UNIVERSITI PUTRA MALAYSIA

EFFECTS OF *MITSUOKELLA JALALUDINII* AND *LACTOBACILLUS* CULTURES SUPPLEMENTATION ON THE PERFORMANCE AND NUTRIENT UTILIZATION OF BROILER CHICKENS

LEE HOOI CHING

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To my family

beloved late mum, my dad,

Hooi Ming, Hooi Leong and Hooi Fong

whose love and support make all things possible

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: **Associate Professor Norhani Abdullah, Ph.D.**

Faculty: **Science and Environmental Studies**

A study on the preservation techniques of *Lactobacillus* cultures was conducted by freeze-drying the cultures in different formulations of protectants and storage at -20°C, 4°C and room temperature for 1 to 6 months. Among the six formulations used, cultures preserved with 10% sucrose or 10% skim milk remained unchanged in their viability after freezing and after freeze-drying. All the freeze-dried formulations showed no viability losses after 1 month and 6 months of storage at -20° C, while a little loss at 4°C after 6 months of storage was observed. The viability in all the formulations was reduced after 1 month of storage at room temperature, in particular cultures stored in 10% skim milk. There was no viable count for all the formulations at 10^{-1} dilution, except for samples stored in 10% sucrose (6×10^3 CFU/ml) after 6 months of storage at room temperature.

A feeding trial using 480 one-day-old broiler chicks and eight diets was conducted for 6 weeks. The diets were designed to determine the effects of *Mitsuokella jalaludinii* (a new phytase-producing bacterial species) and *Lactobacillus* cultures (as probiotics) on minerals (Ca, P, Zn, Cu and Mn) and nitrogen utilization, and growth performance of

broilers fed either basal diet containing 0.49% (starter diet) or 0.45% (grower diet) available P (aP)(normal diet) or low-available P diet containing 0.25% (starter diet) or 0.23% (grower diet) aP. Body weight (BW) gain and feed conversion ratio (FCR) of broilers fed basal diet supplemented with *Lactobacillus* (BL) were significantly improved ($P<0.05$) from 1 to 42 days of age, but the feed intake was not affected. Supplementation of freeze-dried *M. jalaudinii* culture to chickens fed low-aP diet with or without *Lactobacillus* cultures significantly improved the BW gain, feed intake and FCR of broilers after 21 and 42 days of feeding when compared to those fed low-aP diet. The FCR of broilers fed low-aP diet supplemented with both *M. jalaludinii* and *Lactobacillus* was significantly improved ($P<0.05$) and the chickens had higher BW gain when compared to those fed low-aP diet supplemented with *M. jalaludinii* culture only. Mortality rate was not affected with the supplementation of *M. jalaludinii* or *Lactobacillus* cultures. No significant differences were found in the weight (% BW) of liver, spleen, bursa, gizzard, proventiculus and ileum of broilers given the different dietary treatments. Supplementation of *M. jalaludinii* and *Lactobacillus* cultures significantly reduced ($P<0.05$) the abdominal fat deposition in broilers as compared to chickens fed basal diet. Broilers fed diets supplemented with *Lactobacillus* cultures had lower ($P<0.05$) serum cholesterol level. *Mitsuokella jalaludinii* culture supplementation to chickens fed low-aP diet significantly increased ($P<0.05$) the digestibility of DM, P, Ca, Zn, and N in 18 to 20-day-old chicks. *Lactobacillus* cultures supplementation to basal diet significantly improved ($P<0.05$) the digestibility of Cu and Ca of broilers. Supplementation of *M. jalaludinii* culture to chickens fed low-aP diet significantly increased ($P<0.05$) the tibia DM, ash, P and Ca (% of DM) and plasma P and Ca concentrations, but Mn concentration in tibia ash was reduced.

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

KESAN PENAMBAHAN KULTUR *MITSUOKELLA JALALUDINII* DAN *LACTOBACILLUS* KE ATAS PRESTASI DAN PENGHADAMAN NUTRISI AYAM PEDAGING

Oleh

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Pengerusi: **Profesor Madya Norhani Abdullah, Ph.D.**

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Satu kajian mengenai teknik mengekalkan kultur *Lactobacillus* telah dijalankan dengan kaedah pengeringan beku di dalam beberapa jenis pelindung dan disimpan pada suhu -20°C, 4°C dan suhu bilik selama 1 hingga 6 bulan. Di antara 6 rumusan yang digunakan, rumusan kultur 10% sukrosa atau 10% susu skim dapat mengekalkan kemandirian selepas pembekuan dan pengeringan beku. Kesemua rumusan yang disimpan pada -20°C menunjukan tiada kehilangan kemandirian selepas 1 dan 6 bulan, tetapi sedikit kehilangan kemandirian pada 4°C selepas 6 bulan. Kemandirian dalam kesemua rumusan yang disimpan pada suhu bilik berkurangan selepas 1 bulan, terutamanya kultur dengan 10% susu skim. Tiada pertumbuhan dikesan pada 10^{-1} untuk semua rumusan, kecuali sampel yang dirumus dengan 10% sukrosa (6×10^3 CFU/ml) selepas 6 bulan disimpan pada suhu bilik.

