



UNIVERSITI PUTRA MALAYSIA

**MODULATORY EFFECTS OF LYSINE AND METHIONINE IN DIETS ON
GROWTH AND TRANSCRIPTS ASSOCIATED WITH IMMUNITY GENES
IN BROILER**

LEE CHAI YAN

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By

LEE CHAI YAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Science**

June 2019

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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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June 2019

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The poultry industry is one of the most advanced livestock industries in the world. Feed formulation is highly emphasized because the cost of feed is the biggest proportion (65-70%) of the production cost. One of the most economical ways to reduce feed cost is by efficiently meeting the nutritional requirement of the broilers. Alternative feed formulation includes supplementing amino acids with reduced levels of dietary crude protein which is more economical as it can reduce the cost of feed. In this study, the effects of amino acids supplementation of lysine and methionine in a low crude protein diet on the growth performance and expression of immunity genes including mucin 2 (MUC2), SLC11A1 (SLC), gallinacin 6 (GAL6) and liver-expressed antimicrobial peptide (LEAP-2) were investigated to select the best feed formulation for the broilers. These immunity genes were chosen as they can provide natural resistance to diseases and pathogens. The gene expression is highly regulated by amino acids that are basic constituents of cellular protein whereby different variations of amino acids in the diet can affect the gene expression. A total of 300 broilers (Cobb500) were tested with ten different dietary treatments. The chickens were fed with two types of diet; starter and finisher and the effects on growth performance were determined. The lysine and methionine levels were manipulated to high, standard and low based on the recommended diet for Cobb500 broilers. Treatment one is the control that consists of diet with standard levels of lysine, methionine and crude protein as recommended by Cobb-Vantress for Cobb500 broilers. Following that, ribonucleic acid (RNA) was extracted from the small intestine (jejunum), spleen and liver and used to study the gene expression of the immunity genes with real-time polymerase chain reaction (qPCR) using SYBR Green assay. The results from the growth performance of the broilers showed that increasing lysine by 0.2% in a low crude protein diet gave the best results in terms of feed conversion ratio of 1.96 ± 0.11 . Meanwhile, gene expression studies on MUC2 gene in the jejunum showed overall upregulation across all dietary treatments with treatment 3 (T3) showing the highest expression with increment of 3.8-fold as compared to the

control. This indicates that the mucins are not only important as a defence mechanism but also display a capacity to uptake nutrients for the rest of the body making it sensitive to dietary intake of amino acids and crude protein. Other genes expressed in spleen and liver such as SLC, GAL6 and LEAP-2 were mostly down regulated across the treatment groups as compared to the control group. These three genes offer innate immunity which recognizes different types of pathogens to prevent infection and may not be as sensitive to dietary intake of lysine and methionine as compared to MUC2 gene. In conclusion, treatment with increased lysine content by 0.2% with normal level of methionine performed better in a low crude protein diet in terms of having the lowest feed conversion ratio and highest upregulation of MUC2 gene.



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

**KESAN MODULASI LISINA DAN METIONINA DI DALAM DIET PADA
PERTUMBUHAN DAN TRANSKRIP BERKAITAN DENGAN GEN
KEIMUNAN DALAM AYAM PEDAGING**

