UNIVERSITI PUTRA MALAYSIA

STUDIES ON THE MECHANISMS AND UTILIZATION OF PROBIOTICS (DIRECT-FED MICROBIALS) IN BROILERS

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STUDIES ON THE MECHANISMS AND UTILIZATION OF PROBIOTICS (DIRECT-FED MICROBIALS) IN BROILERS

By

JIN LIZHI

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STUDIES ON THE MECHANISMS AND UTILIZATION OF PROBIOTICS (DIRECT-FED MICROBIALS) IN BROILERS

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May 1996

Chairman: Prof. Dato' Dr. SYED JALALUDIN SYED SALIM

Faculty : Veterinary Medicine and Animal Science

A series of experiments were conducted to isolate and characterize suitable intestinal microbes as direct-fed microbials and to investigate their effects on broiler and the mechanisms associated with their utilization.

The major types of bacteria found in the duodenum and jeju-ileum of chicken were *Lactobacillus*, *Streptococcus* and *E. coli*. In the cecum, ten groups of bacteria, namely, *Streptococcus*, *Staphylococcus*, *Lactobacillus*, *E. coli*, anaerobic coccus, *Eubacterium*, *Propionibacterium*, *Clostridium*, *Fusobacterium* and *Bacteroides* were isolated and identified. The main *Lactobacillus* species in chicken intestine were *L. acidophilus*, *L. fermentum* and *L. brevis*

In vitro adhesion study demonstrated that 12 out of the 46 intestinal *Lactobacillus* isolates showed moderate to good ability to adhere to the ileal epithelial

cell of chicken. Temperature (between 4°C and 42°C) did not have an effect on the attachment of the *Lactobacillus* isolates to the ileal epithelial cell. Incubation (contact) time of 30 min was found to be insufficient for the attachment of bacterial to the ileal epithelial cell, but contact time extended beyond 1 h did not improve the ability of the bacteria to adhere to the ileal epithelial cell. The pH values of suspending buffer for the *Lactobacillus* strains, ranging from 4 to 7, did not have any significant effect on the attachment of the bacteria to the ileal epithelial cell, but pH 8 reduced significantly ($P<0.05$) the attachment of bacteria. The growth of the 12 adherent *Lactobacillus* isolates was inhibited only slightly by the inclusion of 0.3% chicken bile in the culture medium, and they all showed a tolerance (in varying degrees) to low pH values (1.0 or 2.0).

Antagonistic study indicated that all the 12 adherent *Lactobacillus* isolates were able to inhibit the growth of the five strains of salmonella, viz., *S. enteritidis* 935/79, *S. pullorum*, *S. typhimurium*, *S. blockley* and *S. enteritidis* 94/448, and the three strains of *E. coli* (i.e. *E. coli* O1:K1, O2:K1 and O78:K80) in varying degrees in vitro. Inhibition of the pathogenic bacteria was probably due to the production of organic acids by the *Lactobacillus* isolates. In vitro study on competitive exclusion toward pathogenic bacteria showed that *L. acidophilus* I 26 significantly reduced ($P<0.05$) the attachment of *S. pullorum* to ileal epithelial cell in the tests for exclusion and competition but not in the displacement tests. *L. fermentum* I 25 was found to have some ability to reduce the attachment of *S. typhimurium* to ileal epithelial cell

under the conditions of exclusion ($P<0.08$), competition ($P<0.09$), but not displacement. However, both *L. acidophilus* I26 and *L. fermentum* I 25 were unable to reduce the adherence of *S. enteritidis* and three strains of *E. coli* to ileal epithelial cell under the conditions of exclusion, competition and displacement. In the in vivo experiment, treatment of chicks with *L. acidophilus* I 26 reduced the number of *S. pullorum* in the ileum and cecum 5 days after challenge with salmonella, and in the cecum 10 days after challenge. Treatment of chicks with a mixture of 12 adherent *Lactobacillus* isolates reduced significantly the mean number of *S. pullorum* in their duodenum, ileum and cecum 5 days after being challenged by salmonella, and in their cecum 10 days after challenge. Treatment with *L. acidophilus* I 26 did not reduce significantly the population of *E. coli* in the duodenum, ileum and cecum 5 days or 10 days after being challenged with *E. coli* O2:K1. On the other hand, there was a significant reduction in the number of *E. coli* in the duodenum, ileum and cecum of chicks treated with a mixture of *Lactobacillus* 5 days and 10 days after challenge.