Kajian pemakanan yang melibatkan 480 ekor anak ayam berumur satu hari dan 8 jenis diet telah dijalankan selama 6 minggu. Makanan ayam tersebut dirumus untuk menentukan kesan *Mitsuokella jalaludinii* (bacteria spesis baru yang menghasilkan fitase) dan *Lactobacillus* (sebagai probiotik) dari segi penggunaan mineral (Ca, P, Zn,

Cu dan Mn) dan nitrogen, dan prestasi ayam yang diberi makanan basal yang mengandungi 0.49% atau 0.45% fosforus ataupun mengandungi 0.25% atau 0.23% fosforus (rendah-aP). Keputusan menunjukkan ayam yang diberi makanan basal yang ditambah dengan *Lactobacillus* (BL) dari umur 1 hingga 42 hari mengalami peningkatan penambahan berat badan (BW) dan kadar penukaran makanan (FCR) yang signifikan ($P<0.05$), tetapi jumlah pengambilan makanan tidak terjejas. Penambahan kultur *M. jalaludinii* ke dalam makanan rendah-aP yang ada atau tiada *Lactobacillus* meningkatkan prestasi penambahan BW, pengambilan makanan dan FCR ayam berumur 21 dan 42 hari apabila dibandingkan dengan ayam yang diberi makanan rendah-aP. Ayam yang diberi makanan rendah-aP dengan *M. jalaludinii* dan *Lactobacillus* menunjukkan peningkatan prestasi FCR yang signifikan ($P<0.05$) dan penambahan BW yang lebih tinggi apabila dibandingkan dengan ayam yang diberi diet rendah-aP dengan kultur *M. jalaludinii* sahaja. Penambahan *M. jalaludinii* atau *Lactobacillus* tidak mempengaruhi kadar kematian ayam. Didapati tiada perbezaan yang signifikan dalam berat (% BW) hati, limpa, bursa, tembolok, proventikulus dan ileum ayam untuk kesemua jenis pemakanan. Timbunan lemak abdominal ayam yang diberi makanan dengan *M. jalaludinii* dan *Lactobacillus* berkurangan ($P<0.05$) apabila dibandingkan dengan ayam yang diberi makanan basal. Ayam yang diberi makanan dengan kultur *Lactobacillus* mempunyai kandungan kolesterol serum yang rendah ($P<0.05$). Penghadaman jisim kering (JK), P, Ca, Zn dan N untuk ayam berumur 18 ke 20 hari ditingkatkan dengan penambahan kultur *M. jalaludinii* ke dalam makanan rendah-aP. Penambahan kultur *Lactobacillus* ke makanan basal meningkatkan penghadaman Cu dan Ca ayam ($P<0.05$). Penambahan kultur *M. jalaludinii* ke dalam makan rendah-aP meningkatkan JK, abu, P dan Ca (% DM) tulang tibia dan kandungan P dan Ca plasma, tetapi kandungan Mn dalam tulang tibia berkurangan.

ACKNOWLEDGEMENTS

Without the encouragement and participation of many people, this project would never have been accomplished. My personal and very deep appreciation goes to the chairman of the supervisory committee, Associate Professor Dr. Norhani binti Abdullah for her expert guidance and support throughout the course of the project. Also special thanks and appreciation to the supervisory committee members, Professor Dr. Ho Yin Wan and Associate Professor Dr. Zulkifli Idrus who have given me invaluable suggestions and advices.

Very special thanks are extended to the members of the Digestive Microbiology Unit, Institute of Bioscience: Tongsuk, Darlis, Chin Chin, Kalavathy, Madam Haw, Siddiq, Kak Latiffah, Vicky, En. Khairul, and Mr. Jivan. who have been very helpful to me. I am also indebted to Mr. Nagaya and Mr. Paimon for helping me in the field and to Mr. Ibrahim Mohsin, from the Nutrition Laboratory, for his technical assistance and co-operation. My personal sincerest appreciation and thanks to Lan Ganqiu who unselfishly shared with me his knowledge and has helped me in so many ways. I also wish to thank all my friends, especially Pit Kang, Albert and Max for sharing with me a joyful stay here.