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Industri poltri merupakan salah satu daripada industri ternakan yang termaju di dunia. Formula pemakanan diberi penekanan kerana kos bahan makanan membentuk nisbah yang terbesar (65-70%) dalam kos pengeluaran. Salah satu cara yang paling berekonomi untuk mengurangkan kos pemakanan adalah dengan memenuhi keperluan nutrien ayam pedaging dengan berkesan. Formulasi pemakanan alternatif termasuk menambah diet tambahan asid amino sambil mengurangkan diet protein kasar yang lebih berekonomi kerana boleh mengurangkan kos bahan makanan. Kesan penambahan asid amino iaitu lisina dan metionina dalam diet rendah protein kasar pada prestasi pertumbuhan dan ekspresi gen keimunan termasuk mucin 2 (MUC2), SLC11A1 (SLC), gallinacin 6 (GAL6) dan peptida antimikrob terungkap di hati (LEAP-2) disiasat untuk memilih formulasi yang terbaik untuk ayam pedaging. Gen-gen immuniti tersebut dipilih kerana mampu memberi ketahanan semula jadi terhadap pelbagai jenis penyakit dan patogen. Ekspresi gen adalah sangat terkawal oleh asid amino yang merupakan jujuk asas protein sel di mana variasi berbeza asid amino boleh memberi kesan terhadap ekspresi gen. Sejumlah 300 ayam pedaging (Cobb500) diuji dengan sepuluh jenis diet berlainan. Ayam diberikan dua jenis diet; pemula dan penamat dan kesan terhadap prestasi pertumbuhan ditentukan. Paras lisina dan metionina dimanipulasi pada paras tinggi, standard dan rendah mengikut diet yang disyorkan untuk ayam pedaging Cobb500. Rawatan satu merupakan kawalan yang terdiri daripada diet dengan paras lisina, metionina dan protein kasar standard yang disyorkan oleh Cobb-Vantress untuk ayam pedaging Cobb500. Selepas itu, asid ribonukleik (RNA) telah diekstrak dari usus kecil (jejunum), limpa dan hati ayam dan digunakan untuk mengkaji ekspresi gen keimunan dengan tindak balas berantai polimerase masa nyata (qPCR) menggunakan asai SYBR Green. Keputusan prestasi pertumbuhan ayam pedaging menunjukkan bahawa pertambahan 0.2% lisina dalam diet rendah protein kasar memberi hasil yang terbaik terutamanya pada nisbah pertukaran makanan iaitu sebanyak 1.96 ± 0.11 . Sementara itu, kajian ekspresi gen MUC2 dalam jejunum menunjukkan peningkatan dalam semua rawatan diet dengan

peningkatan ekspresi tertinggi pada rawatan 3 (T3) sebanyak 3.8 kali ganda berbanding dengan kawalan. Ini menunjukkan bahawa mucin bukan sahaja penting sebagai mekanisma pertahanan tetapi juga memaparkan keupayaan pengambilan nutrien untuk seluruh badan, membuatkan ia sensitif kepada pengambilan diet asid amino dan protein kasar. Gen lain yang diekspresi di limpa dan hati seperti SLC, GAL6 dan LEAP-2 kebanyakannya menunjukkan penurunan pengekspresan gen kawal atur apabila dibandingkan dengan kumpulan kawalan. Ketiga-tiga gen tersebut memberi keimunan semula jadi yang mengenal pasti pelbagai jenis patogen untuk mengelakkan jangkitan dan tidak begitu sensitif kepada pengambilan diet lisina dan metionina berbanding dengan gen MUC2. Kesimpulannya, rawatan dengan peningkatan 0.2% lisina dengan paras standard metionina dalam diet rendah protein kasar menunjukkan prestasi yang lebih bagus terutamanya mempunyai nisbah penukaran makanan yang terendah dan pengekspresan gen kawal atur MUC2 yang paling tinggi.



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LIST OF ABBREVIATIONS

~	approximately
β	beta
°C	degree Celcius
<	less than
%	percent
×	times
μg	microgram
μL	microlitre
μM	micromolar
A	absorbance
AMPs	antimicrobial peptides
ANOVA	analysis of variance
bp	base pairs
BWG	body weight gain
cDNA	complementary deoxyribonucleic acid
CEAA	conditionally essential amino acids
cm	centimetre
CMI	cell-mediated immunity
CP	crude protein
CPO	crude palm oil
Cq	quantification cycle
DNA	deoxyribonucleic acid
E	efficiency
EAA	essential amino acids
EDTA	ethylenediaminetetraacetic acid
ESTs	expressed sequence tags

FCR	feed conversion ratio
FI	feed intake
g	gravity
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GIT	gastrointestinal tract
GR	growth rate
IB	infectious bronchitis
IBD	infectious bursal disease
IBW	initial body weight
mg	milligram
mL	millilitre
mRNA	messenger ribonucleic acid
n	sample size
ND	Newcastle disease
NEAA	non-essential amino acids
ng	nanogram
nm	nanometre
nM	nanomolar
NRC	National Research Council
NTC	no template control
OD	optical density
PCR	polymerase chain reaction
qPCR	real-time polymerase chain reaction
R^2	coefficient of determination
RES	reticuloendothelial system
RIN	RNA integrity number
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid

