KAJIAN *DIRECT-FED MICROBIALS* TERHADAP FERMENTASI RUMEN, PROFIL EKSPRESI GEN SISTEM IMUN DAN PRODUKTIVITAS KAMBING PERAH

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RINGKASAN

Penggunaan *direct-fed microbials* (DFM) telah menjadi pendekatan potensial dalam meningkatkan fermentasi rumen dan produktivitas ternak ruminansia serta memodulasi profil ekspresi gen yang terlibat dalam sistem imun. DFM merupakan aditif pakan berbasis mikroorganisme hidup seperti bakteri asam laktat (BAL) dan yeast yang berfungsi memperbaiki fermentasi rumen, menstabilkan mikroflora rumen, serta meningkatkan efisiensi pencernaan dan performa ternak. Namun eksplorasi DFM dari mikroba sumber lokal khas Indonesia masih terbatas. Penelitian ini diarahkan untuk mengevaluasi potensi isolat BAL dan yeast yang sudah didapatkan sebelumnya dari Ikan frementasi (Budu), dalam meningkatkan fermentasi rumen, profil ekspresi gen sistem imun, dan produktivitas kambing perah. Rangkaian penelitian dilakukan dalam tiga tahap utama.

Penelitian tahap pertama menggunakan rancangan acak lengkap (RAL) dengan 9 perlakuan dan tiga ulangan. Lima isolat BAL (isolat MKS4, MKS6, MKS8, MKS12 dan MKS22) dan tiga isolat yeast (isolat SCB, SCL dan SC) diuji melalui fermentasi rumen *in-vitro* terhadap ransum komplit (70:30 hijauan:konsentrat). Inokulum BAL mengandung 1,02 x 10^{11} CFU/ml dan yeast mengandung 1,5 x 10^{10} CFU/ml. Berdasarkan hasil analisis, perlakuan P4 (isolat MKS12) dan P6 (isolat SCB) menunjukkan kinerja terbaik, ditandai dengan perbedaan yang signifikan dalam degradabilitas bahan kering (DBK), degradabilitas bahan organik (DBO) (masing-masing 70,29% dan 71,16% untuk P4 serta 67,64% dan 65,73% untuk P6), produksi gas total (150-200 ml), VFA total dan NH_3 (121,67 mM dan 16,83 mg/dl untuk P4, serta 96,67 mM dan 13,42 mg/dl untuk P6) ($P<0,05$). Sementara itu, pH rumen berada dalam kisaran optimal (6,8-7,1). Berdasarkan analisis BLAST, isolat MKS12 memiliki tingkat kemiripan sebesar 100% dengan *Schleiferilactobacillus harbinensis* strain LH991, dan isolat SCB tingkat kemiripan sebesar 100% dengan *Pichia kudriavzevii* strain B-5P.

Penelitian tahap kedua menggunakan rancangan acak lengkap (RAL) dengan susunan faktorial 3 × 4 dan tiga ulangan. Faktor A merupakan kombinasi dua jenis DFM yaitu BAL *(S. harbinensis* strain LH991) dan yeast (*P. kudriavzevii* strain B-5P), dengan tiga proporsi berbeda yaitu 1:1, 1:3, dan 3:1, sementara faktor B merupakan dosis DFM yang diberikan (berdasarkan rasio v/v) yang terdiri dari empat level yaitu (1%, 2%, 3% dan 4%) yang diuji secara *in-vitro* untuk melihat efek terhadap fermentasi rumen dan populasi mikroba rumen. Masing-masing mikroba mengandung 1 x 10^{10} CFU/ml. Kombinasi DFM tidak menunjukkan

pengaruh signifikan terhadap fermentasi rumen dan kecernaan pakan (P>0,05), namun rasio 1:1 (A1) meningkatkan produksi butirat, iso-butirat, dan iso-valerat secara signifikan (P<0,05). Penggunaan dosis DFM hingga 4% (B4) secara signifikan meningkatkan konsentrasi NH_3, VFA total, propionat dan butirat, serta menurunkan rasio asetat terhadap propionat (A:P) (P<0,05). Degradabilitas pakan *in-vitro* juga meningkat seiring dengan peningkatan dosis DFM, terutama pada level 4%, yang menunjukkan peningkatan nyata pada DBK, DBO, dan degradabilitas serat kasar (DSK) (P<0,05). Kombinasi DFM berpengaruh terhadap mikroba tertentu terutama meningkatkan populasi *Prevotella ruminicola* dan *Butyrivibrio fibrisolvens* (P<0,05), meskipun tidak memengaruhi populasi mikroba target lainnya. Penggunaan dosis DFM hingga 4% meningkatkan populasi relatif mikroba rumen seperti, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Treponema bryantii*, *Selenomonas ruminantium*, dan *Prevotella ruminicola* serta menurunkan populasi metanogen (P<0,05).