Finally, I would like to express my deepest appreciation and thanks to my family for their unconditioned love, encouragement and support throughout my study.

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

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Poultry products are important sources of protein for consumers in the world currently and also will be in the future. Poultry production forms a major component of the livestock industry. There are two major types of poultry productions i.e., the production of meat from broilers and the production of eggs from layers.

Over the past several decades, tremendous improvements in the efficiency of nutrient utilization for poultry had been achieved. While the individual bird has become more efficient in the conversion of nutrients to meat and eggs, the large increase in animal units has led to an overall increase in environmental problems in waste management and minerals deposition.

Phosphorus is an essential element for growth and survival of poultry. In diets containing plant ingredients, such as corn and soybean meal, about 60 - 80% of the total phosphorus exist as phytate phosphorus that is bound to phytic acid (Nelson *et al.*, 1968). Phytate phosphorus is not well absorbed by the birds due to the absence or scarcity of the enzyme phytase in their intestines (Simons *et al.*, 1990). Inorganic or nonphytate phosphorus is therefore added in the feed to meet the demands. Undigested phytate phosphorus is excreted in the faeces, and excess phosphorus can be washed into waterways and accumulate in ponds and lakes. This would result in rapid algae growth, which affect the amount of oxygen in water (eutrophication) and reduce the survival rate of living organisms in the water (Edwards and Daniel, 1992).

To alleviate the problems caused by undegradable phytate compounds, phytase enzyme as feed supplement has been used in a number of studies (Simons *et al.*, 1990; Denbow *et al.*, 1995; Sebastian *et al.*, 1996a,b). The results from these studies indicated that phytase enzyme could increase P availability from phytate and hence increase phytate utilization by the birds.

The issue on the use of antibiotics has been long debated in poultry production. For a high level of economic efficiency, poultry are raised under intensive production systems in densely populated colonies or flocks. The chickens are stressed by various factors and these tend to create an imbalance in the intestinal microflora and lowering of body defence mechanism (Jin *et al.*, 1997). Under such systems, antimicrobial feed additives such as antibiotics and synthetic antimicrobial agents are often used to suppress or eliminate harmful organisms in the intestine and to improve growth rate and feed efficiency. However, the continued use of antibiotics at sub-therapeutic levels in animal feeds may result in the development of drug-resistant microorganisms in human (Smith and Crabb, 1957). In 1985, Frost claimed that antibiotics used in animal production have potential risk to public health. As a consequence, an alternative to antibiotics is sought in poultry production and has become an area of great interest. One alternative is the use of living microbes known as probiotics as feed supplement. The bacteria, *Lactobacillus* spp. have been successfully used as a probiotic (Jin *et al.*, 1996c, 1998).

Probiotics can be fed to the animal in various forms. They can be in a dehydrated culture form, which is normally produced by freeze-drying. However, some bacteria may be very sensitive to this technique as they show a poor survival

rate, probably due to the freezing and subsequent drying treatment (Bozoglu and Gurakan, 1989). In order to improve the survival rate of the cultures, some protectants are usually added during the freeze-drying process (Potts, 1994). Protectants that are commonly used are skim milk, glucose, sucrose and glycerol.

1.2 Objectives

Separate studies on the effects of phytase enzyme from *Mitsuokella jalaludinii* (a new phytase-producing bacterial species) or of *Lactobacillus* spp. as a probiotic on performances and nutritive value of feed in broilers and layers have been conducted (Jin, 1996; Lan *et al.*, 1999; Kalavathy *et al.*, 2002). However, the effects of feeding both phytase enzyme and *Lactobacillus* cultures in broilers have not been investigated. Also the best preservation technique for the *Lactobacillus* spp. has not been studied. Therefore, the objectives of the present study were to develop preservation techniques in maintaining the viability of *Lactobacillus* cultures and to determine the effects of phytase on phosphorus utilization with or without *Lactobacillus* supplementation in broilers. *Mitsuokella jalaludinii* from the rumen of cattle (Lan *et al.*, 1999) as the source of phytase enzyme and 12 strains of *Lactobacillus* (Jin *et al.*, 1996a) as a probiotic were used. All bacterial strains were maintained in the Digestive Microbiology Unit, Institute of Bioscience, Universiti Putra Malaysia.

