalami penurunan dengan pemberian EO pinus pada dosis 100 dan 200 mg/l ($P<0,05$), sedangkan metanogen hidrogenotropik tidak dipengaruhi secara signifikan namun jumlahnya lebih rendah dibanding kontrol pada pemberian 100 mg/l dan lebih tinggi dari kontrol pada pemberian 200 mg/l. Dari hasil penelitian dapat disimpulkan bahwa pemberian EO pinus pada dosis 100 mg/l merupakan kandidat aditif pakan yang paling optimum yang mampu menurunkan metan tanpa mengganggu proses fermentasi pakan sintesis VFA dan sintesis mikrobia rumen.

Kata kunci: Metan, Metanogen, Metagenomik, Rumen, Essential oil

ABSTRACT

MOLECULAR STUDY OF RUMEN METHANOGENS AND EVALUATION OF METHANE PRODUCTION IN RUMINANT AS IMPACT OF PLANT ESSENTIAL OIL ADDITION

This study was conducted to find out a candidate of feed additive for mitigating of methane production based on essential oil (EO). The selection was done among raw materials of EO source and commercial distillates of EOs. The effect of EOs on methane production, parameters of fermentation, nutrient digestibility, the activity of hydrolytic enzymes and also diversity and abundance of methanogens were used as consideration in this selection.

Four raw material of EO sources i.e. cardamom (*Amomum compactum)*, cutcherry (*Kaempferia galanga* L.), red ginger (*Zingiber officinale Var.Rubrum*), and cumin (*Cuminum cyminum*), and four of distillates EOs i.e. fennel (*Foeniculum vulgare* (Mill)), pine (*Pinus merkusii* (Jungh. and de Vriese)), citronella fragrance (*Cymbopogon nardus* (L.) Rendle), and cajeput (*Melaleuca leucadendra* (L.)) were used as sample in this research. Nutrient content of raw materials EO sources was analyzed according to the proximate method whereas essential oil contents were determined by Stahl distillation. The active component of EOs was analyzed using gas chromatography-mass spectrometry (GCMS*)*. In vitro gas production technique was used as a simulation of feed fermentation in the rumen. Each raw material and distillates EO were added and mix with feed, the substrate for in vitro fermentation, to meet the doses of EO 0, 25, 50, 75, and 100 mg/l of media for the lower doses and at 0, 100, 200, 400, and 800 mg/ l for the higher doses. Feed consisted of *Pennisetum purpureum*, rice bran and wheat pollard in ratio of 60:20:20 dry matter bases. Nutrient content of the feed was crude protein 13.4%, crude fiber 21.44% and non nitrogen extract 46.50%. Fermentation was incubate at 39ºC for 24 h.

Collected data included methane production, parameter of fermentation (pH, gas production, concentration of volatile fatty acid (VFA), acetate, propionate, butyrate, concentration of ammonia, number of protozoa, microbial protein synthesis, and nutrient digestibility of dry matter, organic matter, crude protein, and crude fiber, and also activity of amylase, endoglucanase, and protease. The experiment were arranged on the completely randomized design with 4x5 factorial treatments. Data were statistically analyzed using ANOVA. When significantly differencees followed by post hoc of Duncan's Multiple Range test (DMRT). Data of methanogen diversity and abundance were collected from fermentation with addition of optimum treatment. The diversity of methanogens were studied using two methods which was terminal restriction fragment length polymorphism (TRFLP) method based on *mcrA* gen encode methyl-Co M reductase, and metagenomic analysis of sequencing product of hypervariable regions V4, encode 16S rRNA gene of archaea using of *next-generation* sequencing (NGS) with Illumina platform. Quantitative polymerase chain reaction (qPCR) was used to analyzed the abundance of active methanogen based on *mcrA* gene expression for total of methanogen, *mer* gene for hydrogenotroph, *mtaA1* gene for methylotroph, *acs2* gene for acetoclastic. *Unimet* gene or archaea was used as gene *reference*. Obtained data of methanogens abundance were analyzed with analysis of variance independent sample T-Test.

Result data showed that the EO content of raw material of cardamom, cutcherry, red ginger and cumin respectively were 3.88%, 3.09%, 2.89% and 2.20%, whereas active component of each raw material were *eucalyptol* (68.75%), *hexadecane* (64.98%), *zingiberene* (31.46%) *carvone* (86.04%). Component distilate EO of cardamom was *trans-anethole* (68.76%), pine *α-pinene* (24.49%), *2,6,-dimethylundecane* (19.51%) and *2-methyl-3-ethylheptane* (19.44%), citronella fragrance *geraniol* (22.70%) and *citronella* (15.57%), and cajuput EO was *eucalyptole* (43.42%). Addition of raw material EO at doses of 0-100 mg/l did not effect on pH, ammonia, synthesis of microbial protein, butyrate, ratio of acetate:propionate, digestibility of dry matter and organic matter, and also the activity of protease. Raw material doses also did not influence the pH, methane production/feed dry matter, ammonia, of microbial protein synthesis, total VFA, acetate, propionate, butyrate, ratio of acetate:propionate, and activity of endoglucanase. Methane production was different among fermentation with different of raw material of EO source as well as methane production/feed dry matter, volume of gas production, number of protozoa cells, crude protein digestibility and crude fiber digestibility ($P<0.01$). The increasing doses of *raw materials* addition increase methane and gas production, cell protozoa number, crude fiber digestibility ($P<0.01$) and organic matter digestibility ($P<0.05$), and also decreased crude protein digestibility ($P<0.01$). Distillates EO at lower doses (0-100 mg/l) did not reduced methane production. There was different pattern of distillates EO on parameter of fermentation, VFA, nutrient digestibility and hydrolytic enzymes. The significant effect of distillate EO on measured data was showed by addition at high doses (0-800 mg/l). Data pH increased with increasing of doses of distillate EO ($P<0.01$). Methane production, volume gas production, cell protozoa number, microbial protein synthesis, dry matter, organic matter and crude fiber digestibility, were decreased ($P<0.01$) by addition of distillate EO with different manner among EOs. Addition at doses 200 mg/l and above, not only reduced methane production but also have detrimental effect on gas production, microbial protein synthesis, digestibility of dry matter, organic matter and crude fiber. Total of VFA, acetate, propionate, butyrate and ratio of acetate:propionate did not affected by addition of distillate EO at high doses. The greatest of methane reduction were occurred at addition of pine EO at level 100 and 200 mg/l. The methane reduction at both doses were 31.55 and 40.02% of control. Result of TRFLP sequence analysis showed diversity of methanogen was affected by addition of pine EO at doses 100 and 200 mg/l. *Methanomicrobiales* was the dominant methanogen of all treatment even though in different proportion. While metagenomics analysis of NGS sequence showed *Metanobrevibacter* was the dominant methanogen in fermentation control and treatment of 200 mg/l. At the other hand *Methanomassiliicoccaceae* was the dominant methanogen of fermentation with pine EO treatment of 100 mg/l. Total active methanogen decreased by addition of pine EO at doses 100 and 200 mg/l, likewise acetoclastic and methylotropic methanogens ($P<0.05$). Active hydrogenothropic methanogens of fermentation with 100 mg/l pine EO was lower than control, whereas at doses 200 mg/l those methanogens tend to be higher than control. In conclusion, addition of pine EO at doses 100 mg/l is the most effective as a candidate of feed additive to reduce methane with no detrimental effect on nutrient digestibility, VFA and microbial protein synthesis.

Keywords: Methane, Methanogen, Metagenomic, Rumen, Essential oil