Penelitian tahap ketiga dilakukan secara *in-vivo* dengan menggunakan 20 ekor kambing perah Sapera, yang dibagi kedalam dua kelompok perlakuan yaitu kelompok kontrol (G1) dan kelompok DFM (G2). DFM mengandung konsentrasi masing-masing sebesar 1,2 x 10^{11} CFU/ml yang diberikan sebanyak 10 ml/ekor/hari secara oral selama 8 minggu. Hasil menunjukkan bahwa pemberian DFM tidak mengubah konsumsi pakan (P>0,05) namun secara keseluruhan meningkatkan produksi susu harian serta kualitas susu (P<0,05). Segi aspek ekonomi, pemberian DFM meningkatkan efisiensi pakan, IOFC dan efisiensi ekonomi (P<0,05). Hasil sekuensing RNA menggunakan NGS diperoleh 220 gen differensial yang terekspresi yang signifikan (FDR<0,05), terdiri atas 135 gen *upregulated* dan 85 gen *downregulated*. Analisis *gene ontology* (GO) proses biologis menunjukkan bahwa pemberian DFM cenderung memengaruhi ekspresi gen yang terlibat dalam sistem imun ternak.

Analisis GO menunjukkan kecenderungan peningkatan signifikan ekspresi gen jalur proses biologis pada kategori *immune system process, immune response*, *defense response to bacterium* dan *antimicrobial humoral response* (P<0,001), yang menandakan aktivasi kekebalan bawaan dan adaptif, termasuk produksi protein antimikroba, sekresi molekul imun, penghambatan patogen dan penurunan inflamasi. Hasil analisis KEGG mendukung temuan ini dengan kecenderungan aktivasi jalur *Staphylococcus aureus infection, NK cell-mediated cytotoxicity*, dan *hematopoietic cell lineage* (P<0,001), serta munculnya jalur imunoregulator seperti *asthma, rheumatoid arthritis* dan *salivary secretion* (P<0,01) yang mencerminkan peran DFM dalam menjaga keseimbangan imun dan homeostasis. Volcano plot menunjukkan signifikansi (P<0,05) upregulasi gen-gen protektif seperti *GBP, cathelicidins, S_100, trypsin*, dan *amidase,* serta downregulasi *TACC, proteasome* dan *MHC 1* sebagai bentuk regulasi inflamasi dan penekanan aktivitas stress. Secara keseluruhan, DFM tidak hanya meningkatkan kesehatan saluran cerna dan menghambat patogen, tetapi juga diduga berperan sebagai imunomodulator yang memperkuat sistem pertahanan tubuh melalui regulasi ekspresi gen imun secara sistemik dan mukosal.

Berdasarkan hasil penelitian, didapatkan dua isolat unggul kandidat DFM yang berasal dari ikan fermentasi (Budu) yaitu *Schleiferilactobacillus harbinensis* strain LH991 dan *Pichia kudriavzevii* strain B-5P yang memberikan pengaruh fermentasi di rumen pada rasio 1:1 dengan dosis 4% serta meningkatkan produktivitas dan cenderung memodulasi profil ekspresi gen sistem imun pada kambing perah

Sapera. DFM juga menunjukkan perannya sebagai agen imunomodulator potensial. Temuan ini mendukung pendekatan nutrigenomik sebagai strategi baru dalam peningkatan kinerja ternak ruminansia secara lebih optimal, sekaligus memperkuat potensi pemanfaatan sumber daya mikroba lokal dalam pengembangan teknologi pakan fungsional.

Kata Kunci: bakteri asam laktat, ekspresi gen sistem imun, fermentasi rumen, kambing perah Sapera, produktivitas, yeast

A STUDY ON THE EFFECTS OF DIRECT-FED MICROBIALS ON RUMEN FERMENTATION, IMMUNE SYSTEM GENE EXPRESSION, AND PRODUCTIVITY IN DAIRY GOATS

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SUMMARY

The use of direct-fed microbials (DFM) has emerged as a promising approach to enhance rumen fermentation and ruminant productivity, as well as to modulate gene expression profiles associated with the immune system. DFM are feed additives consisting of live microorganisms, such as lactic acid bacteria (LAB) and yeast, which function to improve rumen fermentation, stabilize ruminal microflora, and increase digestive efficiency and animal performance. However, exploration of DFM derived from local microbial sources, particularly indigenous to Indonesia, remains limited. This study was designed to evaluate the potential of LAB and yeast isolates previously obtained from fermented fish "Budu" in improving rumen fermentation, immune-related gene expression, and the productivity of dairy goats. The research was conducted in three main stages.

