KAJIAN BAKTERI ASAM LAKTAT ISOLAT ASAL AMPAS SUSU KEDELAI SEBAGAI PROBIOTIK POTENSIAL PENGHASIL SELULASE DAN FITASE SERTA APLIKASINYA PADA BROILER

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RINGKASAN

Ampas susu kedelai (ASK) memiliki potensi sebagai pakan ayam broiler namun pemanfaatannya masih rendah (10% dalam ransum broiler) karena mengandung serat kasar dan asam fitat yang tinggi. Penelitian ini bertujuan untuk mendapatkan isolat BAL selulolitik dan fitatolitik dari ASK dan menguji kemampuannya sebagai kandidat probiotik untuk meningkatkan daya guna ASK sebagai bahan pakan unggas.

Penelitian ini terdiri dari tiga tahap. **Tahap pertama** yaitu isolasi BAL yang bersifat selulolitik dan fitatolitik dari ASK. Tahap ini menggunakan metode deskriptif dengan melakukan pengamatan aktivitas selulase dan fitase secara kualitatif dan kuantitatif. **Tahap kedua** yaitu pengujian kemampuan BAL sebagai kandidat probiotik dan identifikasi BAL terpilih. Penelitian ini menggunakan metode eksperiman. Rancangan yang digunakan adalah rancangan acak lengkap (RAL) dengan empat isolat BAL (yang terpilih dari tahap satu) sebagai perlakuan dan lima ulangan. Peubah yang diamati adalah ketahanan terhadap pH lambung (pH 2,5) selama 3 jam dan 6 jam, ketahanan terhadap garam empedu 0.3% dan 0.6%, hidrofobisitas pada usus dan daya hambat terhadap bakteri patogen. Kandidat BAL terpilih berdasarkan analisis TOPSIS kemudian dikarakterisasi secara morfologi dan diidentifikasi secara molekuler. **Tahap ketiga** yaitu aplikasi penggunaan BAL sebagai probiotik penghasil selulase dan fitase (yang terpilih pada tahap dua) pada broiler yang mendapat ransum mengandung ASK. Metode yang digunakan yaitu metode eksperimen. Rancangan yanag digunakan adalah RAL pola faktorial dengan dua faktor dan tiga ulangan. Faktor A yaitu persentase penggunaan ASK dalam ransum broiler (A1: 15% ASK, A2: 20% ASK dan A3: 25% ASK) dan faktor B yaitu dosis probiotik (B1: $3x10^8$ CFU/mL; B2: $3x10^{10}$ CFU/mL dan B3: $3x10^{12}$ CFU/mL). Peubah yang diamati adalah koloni BAL di usus halus, selulase dan fitase di usus halus, morfologi usus (tinggi villi, lebar villi dan rasio tinggi villi dan kedalaman kripta), performa pertumbuhan (konsumsi ransum, pertambahan bobot

badan, konversi ransum), performa karkas (bobot hidup, persentase lemak abdominal, berat organ dalam (hati, empedu, jantung, proventikulus, ventrikulus, pankreas), bobot karkas, persentase karkas dan kolesterol daging paha bawah ayam broiler.

Hasil penelitian **tahap pertama** didapatkan 56 isolat bakteri yang telah dimurnikan. Selanjutnya, dilakukan pengujian BAL menggunakan MRSA+$CaCO_3$, sehingga diperoleh 21 isolat BAL berdasarkan pembentukan zona bening. Isolat yang didapat kemudian dilakukan skrining penghasil selulase dan fitase secara kualitatif dan kuantitatif. Skrining kualitatif dilakukan untuk mendeteksi isolat yang mampu memproduksi selulase dan fitase ekstraseluler berdasarkan pembentukan zona bening dan dilanjutkan dengan skrining kuantitatif. Skrining kuantitatif selulase dan fitase dilakukan untuk mengetahui konsentrasi selulase dan fitase enzim ektraseluler. Sebanyak 13 isolat dari 21 isolat BAL mampu menghasilkan selulase dan fitase namun dipilih 4 isolat yang menghasilkan selulase dan fitase tertinggi berdasarkan Analisa TOPSIS yaitu isolat AS15, AS21, AS28 dan AS44.

Hasil penelitian **tahap kedua** diperoleh, isolat AS15 berpengaruh sangat nyata ($P<0,01$) lebih tinggi dari AS21, AS28 dan AS44 terhadap uji probiotik yaitu dilihat dari daya tahan AS15 terhadap pH lambung sebesar 94.44 % setelah 3 jam dan 90.86% setelah 6 jam, tahan terhadap 0.3% (88.07%) dan 0.5% (81.24%) garam empedu, memiliki hidrofobisitas terhadap usus (91.24±0.429), zona hambat terhadap pertumbuhan bakteri patogen yakni *Escherichia coli* (22.25 mm), *Salmonella enteritidis* (13.30 mm), *Staphylococcus aureus* (15.15 mm). Berdasarkan analisis TOPSIS, isolat AS15 merupakan kandidat yang memiliki nilai preferensi tertinggi sebagai probiotik dan diidentifikasi molekuler sebagai *Lactobacillus casei* AS15.

