UNIVERSITI PUTRA MALAYSIA

EFFECTS OF FEEDING METABOLITES FROM *LACTOBACILLUS PLANTARUM* STRAINS ON LIPID METABOLISM, GUT MORPHOLOGY AND GROWTH PERFORMANCE OF BROILER CHICKENS

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By

NGUYEN TIEN THANH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

July 2008

DEDICATION

Dedicated to my Dearest Mother, my beloved family and my brother and sisters

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

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Chairman: **Associate Professor Loh Teck Chwen, PhD**

Faculty: **Agriculture**

Four experiments were conducted to study the effects of feeding metabolite combinations produced from six strains of *L. plantarum* on the performance of broiler chickens. The inhibitory activity of different combinations of metabolites produced by locally isolated *L. plantarum* against various pathogens was studied in the first experiment. Sixty-three combinations of metabolites obtained from 6 strains of *L. plantarum*: UL4, TL1, RS5, RI11, RG14 and RG11 were equally and homogenously mixed. The inhibitory activity was then determined against 5 selected indicators, which are *E. coli*, *L. monocytogenes*, *S. typhimurium*, Vancomycin resistant enterococci (VRE) and *Pediococcus acidilactici.* The inhibitory activity was measured based on the diameter of inhibitory zone. Four combinations with the highest inhibitory scores were identified. The combination of four strains RS5, RI11, RG14 and RG11 has given the highest score, followed by the combinations of TL1, RG14 and RG11 strains, combinations of TL1, RI11 and RG11 strains, and combinations of TL1, RS5, RI11 and RG14 strains. These results indicate that

different combinations of metabolites had different antibacterial activity, which could be used in food and feed industries. Combinations of different metabolites further enhance the antimicrobial activity.

Four combinations of metabolites with the highest inhibitory activity were used in the following experiment to study the performance of broiler chickens. A total of 432 day-old male Ross broiler chicks were raised to 42 days of age in deep litter system. Each pen consisted of 12 chicks and was randomly allocated to the open house with wood shavings litter. The birds were vaccinated (IB-ND live vaccine, Fort Dodge, USA) against infectious bronchitis (IB) and Newcastle disease (ND). The birds were also vaccinated with IBD vaccine (MyVac UPM93, Malaysia) against infectious bursal disease (IBD) on day 14. Wing band was applied to all of the birds for identification. Water and feed were provided *ad libitum*. The feed intake and body weight were recorded weekly. The starter and finisher diets were offered to the birds from 0 - 21 and 22 - 42 days of age, respectively. The dietary treatments consisted of: (i) corn-soybean based diet without antibiotic (-ve control) diet; (ii) basal diet with neomycin and oxytetracyclin (+ve control); (iii) basal diet supplemented with 0.3% metabolite combinations of *L. plantarum* RS5, RI11, RG14 and RG11 (Com3456); (iv) basal diet supplemented with 0.3% metabolite combinations of *L. plantarum* TL1, RG14 and RG11 (Com256); (v) basal diet supplemented with 0.3% metabolite combinations of *L. plantarum* TL1, RI11 and RG11 (Com246); (vi) basal diet supplemented with 0.3% metabolite combinations of *L. plantarum* TL1, RS5, RG14 and RG11 (Com2456). Greater final body weight (BW), weight gain (WG), average daily gain (ADG), volatile fatty acids (VFA) and lower feed conversion ratio (FCR) were significantly ($p<0.05$) found in the birds fed with the 4 metabolite combinations.

Supplementation of metabolites combination also increased faecal lactic acid bacteria (LAB) population, villi height of small intestine and lowered plasma and meat cholesterol and faecal *Enterobacteriaceae* (ENT) population.

In the third experiment, the effect of feeding different dosages of Com3456 obtained from the second experiment on the performance of broiler chickens was studied. A total of 504 day-old male Ross broiler chicks were grouped into 7 treatments and offered with different diets: (i) standard corn-soybean based diet (negative control); (ii) standard corn-soybean based diet + neomycin and oxytetracycline (positive control); (iii) standard corn-soybean based diet + 0.1% metabolite combination of *L. plantarum* RS5, RI11, RG14 and RG11 strains (Com3456); (iv) standard corn-soybean based diet + 0.2% of Com3456; (v) standard corn-soybean based diet + 0.3% of Com3456 (vi) standard corn-soybean based diet + 0.4% of Com3456 and (vii) standard corn-soybean based diet + 0.5% of Com3456. Supplementation of Com3456 with different dosages improved growth performance, reduced ENT and increased LAB count, lowered plasma and meat cholesterol, and increased villi height of small intestine and volatile fatty acids (VFA). Nevertheless, abdominal fat deposition was not affected by Com3456 metabolites. Only slight increase of antibody titers against Newcastle disease, infectious bronchitis and infectious bursal disease was found in those birds supplemented with the metabolites. Treatment with 0.2% Com3456 had the best results with minimal cost, especially in terms of growth performance, FCR and plasma and meat cholesterol reduction among other dosages. These results indicate that 0.2% is optimum dosages to be included in the diets of broiler chickens in order to replace antimicrobial growth promoters (AGP).

