UNIVERSITI PUTRA MALAYSIA

GROWTH PERFORMANCE, GUT MORPHOLOGY AND IMMUNE RESPONSE OF BROILER CHICKENS FED LOW PROTEIN DIETS SUPPLEMENTED WITH LYSINE AND METHIONINE

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Master of Science

May 2015

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

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May 2015

Chairman: Professor Loh Teck Chwen, PhD

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Protein is one of the important nutrients in feedstuff that needs to be met for the basic nutrient requirement of animals. The ideal protein concept contains all amino acids in the exact amount and proportion in order to maintain and fulfill the chicken's requirements and at the same time reduce the feed cost. The present study was conducted to evaluate the effect of feeding a low crude protein diet with lysine and methionine supplementation on broiler performance. Two experiments were conducted in this study. In the first experiments, a total of 288 Cobb500 broiler chickens were used with 8 dietary treatments. The chickens were offered with a starter diet (21% to 18% crude protein) and finisher diet (18% to 15% crude protein) supplemented with three commercial amino acids (L-Lysine, DL-Methionine and L-Threonine). The amino acids in the starter and finisher diets from the different treatment groups were adjusted to similar levels. In continuation from the first experiment, the optimum level of the crude protein diet was used whilst the level of methionine and lysine was manipulated to the high, normal and low levels in the diets. Three hundred Cobb 500 chickens were used in this study with 10 treatments. The chickens were offered a starter diet that consisted of 1.4%, 1.2%, 1.0% lysine and 0.51%, 0.46%, 0.41% methionine in 19% crude protein; whereas the finisher diet contained 1.25%, 1.05%, 0.85% lysine and 0.48% 0.43%, 0.38% methionine in 16% crude protein diets. In the first experiment, reducing dietary crude protein by 2% with amino acid supplementation had a better growth performance, carcass composition, gut morphology and microflora than birds fed with commercial diet. The second experiment reported that high lysine and normal methionine levels in the diets had a greater growth performance, breast meat yield and liver weight as well as lower feed conversion ratio and abdominal fats. Increased methionine levels in the low crude protein diets showed a higher final body weight, weight gain, breast meat yield, liver weight and lower feed conversion ratio, abdominal fat. In conclusion, increasing 0.2% lysine level and maintaining methionine level supplementation in a dietary crude protein reduction by 2% is optimal for maximizing growth performance, absorptive capacity, and immune response in broiler chickens. In addition, it has been widely accepted that the case of the dietary protein level is an economic decision to be made by industrial companies to increase the cost effective benefits.

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

PRESTASI PERTUMBUHAN, MORFOLOGI USUS DAN TINDAKBALAS IMUNISASI AYAM PEDAGING DIBERI MAKANAN DIET RENDAH PROTEIN DENGAN PENAMBAHAN LYSINE DAN METHIONINE

