



UNIVERSITI PUTRA MALAYSIA

**SELECTION OF OLIGOSACCHARIDES AS PREBIOTIC FOR PROBIOTIC
LACTOBACILLUS STRAINS AND THEIR EFFECTS ON PERFORMANCE OF
BROILER CHICKENS**

SAMINATHAN A/L POOTHAN MOOKIAH

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BROILER CHICKENS**

By

SAMINATHAN A/L POOTHAN MOOKIAH

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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May 2011

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Chairman: Professor Ho Yin Wan, PhD

Institute: Bioscience

Currently, a variety of probiotic bacterial strains and prebiotic oligosaccharides have been used singly or in combinations as synbiotics (probiotics + prebiotics) to enhance the health and well-being of the host animals. However, very often, synbiotics are produced without studying and selecting suitable prebiotics for specific probiotic bacterial strains. In the present study, a series of experiments was carried out to investigate the growth of 11 probiotic *Lactobacillus* strains in prebiotic oligosaccharides *in vitro* to select a suitable prebiotic for developing a synbiotic, and to determine the effects of the prebiotic singly and in combination as a synbiotic on the growth performance of chickens *in vivo*. In a preliminary study, the growth of the 11 *Lactobacillus* strains was assessed in 10 commercially available prebiotic oligosaccharides *in vitro*. The results showed that the utilization of oligosaccharides was highly variable among the 11 *Lactobacillus* strains and considerable strain differences (*P*

< 0.05) were observed. Prebiotic isomaltooligosaccharides (IMO) supported good growth for most of the *Lactobacillus* strains, followed by galactooligosaccharides (GOS), gentiooligosaccharides (GTO) and fructooligosaccharides (FOS). Oligosaccharides such as Raftilose L60, Raftilose P95, Raftiline LS, and mannanoligosaccharides (MOS) were poorly utilized by all the *Lactobacillus* strains. The four prebiotic oligosaccharides, IMO, GOS, GTO and FOS, which supported good growth of most of the *Lactobacillus* strains were selected for further studies on the growth kinetics and organic acids (acetic and lactic acids) production of the *Lactobacillus* strains. The results of the growth kinetics study also showed variations in the specific growth rates (μ) and growth patterns of the 11 *Lactobacillus* strains on GOS, IMO, GTO and FOS. Productions of acetic and lactic acids appeared to be growth associated and correlated with the growth patterns of the *Lactobacillus* strains on different prebiotic oligosaccharides. Among the four selected prebiotics, IMO was found to provide the best growth and higher specific growth rates, with generation of high concentrations of lactic and acetic acids of most of the *Lactobacillus* strains, and was therefore considered as a suitable prebiotic for the strains. The efficacy of prebiotic IMO was then evaluated singly and in combination as a synbiotic *in vivo* in a chicken feeding trial.

The results of the chicken feeding trial showed that supplementation of probiotic (0.1% of a mixture of the 11 *Lactobacillus* strains) (PRO), 0.5% prebiotic IMO (PRE05), 1.0% prebiotic IMO (PRE10), synbiotic with 0.5% prebiotic IMO (SYN05) and synbiotic with 1.0% prebiotic IMO (SYN10) could improve body weight, weight gain and feed

efficiency of broiler chickens, and there were no significant differences between the treatments. The supplementation of probiotic, prebiotics and synbiotics also significantly ($P < 0.05$) increased the caecal populations of lactobacilli and bifidobacteria, decreased the caecal *E.coli* and total aerobe populations, increased the caecal VFA and non-VFA concentrations, and lowered the serum total cholesterol, LDL cholesterol and triglycerides concentrations; but there were no significant differences in the relative weights of the organs such as the heart, liver, spleen and bursa as compared to the control. In all the parameters studied, the synbiotics did not show a twofold synergistic effect, although in some parameters (e.g caecal VFA and non-VFA concentrations) there were significant increases when compared to those of probiotic or prebiotic alone. The results of the chicken feeding trial indicated that prebiotic IMO (0.5 or 1.0%) and its synbiotic could be as effective as probiotic in improving the performance of broiler chickens and in conferring other health benefits on the chickens.

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sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMILIHAN OLIGOSAKARIDA SEBAGAI PREBIOTIK UNTUK PROBIOTIK
LACTOBACILLUS STRAINS DAN PENGARUHNYA DALAM PRESTASI
AYAM PEDAGING**

Oleh

SAMINATHAN A/L POOTHAN MOOKIAH

Mei 2011

Pengerusi: Profesor Ho Yin Wan, PhD

Institut: Biosains

Ketika ini, pelbagai strain bakteria probiotik dan prebiotik oligosakarida telah digunakan secara berasingan atau dalam kombinasi sebagai sinbiotik (probiotik + prebiotik) untuk meningkatkan tahap kesihatan haiwan perumah. Namun, sering kali synbiotik dihasilkan tanpa kajian terdahulu bagi memilih prebiotik yang sesuai dan khusus bagi strain bakteria probiotik. Dalam kajian ini, satu siri eksperimen telah dijalankan untuk mengkaji pertumbuhan 11 strain probiotik *Lactobacillus* di dalam prebiotik oligosakarida secara *in vitro* bagi mengenalpasti prebiotik yang sesuai untuk menghasilkan satu sinbiotik (probiotik + prebiotik), dan mengenalpasti kesan prebiotik dan sinbiotik tersebut terhadap prestasi pertumbuhan ayam *in vivo*. Dalam kajian awal, pertumbuhan 11 strain *Lactobacillus* dalam 10 jenis prebiotik oligosakarida yang tersedia secara komersial ditentukan secara *in vitro*. Keputusan kajian menunjukkan terdapat perbezaan yang ketara dalam penggunaan oligosakarida tersebut oleh 11 strain

