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PROBIOTIC EFFECTS OF *LACTOBACILLUS* STRAINS ON CECAL MICROBIOTA AND FERMENTATION, INTESTINAL DEVELOPMENT AND RESPONSE TO HUMAN CONTACT IN BROILER CHICKENS

AMIR MEIMANDIPOUR

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By

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March 2010

Chairman: Professor Mohd Yazid Abdul Manap, PhD

Faculty: Food Science and Technology

The aim of this study was to evaluate the probiotic effects of *Lactobacillus agilis* JCM 1048 and *Lactobacillus salivarius* subsp*. salicinius* JCM 1230 under *in vitro* and *in vivo* conditions. In the first *in vitro* experiment, batch bioreactor system was used to assess the effects of two *Lactobacillus* strains on selected bacterial groups, short chain fatty acids (SCFAs) and lactate concentrations. In this experiment, 24 h incubation was done in a simulated chicken cecum, at two pH values. The addition of *L. agilis* JCM 1048 and *L. salivarius* subsp*. salicinius* JCM 1230 significantly ($P<0.05$) increased number of total anaerobes (19%), lactobacilli (18%) and bifidobacteria (27%) at pH 5.8 while decreased those of aerobes (pH=5.8, 13% & pH=6.7, 9%) and streptococci (22% in both pH values) in (Cc+L) treatment group after 24 h incubation as compared to (Cc) group (Table 3.1). The supplementation with lactobacilli was found to increase the

production of lactate, propionate and butyrate (pH=5.8, 256% & pH=6.7, 246%), while suppressed the concentration of acetate (Table 3.3). Furthermore, pH did not change the formation of butyrate, while the production of acetate and propionate decreased at pH 5.8. The second *in vitro* experiment was also conducted using a batch bioreactor. The microbial groups were determined by using real time polymerase chain reaction (PCR) and SCFAs concentrations were monitored over 24 h batch culture incubation at pH 5.8. The supplementation of cecal content with two strains of *Lactobacillus* significantly ($P<0.05$) increased the number of lactobacilli (8%), bifidobacteria (10%) and *Faecalibacterium prausnitzii* (10%) compared to (Cc+L) group (Table 4.6). *Lactobacillus* supplementation did not affect ($P<0.05$) the number of *E.coli* and *Clostridium butyricum*; however, it significantly ($P<0.05$) decreased those of *Salmonella* (14%). Inclusion of *Lactobacillus* in the vessel containing cecal material significantly ($P<0.05$) increased the production of propionate and butyrate (261%), whereas decreased the amount of acetate (Table 4.8).

The *in vivo* study was conducted to assess the effects of two *Lactobacillus* strains on broiler performance, small intestine development and SCFAs concentration in various segments of the GIT in the chickens under unpleasant physical contact (UPC). The following treatments were applied from day one: (1) chicks exposed to normal human contact fed basal diet

(control); (2) chicks were exposed to UPC and fed basal diet (UPC-BD); (3) chicks were exposed to UPC and fed basal diet supplemented with *Lactobacillus* (UPC-BDL). From day 1 to day 21, the chicks in UPC groups were caught using both hands, placed in plastic crates and moved to a separate room. The chicks were picked up in a group, suspended by legs and swung gently for 30 s once daily. Unpleasant physical contact significantly ($P<0.05$) increased blood plasma concentration in UPC-BD and UPC-BDL groups, respectively 37% and 27% at 2 weeks of age (Table 5.2). Broilers in UPC-BDL treatment group showed significantly ($P<0.05$) higher body weight gain (10%) and better feed conversion ration (7.4%) as compared with UPC-BD treatment group at 7 days of age (Table 5.4). Duodenal villi height was numerically suppressed (7.4%) in UPC-BD treatment as compared with the control group at 14 days of age (Table 5.5). Dietary *Lactobacillus* profoundly increased (11%) duodenal villi height in UPC-BDL birds at 28 days of age. Broilers in UPC-BD treatment showed a significant ($P<0.05$) lower concentration of duodenal lactate (22%), ileal acetate (51%), cecal lactate (16%), cecal acetate (41%), and cecal butyrate (72%) as compared to the control groups at 14 days of age (Table 5.9). However, lactobacilli administration significantly ($P<0.05$) increased the concentration of duodenal lactate (21%), duodenal propionate (23%), cecal lactate (16%), and cecal butyrate (112%) in UPC-BDL treatment group compared to UPC-BD counterparts at similar age. Lactobacilli

supplementation profoundly suppressed ileal and cecal acetate concentration, respectively 51% and 42% in 14 days UPC-BDL treatment group compared with UPC-BD birds. Both duodenal and jejunal pH values significantly ($P<0.05$) increased (6%) in UPC-BD treatment compared with the control group at 14 days of age (Table 5.14). Adding *Lactobacillus* to the diet, significantly ($P<0.05$) lowered duodenal and jejunal pH values in UPC-BDL group as compared with UPC-BD group at 14 and 28 days of age.