In the last experiment, the very low-density lipoprotein (VLDL) lipid profiles, intestinal LAB count and bile salts deconjugation of LAB were studied. The results showed that metabolite combinations supplemented in broilers feed reduced free cholesterol and cholesterol esters in VLDL particles and increased LAB count in digesta of small intestine. The results also suggest that LAB are able to deconjugate bile salts and lead to the increase of cholesterol utilization for synthesizing new conjugated bile salts. These properties of metabolite combinations in the diets of broiler chickens contribute to the reduction of cholesterol in plasma and meat.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PEMBERIAN METABOLIT DARIPADA STRAIN *LACTOBACILLUS PLANTARUM* TERHADAP METABOLISME LIPID, MORPHOLOGI SALUR GASTRO-USUS DAN PRESTASI PERTUNBAHAN AYAM PEDAGING

Oleh

NGUYEN TIEN THANH

July 2008

Pengerusi: **Profesor Madya Loh Teck Chwen, PhD**

Fakulti: **Pertanian**

Empat eksperimen telah dijalankan untuk mengkaji kesan kombinasi metabolit yang dihasilkan daripada 6 strain *L. plantarum* ke atas prestasi ayam pedaging. Aktiviti perencat daripada kombinasi metabolit berlainan yang dihasilkan melalui pemencilan *L. plantarum* terhadap pelbagai patogen telah dikaji dalam eksperimen pertama. Enam puluh tiga kombinasi metabolit diperolehi daripada enam strain *L. plantarum*: UL4, TL1, RS5, RI11, RG14 dan RG11 yang dicampur secara sekata dan homogenus. Aktiviti perencat telah dijalankan ke atas 5 petunjuk terpilih, iaitu *E. coli*, *L. monocytogenes*, *S. typhimurium*, Vancomycin resistan enterococci (VRE) dan *Pediococus acidilactici.* Aktiviti perencat ditindakan berdasarkan kepada diameter zon perencat. Empat kombinasi metabolit dengan skor perencat tertinggi telah dikenal pasti. Kombinasi bagi empat strain RS5, RI11, RG14 dan RG11 menunjukkan skor tertinggi, diikuti dengan kombinasi strain TL1, RG14 dan RG11, kombinasi strain TL1, RGI11 dan RG11, dan kombinasi strain TL1, RS5, RI11 dan RG14. Keputusan ini menunjukkan perbezaan kombinasi metabolit yang berbeza

menyebabkan berbezaan aktiviti antibakteria, maklumat maklumat ini boleh diguna pakai dalam industri makanan manusia dan makanan haiwan. Pada umunia kombinasi metabolit ini akan mempertingkatkan aktiviti antimikrob.

Empat kombinasi metabolit dengan aktiviti perencat tertinggi telah digunakan dalam eksperimen kedua untuk mengkaji prestasi ayam pedaging. Sejumlah 432 ekor ayam pedaging Ross jantan yang diperoleh dari sebuah syarikat tempatan telah diternak dari umur satu hari hingga 42 hari menggunakan sistem sarap. Setiap reban terdiri daripada 12 anak ayam yang ditempatkan secara rawak dalam sistem rumah terbuka dengan menggunakan sarap tebal berasaskan habuk kayu. Ayam disuntikkan dengan vaksin (IB-ND vaksin hidup, Fort Dodge, USA) IBD dan ND. Ayam pedaging ini juga diberikan vaksin IBD (MyVac UPM93, Malaysia) pada hari ke-14. 'Wing banding' juga dikenakan kepada semua ayam bagi memudahkan pengecaman. Air dan makanan dibekalkan secara *ad libitum*. Makanan dan berat badan ditimbang setiap minggu. Makanan permulaan dan penghabisan masing-masing diberikan kepada ayam dari 0-21 dan 22-42 hari. Rawatan terdiri daripada: (i) diet berasaskan jagung-kacang soya tanpa antibiotik (kawalan -ve); (ii) diet ditambahkan antibiotik (kawalan +ve); (iii) diet asas ditambahkan dengan 0.3% kombinasi metabolit *L. plantarum* RS5, RI11, RG14 dan RG11 (Com3456); (iv) diet asas ditambahkan dengan 0.3% kombinasi metabolit *L. plantarum* TL1, RG14 dan RG11 (Com 256); (v) diet asas ditambahkan dengan 0.3% kombinasi metabolit *L. plantarum* TL1, RI11 dan RG11 (Com 246) dan (vi) diet asas ditambahkan dengan 0.3% kombinasi metabolit *L. plantarum* TL1, RI11, RG14 dan RG11 (Com 2456). Kelebihan berat badan akhir, pertambahan berat badan, purata pertambahan berat harian, VFA dan kadar pertukaran makanan yang lebih rendah diperoleh daripada kumpulan ayam

yang diberikan metabolit. Pemberian kombinasi metabolit juga meningkatkan populasi bakteria asid laktik tinja, ketinggian vilus usus kecil di samping merendahkan kolesterol plasma dan populasi ENT tinja.