In the first stage, a completely randomized design (CRD) was used with nine treatments and three replications. Five LAB isolates (MKS4, MKS6, MKS8, MKS12, and MKS22) and three yeast isolates (SCB, SCL, and SC) were evaluated through *in-vitro* rumen fermentation using a total mixed ration (TMR) at a 70:30 forage-to-concentrate ratio. The LAB inoculum contained 1.02×10^{11} CFU/ml and the yeast inoculum contained 1.5×10^{10} CFU/ml. Based on the analysis, treatment P4 (MKS12) and P6 (SCB) showed the best performance, with significant differences in dry matter degradability (DMD) and organic matter degradability (OMD), reaching 70.29% and 71.16% for P4, and 67.64% and 65.73% for P6, respectively. Total gas production ranged from 150–200 ml, while total VFA and NH_3 concentrations were 121.67 mM and 16.83 mg/dl for P4, and 96.67 mM and 13.42 mg/dl for P6 ($P<0.05$). Rumen pH remained within the optimal range (6.8–7.1). BLAST analysis revealed that isolate MKS12 was 100% identical to *Schleiferilactobacillus harbinensis* strain LH991, while SCB matched 100% with *Pichia kudriavzevii* strain B-5P.

The second stage involved a CRD with a 3 × 4 factorial design and three replications. Factor A was the combination of two DFM types (*S. harbinensis* strain LH991 and *P. kudriavzevii* strain B-5P) at three ratios (1:1, 1:3, and 3:1), and Factor B was the DFM dose (v/v) at four levels (1%, 2%, 3%, and 4%), tested *in-vitro* for their effects on rumen fermentation and microbial populations. Each microorganism had a concentration of 1×10^{10} CFU/ml. While DFM combinations did not significantly affect overall rumen fermentation or feed degradability

(P>0.05), the 1:1 ratio significantly increased butyrate, iso-butyrate, and iso-valerate production (P<0.05). DFM dosage up to 4% (B4) significantly enhanced NH_3, total VFA, propionate, and butyrate concentrations, and reduced the acetate-to-propionate ratio (A:P) (P<0.05). Feed degradability also improved with higher DFM doses, particularly at the 4% level, showing significant increases in DMD, OMD, and crude fiber degradation (CFD) (P<0.05). The DFM combination significantly increased the abundance of certain rumen microbes such as *Prevotella ruminicola* and *Butyrivibrio fibrisolvens* (P<0.05), although other microbial populations remained unaffected. DFM doses up to 4% significantly increased relative populations of *Ruminococcus albus, Ruminococcus flavefaciens, Treponema bryantii, Selenomonas ruminantium*, and *Prevotella ruminicola,* while reducing methanogen populations (P<0.05).

The third stage was conducted in-vivo using 20 lactating Sapera dairy goats, randomly assigned to control (G1) and DFM treatment groups (G2). DFM containing 1.2×10^{11} CFU/ml was administered orally at a dose of 10 ml/head/day for eight weeks. The results indicated that DFM supplementation did not affect feed intake (P>0.05), but significantly improved daily milk yield and milk quality (P<0.05). Economically, DFM enhanced feed efficiency, income over feed cost (IOFC), and economic efficiency (P<0.05). RNA sequencing using *next-generation sequencing* (NGS) identified 220 significantly differentially expressed genes (FDR<0.05), including 135 upregulated and 85 downregulated genes. Gene ontology (GO) analysis of biological processes revealed that supplementation with DFM tended to influence the expression of genes involved in the immune system of livestock.

GO biological process analysis showed significant enrichment in *immune system process, immune response, defense response to bacterium,* and *antimicrobial humoral response* categories (P<0.001), indicating the activation of both innate and adaptive immune responses, including the production of antimicrobial proteins, secretion of immune molecules, inhibition of pathogens, and suppression of inflammation. KEGG pathway analysis supported these findings, with activation of pathways such as *Staphylococcus aureus infection, NK cell-mediated cytotoxicity,* and *hematopoietic cell lineage* (P<0.001), as well as the emergence of immunoregulatory pathways like *asthma*, *rheumatoid arthritis* and *salivary secretion* (P<0.01), reflecting the role of DFM in maintaining immune balance and homeostasis. Volcano plot analysis showed significant (P<0.05) upregulation of protective genes such as *GBP, cathelicidins, S100, trypsin,* and *amidase* alongside downregulation of *TACC, proteasome,* and *MHC I*, indicating a modulation of inflammatory regulation and suppression of stress-related activity. Overall, DFM not only improved gastrointestinal health and inhibited pathogens but also appeared to function as an immunomodulator that strengthened systemic and mucosal immune defenses through the regulation of immune-related gene expression.

Based on the findings, two superior DFM candidate isolates from fermented fish (Budu), *Schleiferilactobacillus harbinensis* strain LH991 and *Pichia kudriavzevii* strain B-5P, demonstrated beneficial effects on enhanced rumen fermentation at a 1:1 ratio and 4% dosage, improved productivity, and tended to modulated immune gene expression profiles in Sapera dairy goats. DFM thus demonstrates its potential as a functional immunomodulatory agent. These results support the application of nutrigenomic approaches as a novel strategy for

optimizing ruminant performance while highlighting the potential use of local microbial resources in the development of functional feed technology.

Keywords: immune gene expression, lactic acid bacteria, productivity, rumen fermentation, Sapera dairy goats, yeast