In conclusion, the results of the current study suggested that *L. agilis* JCM 1048 and *L. salivarius* subsp. *salicinius* JCM 1230 have the ability to re-establish proper microbial balance in the chicken cecum. *Lactobacillus* supplementation profoundly reduced total number of aerobes, S*treptococcus*, and *Salmonella* in favor of lactobacilli, bifidobacteria, and *F. prausnitzii* which was coincided by high formation of lactate, butyrate as well as propionate. Although, UPC was not too aversive to decrease broiler performance, but it negatively affected duodenal structural growth in broilers of UPC-BD group at 14 days of age. Furthermore, UPC negatively changed the GIT bacterial metabolic end products during unpleasant handling. Dietary supplementation with *Lactobacillus* ameliorated detrimental effects of UPC on broilers over the course of the experimental period.

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

KESAN PROBIOTIK STRAIN *LACTOBACILLUS* PADA CECAL MIKROBIOTA DAN FERMENTASI, PERKEMBARGAN USUS KECIL DAN RESPON AYAM PEDAGING TERHADAP MANUSIA

Oleh

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Mac 2010

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Tujuan kajian ini adalah untuk menilai kesan-kesan probiotik *Lactobacillus agilis* JCM 1048 dan *L. salivarius* subsp*. salicinius* JCM 1230 dalam keadaan *in vitro* dan *in vivo*. Dalam eksperimen *in vitro* yang pertama, sistem bioreaktor berkelompok telah digunakan untuk menilai pengaruh dua strain *Lactobacillus* terhadap kumpulan bakteria yang terpilih, asid lemak rantai pendek (SCFAs) dan kepekatan laktat. Dalam kajian ini, inkubasi selama 24 jam dalam simulasi cecum ayam telah dijalankan pada dua nilai pH. Penambahan *L. agilis* JCM 1048 dan *L. salivarius* subsp. *salicinius* JCM 1230 telah meningkatkan jumlah bilangan koloni pada keseluruhan anaerob (19%), *Lactobacilli* (18%) dan *Bifidobakteria* (27%) pada pH 5.8 dan juga mengurangkan bilangan aerob (pH=.58, 13% dan pH=6.7, 9%) dan *Streptococci* (22% pada kedua-dua nilai pH) dalam kumpulan rawatan (Cc+L) selepas inkubasi selama 24 jam jika dibandingkan kepada

kumpulan (Cc). Penambahan *Lactobacilli* telah menunjukkan peningkatan terhadap penghasilan laktat, propionat dan butyrate (pH=5.8, 256% and pH=6.7, 246%) dan merendahkan kepekatan asetat. Tambahan lagi, pH tidak mengubah pembentukan butirat, manakala penghasilan asetat dan propionat menurun pada pH 5.8. Eksperimen *in vitro* yang kedua juga dijalankan dengan menggunakan bioreaktor berkelompok. Kumpulan mikrob ditentukan dengan kaedah 'real time' Polymerase Chain Reaction (PCR) dan kepekatan asid lemak rantai pendek (SCFAs) dipantau selepas 24 jam inkubasi kultur berkelompok pada pH 5.8. Penambahan dua strain *Lactobacillus* dalam kandungan cecal telah meningkatkan bilangan *Lactobacillus* (8%), *Bifidobacteria* (10%) dan *Faecalibacterium prausnitzii* (10%) secara signifikan ($P<0.05$) berbanding kumpulan kawalannya (Cc+L). Penambahan *Lactobacillus* tidak mempengaruhi ($P<0.05$) bilangan *E.coli* dan *Clostridium butyricum*; bagaimanapun, ia mengurangkan bilangan *Salmonella* (14%) secara signifikan ($P<0.05$). Rangkuman *Lactobacillus* di dalam vessel mengandungi material cecal telah meningkatkan penghasilan propionat dan butyrat (261%) secara signifikan ($P<0.05$), bagaimanapun telah mengurangkan jumlah asetat.