All the 12 adherent *Lactobacillus* isolates were found to secrete amylase, protease and lipase, either extracellularly or intracellularly, or both extracellularly and intracellarly. Amylase activity in the lower part of small intestine increased when the adherent *Lactobacillus* cultures were fed to the broilers but there was no effect on the activity when commercial lactobacilli were fed. In general, lypolytic and proteolytic activities in the lower small intestine were not affected by feeding either adherent *Lactobacillus* or commercial lactobacilli and *B. subtilis* to the broilers. Duodenal

amylolytic, lypolytic and proteolytic activities were also not affected by the supplementation of either adherent or commercial *Lactobacillus* cultures The bacterial enzyme, β-glucuronidase, in the small intestine and feces was reduced when *L. acidophilus* was fed to the chickens The enzyme activity was also significantly lower in the intestine of birds fed a diet with a mixture of *Lactobacillus* Fecal β-glucosidase activity was lower (P<0 05) in birds fed diets with adherent *Lactobacillus* cultures, but the intestinal enzyme levels were not affected There was no effect on either intestinal or fecal β-glucuronidase and β-glucosidase activities in chicks fed diets with commercial lactobacilli or *B subtilis* cultures

The results of feeding trials showed that chicks fed either a single strain of *L acidophilus* or a mixture of *Lactobacillus* cultures had significantly more body weight gain and better feed conversion rate than control chicks 6 weeks after feeding The birds fed diets with commercial lactobacilli or *B subtilis* for 4 weeks also grew faster than the control, but the incorporation of lactobacilli in the drinking water did not affect the growth of the chicks Supplementing *L. acidophilus* or a mixture of *Lactobacillus* cultures to the diets of chicks did not affect the weights of liver, spleen, bursa, gizzard, duodenum, jeju-ileum and total small intestine 42 days after feeding The weights of the liver, spleen and bursa were also not affected by the incorporation of commercial lactobacilli or *B subtilis* to the diets

Abstrak disertasi yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi syarat untuk mendapatkan ijazah Doktor Falsafah

KAJIAN MENGENAI MEKANISME BERKAITAN DENGAN PENGGUNAAN PROBIOTIK (MIKROB MAKANAN) DALAM AYAM DAGING

Oleh

JIN LIZHI

Mei 1996

Pengerusi : Prof. Dato' Dr. SYED JALALUDIN SYED SALIM

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Satu siri eksperimen telah dilakukan untuk mengasing dan mencirikan mikrob usus yang sesuai untuk digunakan sebagai mikrob makanan dan menyelidik kesan mereka terhadap ayam daging serta mekanisme berkaitan dengan penggunaannya.

Jenis bakteria utama dalam duodenum dan jeju-ileum ayam ialah *Lactobacillus, Streptococcus* dan *Escherichia.* Dalam sekum, sepuluh kumpulan bakteria iaitu, *Streptococcus, Staphylococcus, Lactobacillus, E. coli,* kokus anaerobik, *Eubacterium, Propionibacterium, Clostridium, Fusobacterium* dan *Bacteriodes* telah diasing dan dikenalpasti. Spesies utama *Lactobacillus* dalam usus ayam ialah *L. acidophilus, L. fermentum* dan *L. brevis.*

Kajian penglekatan *in vitro* menunjukkan 12 dari 46 isolat *Lactobacillus* dari usus melekat dengan baik ke sel ileal epitelia ayam. Suhu (antara 4°C dan 42°C)

tidak menjejaskan penglekatan isolat *Lactobacillus* ke sel ileal epitelia. Masa pengeraman (sentuhan) selama 30 min didapati tidak cukup untuk penglekatan bakteria ke sel ileal epitelia, tetapi masa melebihi 1 j tidak meningkatkan keupayaan bakteria melekat pada sel ileal epitelia. Nilai pH pemampan untuk strain *Lactobacillus* dalam jarak 4 ke 7, tidak memberi kesan bererti terhadap penglekatan bakteria ke sel ileal epitelia, tetapi pH 8 merendahkan secara bererti ($P< 0.05$) penglekatan bakteria. Pertumbuhan 12 isolat *Lactobacillus* melekat direncat sedikit oleh 0.3% hempedu ayam dalam media kultur dan kesemua menunjukkan ketahanan terhadap nilai pH yang rendah (1.0 ke 3.0).