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Protein merupakan salah satu nutrien penting dalam pemakanan ternakan sebagai nutrien asas haiwan tersebut. Konsep protein ideal mengandungi semua asid amino dalam jumlah dan nisbah yang tepat untuk mengekalkan dan memenuhi keperluan asas ayam dan pada masa yang sama ia mengurangkan kos makanan. Oleh yang demikian, kajian ini dijalankan untuk menilai kesan pemberian makanan yang mengandungi rendah protein kasar dengan penambahan asid amino terhadap prestasi ayam daging. Dua ujikaji telah dijalankan di dalam kajian ini. Dalam ujikaji pertama, sejumlah 288 ayam daging Cobb500 telah dibahagikan kepada 8 jenis diet. Ayam disajikan dengan diet permulaan (21% hingga 18% protein kasar) dan diet pengakhiran (18% hingga 15% protein kasar) yang ditambah dengan tiga jenis asid amino (L-Lysine, DL-Methionine dan L-Threonine). Asid amino dalam diet permulaan dan diet pengakhiran dalam kumpulan diet yang berbeza telah diselaraskan pada tahap yang sama. Daripada kesinambungan ujikaji pertama, diet yang mengandungi protein kasar yang optimum telah digunakan manakala kepekatan methionine dan lysine telah dimanipulasi kepada kepekatan tinggi, serdahana, dan rendah dalam diet tersebut. 300 ekor ayam Cobb500 telah digunakan dalam kajian ini dengan 10 jenis diet. Ayam telah diberikan diet permulaan yang terdiri daripada 1.4%, 1.2%, 1.0% lysine dan 0.51%, 0.46%, 0.41% methionine dalam 19% protein kasar; manakala diet pengakhiran mengandungi 1.25%, 1.05%, 0.85% lysine dan 0.48%, 0.43%, 0.38% methionine dalam 16% diet protein kasar. Dalam ujikaji pertama, pengurangan protein kasar dalam diet sehingga 2% ditambahkan dengan asid amino menunjukkan prestasi pertumbuhan, komposisi karkas, morfologi usus dan mikroflora yang lebih baik berbanding dengan ayam memakan makanan komersial. Ujikaji kedua melaporkan bahawa paras lysine yang tinggi dan methionine yang normal dalam diet memperolehi prestasi pertumbuhan, hasil daging dibahagian dada, berat hati yang lebih bagus serta nisbah penukaran makanan dan lemak abdomen yang lebih rendah. Peningkatan paras methionine dalam diet rendah protein kasar menunjukkan tinggi berat badan akhir, penambahan berat badan, hasil daging dibahagian dada, berat hati dan rendah nisbah penukaran makanan, lemak dibahagian abdomen berbanding kawalan positif. Kesimpulannya, peningkatan sebanyak 0.2% paras lysine dan pengekalan paras methionine ditambah dalam diet protein kasar yang dikurangkan sebanyak 2% adalah optimum bagi memaksimumkan

prestasi pertumbuhan, keupayaan menyerap, dan tindak balas imun dalam ayam daging. Di samping itu, ia telah diterima dengan meluas bahawa kes tahap protein pemakanan adalah satu keputusan ekonomi yang akan dibuat oleh syarikat-syarikat industri untuk meningkatkan faedah kos efektif.

ACKNOWLEDGEMENTS

I owe a debt of gratitude to those who are so generous with their time and expertise. There are so many people whom I deeply respect and whose efforts are amazing during my studies.

Firstly, I would like to attribute my deepest appreciation and sincere gratitude toProfessor Dr. Loh Teck Chwen, chairperson of the supervisory committee for granting Graduate Research Assistantship and his beneficial advice, guidance, scientific critism and invaluable suggestion during the study. He gets my highest amount of praise for his professionalism and dedicated role as a supervisor. He also gives me the freedom to make choice and to think without boundaries to achieve good results in my studies.

Besides, I would also extend my gratefulness and appreciation to members of the supervisory committee: Dr Anjas Asmara Samsuddin and Associate Professor. Dr. Foo Hooi Ling for their guidance, concern, patience, inspiration, support and constructive comments throughout the course of the study. The caring and easy going characteristics make me feel comfortable to approach them.

Deep appreciation is also extended to School of Graduate Studies, Universiti Putra Malaysia for granting Graduate Research Fellowship and providing excellent service and support. Specially thanks to Associate Professor. Dr. Foo Hooi Ling for giving me permission to use her laboratory in Biotechnology and Biomolecular Science 3 and Institute of Bioscience to analyse my samples. I wish to thank all lecturers and staffs from the Department of Animal Science, Faculty of Agriculture, UniversityPutra Malaysia for their support.

Sincere thanks and appreciation is also extended to my institution, Institute of Tropical Agriculture, UPM for the supports. Finally, I am deeply grateful to all my family and friends who gave me endless love and support me when I happened to be down and depressed. Special thanks go to En. Shazali B. Abu Hassan, Pn. Zaleha Abdullah, Nurmaya Rafeah Bt. Shazali and also my friends in high places.