SAS	Statistical Analysis Software
SD	standard deviation
SPSS	Statistical Package for the Social Sciences
T	treatment
V	volt
VNTR	variable number tandem repeat



CHAPTER 1

INTRODUCTION

One of the most advanced industries in the livestock industry is poultry. In terms of technological knowledge, it is growing more rapidly as compared to any other animal species. One of the highly perfected science in poultry industry is feed formulation to ensure maximum intake of energy and protein for growth while reducing excessive nutrient feeding. The main reason is because feed contributes to a large proportion of the cost in poultry production. In Malaysia, feed ingredients are mostly imported while additives such as vitamins, minerals and amino acids are fully imported (Loh, 2002). Amino acids are one of the highly studied feed supplements as they are essential for the health and growth of the animal. The main ingredient contributing as crude protein in the broiler diet is soybean meal which is required in large amounts. A reduction of 3% in crude protein while supplementing amino acids which is added in very small amounts can reduce the cost significantly without affecting the health and growth of the broilers (Kamran et al., 2004).

There are 20 different amino acids commonly found in cells and they function as building blocks of protein which contributes primarily to the structure and function of cells. Deficiency of even one amino acid may have a detrimental effect on the growth or immunity of the animal. However, the current knowledge about the amino acid-dependant control of gene expression in broilers is still very limited. Among the essential amino acids in the chicken diet, methionine and lysine were found to be the two most limiting amino acids (Fernandez et al., 1994). They were chosen to be studied in this research as feed supplement in the broiler diet. The effect of the diet on the growth and expression of immunity genes in the small intestine, spleen and liver were studied. The supplementation of amino acids in poultry feed supports the reduction of dietary crude protein to meet the requirements of the birds more accurately and can help in reducing the cost of feed formulation while still maintaining the health and growth of the birds (Kamran et al., 2004).

There are many proteins involved in the immunity of the chicken since it is always confronted with pathogenic and commensal microorganisms such as chemokines, interleukins and transcription factors. The mucin glycoprotein is part of the defensive system in chickens and the most important constituent of the mucus layer protecting the epithelium of the intestinal tract in chickens. The mucin (MUC) gene consists of 13 members and is divided into two types which are the secreted and membrane anchored mucins (Montagne et al., 2004). Mucin 2 (MUC2) gene is a type of secretory mucin that is in the small intestine of the chickens.

Apart from mucins, another gene known as SLC11A1 (SLC) gene previously known as natural resistance-associated macrophage protein 1 (NRAMP1) has been shown to confer natural resistance against *Salmonella typhimurium*, *Mycobacteria* and *Leishmania donovani* (Vidal et al., 1995). NRAMP1 encodes a novel integral membrane protein and

plays a crucial role at host-parasite interface and is found in prokaryotes and eukaryotes. SLC gene also plays a big role in the immunity since it provides natural resistance towards various types of pathogens without the help of vaccines.

Antimicrobial peptides are part of the innate immune system and consists of a diverse range in poultry with their main role of defending against microbial attack (Townes et al., 2004). Among them, gallinacin 6 (GAL6) and chicken liver-expressed antimicrobial peptide 2 (LEAP-2) were chosen to be studied. Gallinacins are beta- defensins of which are efficient at killing various pathogens (Sugiarto & Yu, 2004). GAL6 in particular shows strong expression in kidneys, liver and gall bladder (Lynn et al., 2004). The LEAP-2 gene was found to be expressed in several organs in chickens including the kidney, lung, liver and small intestine and were able to confer immunity against *Salmonella* (Townes et al., 2004).

In this study, the effects of methionine and lysine supplementation on the growth of the broilers and its effect on the health of the broilers by conferring natural immunity and protecting the broilers from diseases and pathogens were studied.

The specific objectives of this study are:

- 1) To determine the effects of lysine and methionine supplementation on the dietary of the broiler towards growth performance
- 2) To analyse the gene expression of immunity genes which are MUC2, SLC, GAL6 and LEAP-2 in broilers supplemented with different concentrations of lysine and methionine

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Thesis

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