Hasil Penelitian **tahap ketiga** didapatkan adanya interaksi sangat nyata ($P<0,01$) antara dosis probiotik dengan level pemberian ASK dalam ransum broiler pada konsumsi ransum, pertambahan bobot badan, FCR, bobot hidup, persentase lemak abdominal, bobot karkas, kolesterol daging paha bawah ayam broiler, koloni BAL di usus halus, selulase dan fitase di usus halus, tinggi villi, lebar villi dan rasio tinggi villi, kedalaman kripta dan tidak ada interaksi ($P>0,05$) pada berat organ fisiologis (hati, empedu, jantung, proventikulus, ventrikulus, pankreas). Kombinasi pemberian dosis probiotik *L. casei* AS15 ($3x10^{12}$ CFU/mL/ 12,48 log CFU/g) dengan level ASK 25% pada ransum broiler memberikan hasil yang optimal dilihat dari peningkatan koloni BAL, selulase dan fitase usus halus, morfologi usus halus, performa pertumbuhan, performa karkas dan penurunan kolesterol daging paha bawah broiler.

Kesimpulan dari tahap pertama penelitian ini yaitu dari 56 isolat bakteri yang dimurnikan, diperoleh 21 isolat BAL, dan 13 di antaranya mampu menghasilkan selulase dan fitase. Berdasarkan analisis TOPSIS, empat isolat terbaik terpilih, yaitu AS15, AS21, AS28, dan AS44. Kesimpulan tahap kedua yaitu isolat AS15 menunjukkan karakteristik probiotik terbaik dibandingkan AS21, AS28, dan AS44. Isolat ini memiliki ketahanan tinggi terhadap kondisi asam lambung dan garam empedu, hidrofobisitas tinggi, serta aktivitas antagonis kuat terhadap bakteri patogen. Isolat AS15 diidentifikasi sebagai *Lactobacillus casei* AS15. Kesimpulan dari tahap ketiga yaitu kombinasi pemberian dosis probiotik *L. casei* AS15 ($3x10^{12}$ CFU/mL/ 12,48 log CFU/g) dengan level ASK 25% pada ransum broiler memberikan hasil yang optimal dilihat dari koloni BAL di duodenum 11.45 log CFU/g; jejenum 11.41 log CFU/g; ileum 11.44 log CFU/g; selulase di duodenum 22.54 U/mL; jejenum 18,75 U/mL; ileum 25,90 U/mL, fitase di duodenum 9.16 U/mL; jejenum 8.56 U/mL; ileum 6,24 U/mL, tinggi villi di duodenum 1521,66 µm; jejenum 1276.51 µm; ileum 963,31 µm; lebar villi di duodenum 231.95 µm; jejenum 222.86 µm; ileum 237.96 µm, rasio tinggi villi dan kedalaman kripta di duodenum 15.42; jejenum 11.88; ileum 9.14), peningkatan performa pertumbuhan (konsumsi ransum 116.08 g/ekor/hari, pertambahan bobot badan 68.62 g/ekor/hari, konversi ransum 1.69), peningkatan performa karkas (bobot hidup 1755,67 g, persentase lemak abdominal 0.93%, tidak mengganggu berat organ fisiologis, bobot karkas 1397 g, persentase karkas 74,10%) dan penurunan kolesterol daging paha 95.32 mg/100 g.

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Kata kunci: ASK, BAL, *L. casei* AS15, Probiotik, Broiler

A STUDY OF LACTIC ACID BACTERIA FROM SOY MILK WASTE AS POTENTIAL PROBIOTICS PRODUCING CELLULASE AND PHYTASE AND ITS APPLICATION IN BROILERS

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SUMMARY

Soy milk waste (SMW) have potential as broiler chicken feed, but its utilization remains low due to their high crude fiber and phytic acid content. This study aimed to isolate cellulolytic and phytolytic LAB from SMW and test their potential as probiotic candidates to enhance the utility of SMW as poultry feed.

This study consisted of three stages. **The first stage** was the isolation of cellulolytic and phytolytic LAB from SMW. This stage used a descriptive method by observing cellulase and phytase activities qualitatively and quantitatively. **The second stage** was the testing of LAB's ability as probiotic candidates and the identification of selected LAB. This study used an experimental method. The design used was a completely randomized plan (CRD) with four LAB isolates (selected from the first stage) as treatments and five replications. The variables observed were resistance to gastric pH (pH 2.5) for 3 and 6 hours, resistance to 0.3% and 0.6% bile salts, intestinal hydrophobicity, and inhibition against pathogenic bacteria. Lactic acid bacteria candidates were selected based on TOPSIS analysis, then characterized morphologically and identified molecularly. **The third stage** was the application of LAB as a producer of probiotic cellulase and phytase (selected in the second stage) in broilers receiving rations containing SMW. The method used was an experimental method. The design used was a factorial CRD with two factors and three replications. Factor A was the percentage of SMW use in broiler rations (A1: 15% ASK, A2: 20% ASK and A3: 25% ASK) and factor B was the probiotic dose (B1: 3x108 CFU/mL; B2: 3x1010 CFU/mL and B3: 3x1012 CFU/mL). The variables observed were LAB colonies in the small intestine, cellulase and phytase in the small intestine, intestinal morphology (villus height, villus width and ratio of villus height to crypt depth), growth performance (feed consumption, body weight

gain, feed conversion), carcass performance (live weight, abdominal fat percentage, weight of internal organs (liver, node, heart, proventriculus, ventriculus, pancreas), carcass weight, carcass percentage, and lower thigh meat cholesterol.