The specific objectives were:

1. To determine preservation methods to maintain the viability of *Lactobacillus* cultures.
2. To evaluate various diets which contained normal or low level of available phosphorus (aP) with or without *M. jalaludinii* (a new phytase-producing bacterial species) or *Lactobacillus* cultures (probiotic) or both. The parameters measured included;
   - growth performance
   - digestibility of nutrients
   - mineral contents of bone and blood plasma
   - weight or size of various intestinal organs, spleen, liver, and bursa of Fabricus
   - abdominal fat deposition and blood serum cholesterol level.

CHAPTER 2

LITERATURE REVIEW

2.1 Poultry Industry – Broiler Chickens

The poultry industry forms a major component of the livestock industry. It is the most developed animal production system compared to the swine, sheep or cattle industries. At present, the poultry industry in the world continues to expand, especially in Asia. A survey on the world trade in poultry meat forecasted that during the period 1997-2000, poultry consumption would increase significantly in China, countries of the former Soviet Union, India, Pakistan, the Philippines and Malaysia (Martin, 1999). The consumption of poultry meat of these countries is relatively low and offers the largest potential for growth and improvement in terms of management.

2.2 Phosphorus Requirements of Poultry

Phosphorus is an essential mineral required in poultry diets for normal growth and development. In addition to its primary role as a structural constituent of the inorganic portion of the bone, phosphorus is an essential component of organic compounds involved in nearly every aspect of metabolism (Patrick and Schaible, 1980). It plays important roles in the metabolism of the three major nutrient groups: carbohydrates, amino acids and lipids. Phosphorus is present in the nucleic acids, DNA and RNA. It is an essential component of many metabolic coenzymes and is required for the storage of energy as part of phosphorylated glucose compounds and high-energy compounds, such as ATP and ADP (Devlin, 1997).

To meet these numerous metabolic demands, poultry of each species and age require specific quantities of phosphorus in a form that can be readily absorbed and available for utilization. In a broiler chicken, this quantity varies from 0.45g of phosphorus for each 100g of diet it consumes to 0.5g, depending on the age of the broiler as well as the response criteria employed in evaluating the requirement (NRC, 1994). In laying hens, from 0.21 to 0.35% available phosphorus is required in the diet to meet metabolic needs.

2.2.1 Phytic Acid and Availability of Phytate Phosphorus

Poultry feed contains ingredients primarily of plant origin, including cereal grains, cereal by-products, and oilseed meals. Phosphorus is provided in the diet as a natural constituent of these ingredients, and often, supplemental amounts of phosphorus are added as an inorganic salt, such as defluorinated or dicalcium phosphate (DCP). Approximately two thirds of all phosphorus in plant products is present as phytic acid (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) or its salts (Nelson *et al.*, 1968; Simons *et al.*, 1990). Since a major portion of poultry diets consists of plant-derived ingredients, phytic acid is present in a considerable amount in the diet of this animal as shown in Table 1 (Reddy *et al.*, 1982; Ravindran *et al.*, 1995). For example, poultry diets based on corn and soybean meal typically contain 0.80% to 0.90% phytic acid (or 0.22% to 0.25% phytate-phosphorus). However, phytate phosphorus is essentially unavailable to monogastric animals such as swine and poultry because they lack the enzyme phytase in their digestive tract, which is necessary to hydrolyse the compound and release the phosphorus for absorption and utilization. The inability to utilize phytic acid phosphorus results in a substantial loss

of nutritional value and may create a significant pollution problem when the manure containing the residual phosphorus is applied to land.

As a reactive anion, phytic acid forms a wide variety of insoluble salts with divalent and trivalent cations, therefore reducing the availability of Cu^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Fe^{3+}, and Ca^{2+} in the monogastric animals (Figure 1; Erdman, 1979). These salts of phytic acid can be termed as phytin. Phytic acid is also known to form complex with proteins as well as starch and consequently reducing their availability (Figure 2; Thompson, 1986). Furthermore, phytic acid also reduces the activity of pepsin, trypsin and amylase (Sebastian *et al.*, 1998).

Table 1: Phytic acid and phytate phosphorus concentration in different morphological components of cereals*

Cereal	Sample	Phytic acid (%)	Phytate phosphorus (%)
Corn (Maize)	Commercial hybrid	0.89	0.25
	Endosperm	0.04	0.01
	Germ	6.39	1.80
	Hull	0.07	0.02
Wheat	Soft	1.14	0.32
	Endosperm	0.004	0.001
	Germ	3.91	1.10
	Hull	0.00	0.00
	Aleurone	4.12	1.16
Rice	Brown	0.89	0.25
	Endosperm	0.01	0.004
	Germ	3.48	0.98
	Pericarp	3.37	0.95
Soybean meal			0.39

* Source: Reddy *et al.* (1982) and Ravindran *et al.* (1995).