Kajian secara *in vivo* telah dijalankan untuk menentukan kesan dua strain *Lactobacillus* terhadap prestasi ayam ternak, perkembangan usus kecil dan kepekatan SCFAs pada pelbagai segmen GIT pada ayam dibawah

interaksi fizikal tidak menyenangkan (UPC). Rawatan berikutnya dijalankan sejak hari pertama: (1) anak-anak ayam didedahkan kepada diet makanan asas dan interaksi dengan manusia secara biasa (kawalan); (2) anak-anak ayam didedahkan dengan UPC dan diet makanan asas (UPC-BD); (3) anak-anak ayam didedahkan kepada UPC dan diet makanan asas ditambah dengan *Lactobacillus* (UPC-BDL). Dari hari pertama sehingga hari ke 21, anak-anak ayam yang dalam kumpulan UPC ditangkap menggunakan dua belah tangan, seterusnya disimpan di dalam tong plastik dan dipindahkan ke bilik berasingan. Ayam tersebut di ambil secara berkumpulan, digantung pada bahagian kaki dan dihayun secara perlahan selama 30 saat sekali setiap sehari. UPC telah menyebabkan peningkatan secara signifikan 37% dan 27% pada kepekatan plasma darah pada kumpulan ayam rawatan UPC-BD dan UPC-BDL masing-masing pada umur yang ke-14 hari. Ayam ternakan dalam kumpulan UPC-BDL menunjukkan penambahan berat badan yang tinggi (10%) secara signifikan ($P<0.05$) dan pertukaran catuan makanan yang lebih baik (7.4%) jika dibandingkan pada kumpulan UPC-BD pada umur hari yang ke-7. Ketinggian vilus duodenal telah disekat dari segi bilangan (7.4%) di dalam ayam-ayam dalam kumpulan olahan UPC-BD jika dibandingkan pada kumpulan kawalan pada usia yang ke-14 hari. Diet *Lactobacillus* meningkatkan ketinggian vilus duodenal (11%) pada kumpulan ayam rawatan UPC-BDL pada usia 28 hari. Ayam- ayam ternak dalam rawatan

UPC-BD secara signifikan (*P*<0.05) mempunyai kepekatan rendah pada duodenal laktat (22%), ileal asetat (51%), cecal laktat (16%), cecal asetat (41%), dan cecal butirat (72%) jika dibandingkan pada kumpulan kawalan pada usia yang ke-14 hari. Bagaimanapun, perlaksanaan lactobacilli dalam kajian ini secara signifikan telah meningkatkan kepekatan duodenal laktat (21%), duodenal propionat (23%), cecal laktat (16%), dan cecal butirat (112%) dalam kumpulan rawatan UPC-BDL jika dibandingkan pada kumpulan rawatan UPC-BD pada usia yang sama. Penambahan *Lactobacilli* ternyata telah menyekat kepekatan ileal dan cecal asetat masing-masing pada 51% dan 42% pada usia 14 hari dalam kumpulan rawatan UPC-BDL jika dibandingkan pada kumpulan ayam rawatan UPC-BD. Nilai pH pada kedua-dua duodenal dan jejunal meningkat (6%) secara signifikan (*P*<0.05) pada kumpulan rawatan UPC-BD jika dibandingkan pada kumpulan kawalan pada usia 14 hari. Penambahan *Lactobacillus* didalam diet makanan ayam secara signifikan (*P*<0.05) telah merendahkan nilai pH pada duodenal dan jejunal pada kumpulan UPC-BDL jika dibandingkan kepada kumpulan UPC-BD pada usia 14 dan 28 hari.

Kesimpulannya, keputusan-keputusan dalam kajian terbaru ini mencadangkan bahawa dua *L. agilis* JCM 1048 dan *L. salivarius* subsp. *salicinius* JCM 1230 mempuyai kemampuan untuk memantapkan semula keseimbangan mikrob yang sempurna dengan pembentukan laktat dan

juga propionat, dan merangsang bakteria penghasil butyrate terutamanya *F. prausnitzii* untuk menghasilkan butyrat dalam cecum ayam. Sungguhpun kaedah UPC tidak begitu di gemari dalam mengurangkan prestasi ayam ternak, tetapi ianya memberi kesan negatif terhadap pertumbuhan struktur duodenal dalam ayam-ayam ternak kumpulan UPC-BD pada usia 14 hari. Tambahan lagi, UPC mengubah secara negatif terhadap hasil akhir metabolik bakteria GIT semasa pengendalian yang kurang menyenangkan. Penambahan diet makanan dengan *Lactobacillus* memulihkan kesan penjejasan UPC pada ternakan-ternakan sepanjang tempoh eksperimen.

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and the rest of my family who provided the support that helped me to make it through the tough times.

I certify that a Thesis Examination Committee has met on 10 of March to conduct the final examination of Amir Meimandipour on his thesis entitled **"Probiotic effects of *Lactobacillus* strains on cecal microbiota and fermentation, intestinal development and response to human contact in broiler chickens"** in accordance with the Universities and University colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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Date: 13 May 2010

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 currently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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AMIR MEIMANDIPOUR
Date: 10 March 2010

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