Lactobacillus ($P < 0.05$). Kebanyakan strain *Lactobacillus* mencapai pertumbuhan yang baik dalam prebiotik isomaltooligosakarida (IMO), diikuti dengan galaktooligosakarida (GOS), gentiooligosakarida (GTO) dan fruktooligosakarida (FOS). Oligosakarida seperti Raftilose L60, Raftilose P95, Raftiline LS, dan mannanoligosakarida (MOS) kurang diguna oleh semua strain *Lactobacillus*. Keempat-empat prebiotik oligosakarida, IMO, GOS, GTO dan FOS, yang menyokong pertumbuhan yang baik kebanyakan strain *Lactobacillus* dikaji lanjut untuk pertumbuhan kinetik dan penghasilan asid organik (asid asetik dan laktik) strain *Lactobacillus*. Hasil dari kajian kinetik pertumbuhan juga menunjukkan variasi dalam kadar pertumbuhan spesifik (μ) dan pola pertumbuhan 11 strain *Lactobacillus* dalam GOS, IMO, GTO dan FOS. Penghasilan asid asetik dan laktik berkorelasi dengan pola pertumbuhan strain *Lactobacillus* dalam prebiotik oligosakarida yang berbeza. Di antara keempat-empat prebiotik yang dikaji, IMO didapati dalam memberi pertumbuhan yang terbaik dan kadar pertumbuhan spesifik yang tertinggi, dengan penghasilan asid laktik dan asid asetik yang berkepekatan tinggi bagi kebanyakan strain *Lactobacillus*, dan dianggap sebagai prebiotik yang paling sesuai. Tahap keberkesanan prebiotik IMO kemudian dikaji secara tunggal dan dalam kombinasi sinbiotik secara *in vivo* dalam kajian pemakanan ayam.

Keputusan kajian pemakanan ayam menunjukkan bahawa suplementasi probiotik (0.1% campuran 11 strain *Lactobacillus*) (PRO), 0.5% prebiotik IMO (PRE05), 1.0% prebiotik IMO (PRE10), sinbiotik dengan 0.5% IMO prebiotik (SYN05) dan sinbiotik dengan 1.0% prebiotik IMO (SYN10) dapat meningkatkan berat badan, kadar pertumbuhan dan kadar pertukaran makanan ayam. Suplementasi probiotik, prebiotik dan sinbiotik juga

meningkatkan populasi *Lactobacillus* dan bifidobakteria dalam sekum, menurunkan populasi *E.coli* dan bakteria aerob dalam sekum, meningkatkan kepekatan VFA dan non-VFA sekum, dan menurunkan paras “total” kolesterol, “low density lipoprotein” kolesterol dan trigliserida di dalam serum secara signifikan, tetapi, tiada perbezaan yang signifikan pada berat relatif organ-organ seperti jantung, hati, limpa dan bursa berbanding dengan ayam dalam kumpulan kawalan. Dalam kesemua parameter yang dikaji, sinbiotik tidak menunjukkan kesan sinergis dua kali ganda, walaupun dalam beberapa parameter (contohnya kepekatan VFA dan non-VFA sekum) terdapat peningkatan yang signifikan apabila dibandingkan dengan probiotik atau prebiotik sahaja. Keputusan kajian menunjukkan bahawa prebiotik IMO (0.5 or 1.0%) dan sinbiotik memberi kesan yang sama seperti probiotik dalam meningkatkan prestasi ayam, dan dalam memberi manfaat kesihatan yang lain kepada ayam.

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I certify that a Thesis Examination Committee has met on 9 May 2011 to conduct the final examination of Saminathan A/L Poothan Mookiah on his thesis entitled "Selection of oligosaccharides as prebiotic for probiotic *Lactobacillus* strains and their effects on performance of broiler chickens" in accordance with the Universities and University College 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 Master of Science.

Members of the Thesis Examination Committee were as follows:

LIANG JUAN BOO, PhD

Fellow Researcher
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Chairperson)

ABDUL RAZAK BIN ALIMON, PhD

Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Internal Examiner)

SHUHAIMI BIN MUSTAFA, PhD

Associate Professor
Halal Products Research Institute
Universiti Putra Malaysia
(Internal Examiner)

ROSLI MD. ILLIAS, PhD

Professor
Faculty of Chemical and Natural Resources Engineering
Universiti Teknologi Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 09 MAY 2011

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Ho Yin Wan, PhD

Professor
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Norhani Abdullah, PhD

Professor
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Member)

Kalavathy Ramasamy, PhD

Associate Professor
Faculty of Pharmacy
Universiti Teknologi Mara
(Member)

Sieo Chin Chin, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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 other institutions.

SAMINATHAN A/L POOTHAN MOOKIAH

Date: 9 May 2011

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