

**POTENSI IMUNOMODULATOR VIRGIN COCONUT OIL (VCO)
SEBAGAI SUPLEMEN PAKAN TERHADAP INFEKSI *Staphylococcus
aureus* PENYEBAB MASTITIS SUBKLINIS PADA KAMBING**

Desy Cahya Widianingrum

Intisari

Pengobatan alternatif diperlukan untuk mengatasi masalah mastitis subklinis dan resistensi *Staphylococcus aureus* terhadap berbagai antibiotik. *Virgin coconut oil* (VCO) memiliki zat aktif yang diduga berperan sebagai imunomodulator dan berfungsi sebagai antibakterial. Penelitian ini dilakukan dengan tujuan untuk melakukan analisis potensi VCO sebagai imunomodulator dan agen protektif terhadap infeksi *Staphylococcus aureus* isolat asal kambing Peranakan Ettawa (PE) dan kajiannya sebagai suplemen pakan ruminansia.

Untuk mencapai tujuan penelitian dilakukan melalui 3 tahap proses penelitian. Tahap pertama dilakukan isolasi, identifikasi dan karakterisasi secara fenotipik maupun genotipik *S. aureus* yang diisolasi dari 110 sampel susu kambing di sentra peternakan kambing PE di wilayah Riau. Penentuan mastitis subklinis pada kambing dilakukan dengan *California Mastitis Test* (CMT). Identifikasi secara fenotipik *S. aureus* dilakukan berdasar pewarnaan Gram, kemampuan memfermentasi *Mannitol Salt Agar* (MSA), uji katalase dan Voges-Proskauer (VP). Sifat resistensi *S. aureus* terhadap berbagai antibiotik dilakukan dengan menggunakan uji difusi menurut Kirby-Bauer. Identifikasi secara molecular dilakukan dengan menggunakan *polymerase chain reaction* (PCR) dengan primer spesies spesifik untuk gen 23SrRNA, gen *nuc*, dan gen *coa*, serta dilakukan konfirmasi melalui *sequencing*. Tahap kedua, penelitian dilakukan untuk mengetahui potensi VCO sebagai agen antimikrobia dan imunomodulator terhadap infeksi *S. aureus*. Potensi sebagai antimikrobia dilakukan melalui uji difusi untuk melihat aktifitas hambatan VCO terhadap pertumbuhan *S. aureus* pada media agar Müller Hinton dan melalui pengamatan *scanning electron microscopy* (SEM). Aktifitas VCO sebagai agen imunomodulator dilakukan melalui uji fagositosis sel-sel makrofag dan sel-sel polimorfonuklear (PMN) terhadap *S. aureus* secara *in vitro*. Uji imunomodulator secara *in vivo* dilakukan pada mencit percobaan yang diinfeksi *S. aureus* dengan pemberian VCO, melalui pemeriksaan leukosit dan diferensial leukosit, uji proliferasi limfosit, aktifitas *superoxide dismutase* (SOD), dan didukung dengan pengamatan histopatologis organ hepar dan ginjal. Tahap ketiga, dilakukan penelitian secara *in vitro* untuk mengetahui efektifitas VCO dengan proteksi dan non-proteksi sebagai bahan suplemen pakan ternak ruminansia.

Berdasarkan hasil penelitian pada tahap pertama, diketahui bahwa kejadian mastitis subklinis pada sentra peternakan kambing PE di Riau dengan *California Mastitis Test* (CMT) adalah 30 dari 110 sampel susu (27,27%). Hasil isolasi dan identifikasi isolat asal susu kambing PE secara fenotipik terdapat 14 isolat diduga *S. aureus*, berdasar pewarnaan Gram, kemampuan memfermentasi mannitol pada

MSA, positif pada uji katalase dan VP. Berdasar uji resistensi diketahui isolat *Staphylococcus* telah resisten terhadap ampicilin, penicillin (78,57%), oxacilin (71,43%) dan masih sensitif terhadap tetracycline (64,29%), erythromycin (71,43%), gentamicin (85,71%). Identifikasi isolat secara genotipik menggunakan primer spesies spesifik 23SrRNA, gen *nuc* dan *coa*, serta analisis *sequencing*, dapat teridentifikasi 5 isolat *S. aureus* (16,67%). Hasil tahap kedua, diketahui bahwa uji dilusi VCO konsentrasi 200 μ L (setara dengan 0,102% asam laurat) mampu menghambat pertumbuhan *S. aureus*. Sediaan VCO berpotensi meningkatkan kemampuan fagositosis *S. aureus* terhadap sel-sel makrofag secara signifikan pada konsentrasi 200 μ L dan fagositosis PMN pada konsentrasi 100 μ L (setara dengan 0,051% asam laurat). Berdasar pengamatan SEM dan aktivitas makrofag terhadap *S. aureus* dengan teknik *fluorescent* diketahui VCO mampu membunuh bakteri dengan merusak dinding sel bakteri tanpa mengganggu sistem kerja makrofag. Percobaan *in vivo* menggunakan tikus percobaan, diketahui bahwa pemberian VCO mampu meningkatkan imunitas tubuh, berdasar respon leukosit, neutrofil, makrofag, proliferasi limfosit, aktivitas SOD serta diteguhkan dengan hasil histopatologi organ hepar dan ginjal yang menggambarkan bahwa VCO mampu memperbaiki sel-sel jaringan setelah diinfeksi dengan *S. aureus*. Hasil tahap ketiga, berdasar pengamatan nilai pencernaan *in vitro* dan kondisi aktivitas rumen, diketahui bahwa ransum dengan penambahan suplemen 250 μ L VCO yang diproteksi dengan 2% formaldehid memperlihatkan hasil terbaik dilihat dari nilai kcBK dan KcBO, pH cairan rumen, proporsi (A+B):P, konsentrasi NH_3 dan konsentrasi protein mikroba. Berdasar keseluruhan hasil penelitian yang telah dilakukan dapat disimpulkan bahwa, suplemen pakan VCO terproteksi berpotensi untuk dapat digunakan sebagai agen imunomodulator dan antimikrobia pada kambing PE dalam melawan mastitis subklinis akibat infeksi *S. aureus*.