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

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LIST OF ABBREVIATIONS

°C	Degree Celsius
µL	Microliter
µm	Micrometer
4MV	4-methyl-valeric acid
AA	Amino Acid
ACN	Acetonitrile
ADG	Average Daily Gain
ANOVA	Analysis Of Variance
AOAC	Association of Analytical Communities
ATP	Adenosine Triphosphate
BW	Body Weight
CF	Crude Fiber
CFU	Colony forming units
cm	Centimeter
-COOH	Carboxyl Group
CP	Crude Protein
CPR	Columbian Plymouth Rock
DAD	Diode Array Detector
dH_2O	Deionized water
DM	Dry Matter
DNA	Deoxyribonucleic acid
EAA	Essential Amino Acid
EDTA	Ethylene Diaminetetraacetic Acid
EE	Ether Extract
ENT	*Enterobacteriaceae*
FCR	Feed Conversion Ratio
FI	Feed Intake
FLD	Fluorescence Detector
FMOC	Fluorenylmethoxycarbonyl chloride
g	Gram
GDP	Gross Domestic Product
GLC	Gas Liquid Chromatography
h	Hours
H	Hydrogen Atom
H_2O_2	Hydrogen Peroxide
H_2SO_4	Hydrogen Sulfuric Acid
HBr	Hydrogen Bromide
HCL	Hydrochloric Acid
HPLC	High Pressure Liquid Chromatography
IBD	Infectious Bursal Disease
IgG	Immunoglobulin G
IgG-HRP	Immunoglobulin G Horseradish Peroxidase
IgM	Immunoglobulin M
IgM-HRP	Immunoglobulin M Horseradish Peroxidase
Kcal	Kilo Calorie
LAB	Lactic Acid Bacteria
LC	Liquid Chromatography
Log_{10} CFU	Logarithm at base of 10
Lys	Lysine
M	Molarity
ME	Metabolizable Energy
MeOH	Methanol
Met	Methionine
mg	Milligrams
mL	Milliliter
mM	Milli Molar
mm	Millimeter
mRNA	Messenger RNA

MRS – agar	Lactobacillus – Agar De Man, Rogosa and Shape
MRS	Man Rogosa Sharpe
N	Nitrogen
Na_2HPO_4	Di-Sodium Hydrogen Phosphate
NaOH	Sodium hydroxide
ND	Not Determine
NEAA	Non- Essential Amino Acid
NH	New Hampshire
$-NH_2$	Amino Group
NH_3	Ammonia
nm	nanometer
OPA	O - phthaldialdehyde
P	Significant level
R	Variable Group
RBF	Round Bottom Flask
RNA	Ribonucleic acid
rpm	Round per minute
SAS	Statistical analysis system
SBM	Soybean Meal
SEM	Standard Error Means
TiO_2	Titanium Oxide
tRNA	Transfer RNA
Ub	Ubiquitin
UPM	University Putra Malaysia
UPP	Proteasome pathway
v/v	Volume Versus Volume
VFA	Volatile Fatty Acid
w/v	Weight Versus Volume
WG	Weight Gain

CHAPTER 1

INTRODUCTION

Agriculture is one of the largest sectorin Malaysian economy and it contributed about 7% to the gross domestic products (GDP) in year 2013 (Abidin *et al.,* 2014). Considering overall performance of the agricultural sector in the year 2012, livestock sector contributed about 11.7%. Among the entire livestock subsector, poultry industry is the most viable industry in Malaysia due to its advancedcommercialization and integration in the production systems (Economic report, 2013).The rapid progress of this sector was partly due to advance technology, genetic improvement and structural changes in the industry.This enhances the private sector to plays an important role in the research and development divisions.