Kajian antagonistik menunjukkan kesemua 12 isolat *Lactobacillus* melekat boleh merencat pertumbuhan lima strain Salmonella iaitu *S. enteritidis* 935/79, *S. pullorum, S. typhimurium, S. blockley* dan *S. enteritidis* 94/448 dan ketiga strain *E. coli* iaitu *E.coli* (O1:K1, O2:K1 dan O78:K80) dengan tahap yang berbeza *in vitro*. Perencatan bakteria patogenik mungkin kerana penghasilan asid organik oleh isolat *Lactobacillus*. Kajian *in vitro* mengenai penyingkiran bersaingan terhadap bakteria patogenik menunjukkan yang *L. acidophilus* I 26 secara bererti ($P<0.05$) menurunkan pengelekatan *S. pullorum* ke sel ileal epitelia dalam ujian penyingkiran dan persaingan tetapi tidak dalam ujian penggantian. *L. fermentum* I 25 dilihat mempunyai keupayaan menurunkan penglekatan *S. typhimurium* ke sel ileal epitelia di dalam keadaan penyingkiran ($P<0.08$), persaingan ($P<0.09$), tetapi tidak untuk penggantian. Walau bagaimanapun, kedua *L. acidophilus* I 26 dan *L. fermentum* I 25 tidak

berupaya untuk menurunkan penglekatan *S. enteritidis* dan ketiga strain *E. coli* ke sel ileal epitelia di dalam keadaan penyingkiran, persaingan dan penggantian. Dalam kajin *in vivo*, rawatan anak ayam dengan *L.acidophilus* I 26 menurunkan jumlah *S. pullorum* dalam ileum dan sekum selepas lima hari dicabar dengan salmonela dan dalam sekum selepas 10 hari dicabar. Anak ayam yang dirawat dengan campuran 12 isolat *Lactobacillus* melekat menunjukkan penurunan secara bererti min jumlah *S. pullorum* dalam duodenum, ileum dan sekumnya selepas lima hari dicabar dengan salmonela, dan dalam sekum selepas 10 hari dicabar. Rawatan dengan *L. acidophilus* I 26 tidak menurunkan populasi *E. coli* dalam duodenum, ileum dan sekum 5 atau 10 hari selepas dicabar dengan *E. coli* O2:K1. Sebaliknya, terdapat penurunan yang bererti ($P < 0.05$) dalam jumlah *E. coli* dalam duodenum, ileum dan sekum anak ayam yang dirawat dengan campuran *Lactobacillus* selepas 5 dan 10 hari dicabar.

Kesemua 12 isolat *Lactobacillus* melekat didapati merembeskan amilase, protease dan lipase samada secara ekstrasel atau intrasel atau kedua-duanya. Aktiviti amilase dalam bahagian bawah usus kecil meningkat bila kultur *Lactobacillus* melekat dimakan oleh ayam daging, tetapi kesan ini tidak dilihat bila laktobasili komersial digunakan. Secara am, aktiviti lipolitik dan proteolitik di bahagian bawah usus kecil tidak terjejas oleh *Lactobacillus* melekat atau laktobasili komersial *dan B. subtilis* bila dimakan oleh ayam daging. Aktiviti amilolitik, lipolitik dan proteolitik tidak juga terjejas oleh penambahan kultur *Lactobacillus* melekat atau komersial. Aktiviti enzim β-glukoronidase bakteria, dalam usus kecil dan tahi dikurangkan bila

L. acidophilus dimakan oleh ayam. Aktiviti enzim juga rendah secara bererti ($P<0.05$) dalam usus ayam yang diberi diet mengandungi campuran *Lactobacillus*. Aktiviti β-glukosidase tahi lebih rendah ($P<0.05$) dalam ayam yang diberi kultur *Lactobacillus* melekat, tetapi tahap enzim usus tidak terjejas. Tiada kesan terhadap aktiviti β-glukoronidase dan β-glukosidase usus atau tahi dalam anak ayam yang diberi diet mengandungi laktobasili komersial atau kultur *B. subtilis*.

Hasil kajian pemberian makanan menunjukkan anak ayam bila diberi makan satu strain *L. acidophilus* atau satu campuran kultur *Lactobacillus* mempunyai peningkatan berat badan yang lebih dan kadar penukaran makanan yang lebih baik bila dibandingkan dengan anak ayam kawalan selepas 6 minggu percubaan. Ayam yang diberi makan laktobasili komersial atau *B. subtilis* selama 4 minggu juga membesar lebih cepat dari ayam kawalan, tetapi pencampuran laktobasili dalam air minuman tidak mempengaruhi pembesaran anak ayam. Penambahan *L. acidophilus* atau satu campuran kultur *Lactobacillus* ke diet anak ayam tidak mempengaruhi berat hati, limpa, bursa, tembolok, duodedum, jeju-ileum dan keseluruhan usus kecil 42 hari selepas pemberian makanan. Berat hati, limpa dan bursa juga tidak dipengaruhi oleh campuran laktobasili komersial atau *B. subtilis* ke dalam diet.