Kata kunci: *Virgin coconut oil*, *Staphylococcus aureus*, antimikrobia, imunomodulator, mastitis, kambing PE,

IMUNOMODULATOR OF VIRGIN COCONUT OIL (VCO) AS FEED SUPPLEMENT FOR *Staphylococcus aureus* INFECTION IN SUBCLINICAL MASTITIS OF GOATS

Desy Cahya Widianingrum

Abstract

Alternative medicine is needed to overcome the problem of subclinical mastitis and *Staphylococcus aureus* resistance to various antibiotics. Virgin coconut oil (VCO) has an active substance which allegedly acted as an immunomodulator and serves as an antibacterial. The aim of this studies were to analyze the potential of VCO as immunomodulator and protective agent against infection of *Staphylococcus aureus* isolated from Ettawa Peranakan goat (PE) and the possibility as a feed supplement of ruminant.

The studies were conducted through 3 stages. The first stage were isolation, identification and characterization of phenotypic and genotypic *S. aureus* isolated from 110 goat milk samples of PE in Riau region. Determination of subclinical mastitis in goats was done with California Mastitis Test (CMT). Phenotypic identification of *S. aureus* was performed by Gram staining, Mannitol Salt Agar (MSA) fermentation, positive reaction on catalase and Voges-Proskauer (VP) tests. The resistance properties of *S. aureus* to various antibiotics were conducted using the Kirby-Bauer diffusion test. Molecular identifications were analysed using polymerase chain reaction (PCR) with specific species primers for 23SrRNA, *nuc* and *coa* genes, and confirmed through sequencing. The second stage of study was conducted to determine the potential of VCO as an antimicrobial agent and immunomodulator against *S. aureus* infection. Potential of VCO as an antimicrobial were performed by dilution test to observe the sensitivity of VCO against *S. aureus* on Müller Hinton agar medium, and through scanning electron microscopy (SEM) observation. VCO activity as an immunomodulatory agent was done through in vitro study by the phagocytosis assays of macrophage cells and polymorphonuclear (PMN) cells against *S. aureus*. In vivo study of immunomodulatory effects were also conducted on mice infected *S. aureus* with VCO treatment, through the observation of leukocytes, lymphocyte proliferation, superoxide dismutase (SOD) activity, and supported by histopathologic observation of hepar and kidney organ. The third stage was conducted by in vitro study to analyze the effectiveness of VCO with protection and non-protection treatment as a feed ingredient supplement in ruminants.

Based on the results of the first study, indicated that the incidence of subclinical mastitis in PE goats in Riau with the CMT were 30 out of 110 samples of milk (27,27%). There were 14 isolates suspected of *S. aureus* based on Gram staining, the ability to fermented MSA, positive reaction to catalase and VP tests. Based on the antimicrobial resistance assays revealed that *Staphylococcus* isolates were resistant to ampicilin, penicillin (78,57%), oxacilin (71,43%) and remained sensitive to tetracycline (64,29%), erythromycin (71,43%), gentamicin (85,71%). Genotypic identification of isolates by using primers specific species 23SrRNA, *nuc*

and *coa* genes and sequencing analysis, could be identified 5 isolates of *S. aureus* (16,67%). The results of the second study remarked that dilution test of VCO 200 μ L (equivalent to 0,102% lauric acid) could inhibit the growth of *S. aureus*. VCO had potentially to improve the ability of *S. aureus* to be phagocytosed by macrophage cells significantly at a concentration of 200 μ L and PMN cells at a concentration of 100 μ L (equivalent to 0,051% lauric acid). By observation of scanning electron microscopy (SEM) and macrophage cells activity against *S. aureus* with fluorescent techniques could be analysed that VCO abled to destroy bacterial cell wall without disturbing the working system of macrophage cells. In vivo experiments using mice were described that VCO had the ability to increase the body immune system of mice, by indicating the response of lymphocytes, neutrophyles, lymphocyte proliferation, and activity of SOD of blood mice after infected by *S. aureus*. Histopathological changes of hepar and renal of mice were illustrated that the VCO abled to repair tissue cells after being infected with *S. aureus*. By in vitro study of the digestibility and rumen activity conditions, could be summerized that the supplement of 250 μ L VCO protected with 2% formaldehyde showed the best results based on the dry and organic matter digestibility, pH of rumen fluid, proportion (A+B):P, NH_3 concentration and microbial protein concentration. Based on the overall research can be concluded that the feed supplement protected VCO has potentially to be used as an immunomodulatory and antimicrobial agent of PE goats to combat subclinical mastitis due to *S. aureus* infection.

Keywords: Virgin coconut oil, *Staphylococcus aureus*, antimikrobial, imunomodulator, mastitis, PE goats