The results of the first stage of research obtained 56 purified bacterial isolates. Next, LAB testing was carried out using MRSA + $CaCO_3$, resulting in 21 LAB isolates obtained based on the formation of clear zones. The isolates obtained were then screened for cellulase and phytase production qualitatively and quantitatively. Qualitative screening was carried out to detect isolates capable of producing extracellular cellulase and phytase based on the formation of clear zones and continued with quantitative screening. Quantitative screening of cellulase and phytase was carried out to determine the concentration of extracellular cellulase and phytase enzymes. A total of 13 isolates from 21 LAB isolates were able to produce cellulase and phytase, but 4 isolates were selected that produced the highest cellulase and phytase based on TOPSIS analysis, namely isolates AS15, AS21, AS28 and AS44.

The results of the second phase of the study obtained, the AS15 isolate had a very significant effect ($P<0.01$) higher than AS21, AS28 and AS44 on the probiotic test, namely seen from the resistance of AS15 to gastric pH of 94.44% after 3 hours and 90.86% after 6 hours, resistant to 0.3% (88.07%) and 0.5% (81.24%) bile salts, has hydrophobicity towards the intestine (91.24±0.429), inhibition zone against the growth of pathogenic bacteria namely *Escherichia coli* (22.25 mm), *Salmonella enteritidis* (13.30 mm), *Staphylococcus aureus* (15.15 mm). Based on the TOPSIS analysis, the AS15 isolate is a candidate that has the highest preference value as a probiotic and is identified molecularly as *Lactobacillus casei* AS15.

The results of the third stage of the study showed a very significant interaction ($P<0.01$) between the probiotic dose and the level of ASK administration in broiler rations on feed consumption, body weight gain, FCR, live weight, abdominal fat percentage, carcass weight, cholesterol in broiler chicken thigh meat, LAB colonies in the small intestine, cellulase and phytase in the small intestine, villus height, villus width and villus height ratio, crypt depth and no interaction ($P>0.05$) on the weight of physiological organs (liver, gallbladder, heart,

proventriculus, ventriculus, pancreas). The combination of probiotic doses of *L. casei* AS15 ($3x10^{12}$ CFU/mL/ 12.48 log CFU/g) with a 25% SMW level in broiler rations provided optimal results seen from the increase in LAB colonies, cellulase and phytase in the small intestine, small intestine morphology, growth performance, carcass performance and a decrease in cholesterol in broiler thigh meat.

The conclusion of the first stage of this study was that from 56 purified bacterial isolates, 21 LAB isolates were obtained, and 13 of them were able to produce cellulase and phytase. Based on TOPSIS analysis, the four best isolates were selected, namely AS15, AS21, AS28, and AS44. The conclusion of the second stage was that the AS15 isolate showed the best probiotic characteristics compared to AS21, AS28, and AS44. This isolate had high resistance to gastric acid and bile salt conditions, high hydrophobicity, and strong antagonistic activity against pathogenic bacteria. The AS15 isolate was identified as *Lactobacillus casei* AS15. The conclusion of the third stage was that the combination of probiotic doses of *L. casei* AS15 ($3x10^{12}$ CFU/mL/12.48 log CFU/g) with SMW level of 25% in the broiler ration provided optimal results seen from LAB colonies in the duodenum 11.45 log CFU/g; jejunum 11.41 log CFU/g; ileum 11.44 log CFU/g; cellulase in duodenum 22.54 U/mL; jejunum 18.75 U/mL; ileum 25.90 U/mL, phytase in duodenum 9.16 U/mL; jejunum 8.56 U/mL; ileum 6.24 U/mL, villus height in duodenum 1521.66 µm; jejunum 1276.51 µm; ileum 963.31 µm; villus width in duodenum 231.95 µm; jejunum 222.86 µm; ileum 237.96 µm, ratio of villus height to crypt depth in duodenum 15.42; jejunum 11.88; ileum 9.14), increased growth performance (feed consumption 116.08 g/head/day, body weight gain 68.62 g/head/day, feed conversion 1.69), increased carcass performance (live weight 1755.67 g, abdominal fat percentage 0.93%, does not interfere with physiological organ weight, carcass weight 1397 g, carcass percentage 74.10%) and decreased thigh meat cholesterol 95.32 mg/100 g.

Keywords: Broiler, LAB, *L. casei* AS15, Probiotic, Soy milk waste