Dalam eksperimen ketiga, kesan dos Com3456 yang berlainan yang diperolehi daripada eksperimen kedua telah dikaji ke atas prestasi ayam pedaging. Sejumlah 504 ekor ayam pedaging Ross jantan dibahagikan kepada 7 kumpulan melalui dan diberikan rawatan pemberian diet yang berbeza seperti berikan: (i) diet kawalan berasaskan jagung-kacang soya (kawalan negatif); (ii) diet kawalan berasaskan jagung-kacang soya + neomycin dan oxytetracyclin (kawalan positif); (iii) diet kawalan berasaskan jagung-kacang soya + 0.1% kombinasi metabolit *L. plantarum* strain RS5, RI11, RG14 dan RG11 (Com3456); (iv) diet kawalan berasaskan jagung-kacang soya + 0.2% kombinasi metabolit Com3456; (v) diet kawalan berasaskan jagung-kacang soya + 0.3% kombinasi metabolit Com3456; (vi) diet kawalan berasaskan jagung-kacang soya + 0.4% kombinasi metabolit Com3456 dan (vii) diet kawalan berasaskan jagung-kacang soya + 0.5% kombinasi metabolit Com3456. Pemberian Com3456 yang berlainan dos meningkatkan prestasi pertumbuhan, merendahkan ENT dan meningkatkan bilangan LAB, merendahkan kolesterol plasma dan daging, serta meningkatkan ketinggian vilus usus kecil dan VFA. Walau bagaimana pun, pertambahan lemak abdomine tidak dipengaruh oleh metabolit. Hanya sedikit peningkatan pada antibodi titer ”Newcastle disease”, ”infection bronchitis” dan ”infectious bursal disease” dijumpai pada ayam yang diberikan metabolit. Rawatan dengan 0.2% Com3456 memberi keputusan yang terbaik, terutamanya dari segi prestasi pertumbuhan, kadar pertukaran makanan dan penurunan kolesterol plasma dan daging dibandingkan dengan dos lain. Keputusan

menunjukkan bahawa 0.2% merupakan optimum untuk menggantikan AGP di dalam diet ayam pedaging.

Dalam eksperimen terakhir, profil lemak VLDL, bilangan LAB dalam usus dan ”bile salt deconjugation” oleh LAB telah dikaji. Keputusan menunjukkan kombinasi metabolit dalam makanan ayam pedaging dapat merendahkan ”free cholesterol” dan ”cholesterol ester” di dalam VLDL serta meningkatkan LAB dalam ”digesta” usus kecil. Keputusan ini juga mencadangkan LAB berupaya untuk ”menyahkonjugat bile salts” serta menggalakkan penggunaan kolesterol untuk mensitesiskan ”bile salts” konjugat baru. Kandungan daripada kombinasi metabolit dalam diet ayam pedaging menyumbang kepada penurunan kolesterol dalam plasma dan daging.

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I certify that an Examination Committee met on 28 July, 2008 to conduct the final examination of Nguyen Tien Thanh on his PhD of Agriculture Science thesis entitled "Effects of Feeding Metabolites from *Lactobacillus Plantarum* Strains on Lipid Metabolism, Gut Morphology and Growth Performance of Broiler Chickens" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 a Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment of the requirements for the degree of PhD of Agriculture Science. The members of the Supervisory Committee were as follows:

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Date: 11 September 2008

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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NGUYEN TIEN THANH
Date:

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3βHSD	3β-hydroxysteroid dehydrogenase/isomerase
4MV	4-methyl-valeric acid
ADG	Average daily gain
ADP	Adenosine diphosphate
AGP	Antimicrobial growth promoters
AKR1C4	3α-hydroxysteroid dehydrogenase
AKR1D1	Δ^{4-3}-oxosteroid-5β-reductase
Apo	Apolipoprotein
ATP	Adenosine triphosphate
AU	Arbitrary unit
BSH	Bile salt hydrolase
BW	Body weight
CA	Cholic acid
CDCA	Chenodeoxycholic acid
CE	Cholesterol esters
CFS	Cell-free supernatant
CFU	Colony-forming unit
Com3456	Metabolite combination from *L. plantarum* RS5, RI11, RG14 and RG11
Com246	Metabolite combination from *L. plantarum* TL1, RI11 and RG11
Com2456	Metabolite combination from *L. plantarum* TL1, RI11, RG14 and RG11
Com256	Metabolite combination from *L. plantarum* TL1, RG14 and RG11
CYP27A1	Sterol 27-hydroxylase