Poultry industry in Malaysia has been self-sufficient since 1984. On the whole, the poultry subsector contributed about of RM10.73 billion, includingpoultry meatworth RM7.07 billion and eggsworthRM3.66 billion. Other than that, 76.14% of the ex-farm output valuealso contributed to the industry (DVS, 2012/2013).Since 1984, the production in the country is more than sufficient to meet domestic demands. In addition, this subsector provides opportunities for poultry products to be exported all over the world and it is expected to integrate and consolidate further to become more efficient and more productive in order to capitalize the growing global export market. However, this subsector is still dependent on imported inputs such as breeding stock and feed grain especially energy and protein sources, feedstuff and animal vaccines that makes it uncompetitive to the changes in world price of such inputs.In the recent years, farmers encountered numerous obstacles due to fluctuation of feed price in the poultry industry (Lepleaideur, 2004). As a result, they are forced to tolerate the increased cost of productionwhich contributes about 75% of feed cost per gram (Conolly, 2012). From the total cost of the feed, 95% is used to meet energy and protein requirements, while 3 to 4% for major mineral, trace mineral and vitamin requirements.Furthermore, 1 to 2%is used tospendfor various feed additives. Feed cost is expected to continue in an upward swing for another 20 years due to a shortage of foodstuffs and biofuel policies (Baker, 2009).

Protein is one of the important nutrients in feedstuff and it is necessary to fulfill the basic nutrient requirement of animals (NRC, 1994). Concentratedproteins include soybean meals and other oilseed meals, cottonseed meals and animal protein sources. Since protein, in general,is one of the most expensive feed ingredients, targeted rations are used inindustries to decrease the quantity of protein in the diet of the broiler chickens. The chickens will be in need ofloweramount of protein as they age. Nevertheless, it may not be cost-effective to have different diets for starters, growers, and finishers for small-scale producers.

In poultry diet, different rations are often used to formulate the broiler's diet andit depends on the production stage of the broiler chickens. Proteins in starter rations are higheras it containsexpensive feed ingredients to fulfill the broiler's requirement. However, protein content for grower and finisher rations can be lower since older broiler chickens have lower protein consumption. Starter diets contains of 24% protein, while grower and finisher diet consists of 20% and 18% protein, respectively (Cheeke, 1991).It is well known for more than 20 years, whereby there are limitations to the amount of free amino acids that can replace the intact protein in order to achievethe maximum weight gain and feed efficiency of the broiler chicks. A vital example is the usage of corn and soybean since the limiting order in corn are lysine, threonine, tryptophan, arginine, valine and isoleucine, sulfur amino acid, phenylalanine, tyrosine, and histidine. Interestingly, the order of limiting amino acids in soybean meal waslysine and valine,threonine, sulfur amino acid, and histidine (Baker, 2009).

Protein is known for its sources of amino acids and an ideal protein concept contains all amino acids in the exact amount and ideal proportion. This helps to maintain and fulfill the chicken requirement at the same time reduce the feed cost (Baker, 2009). However, lately it has been reported that the sulfur amino acids which comprise of methionine and cysteine are limiting in broiler diets (Fatufe and Rodehutscord, 2005). Methionine plays a vital role to maintain proper amino acids balance in the body that stimulates growth, maximizes the carcass yield, reduces carcass fatness and promotes efficient feed intake and reduce production costs (Lamme *et al.*, 2005; Bunchasak, 2009). The second that limiting the amino acid in the broiler chicken's diets is lysine beside methionine, and it is mainly involved in protein deposition such as high lean meat deposition (Bellaver *et al.*, 2002). Unlike lysine, threonine is not only used for protein deposition, but it plays several other vital functions. Threonine is the third that limiting the amino acid in broiler diets and is particularly involved in the maintenance processes and the synthesis of immune protein to the broiler. These limited amino acids should be supplemented in low crude protein diet since the protein level is reduced and the available amino acids in the diet are reduced as well.We carry out this study by putting forward the hypothesis that feeding diet withlow crude protein that is supplemented by optimum level of lysine and methionine will support optimum nutrient requirement of the broiler chickens, leads to improvedperformance of growth, digestibility of nutrient, immune response, morphology of the intestine, and intestinal microflora population of the broiler chickens. Therefore, this study embarks with the objectives:

i. To determine the optimum reduction level of crude protein in the diet while supplemented with lysine, methionine and threonine on growth performance, nutrient digestibility, intestinal morphology, microflora count and volatile fatty acid profile of the broiler chickens.

ii. To identify the optimum level of lysine and methionine supplementation in the low crude protein diet on growth performance, nutrient digestibility, intestinal morphology, microflora count and immunity profile of the broiler chickens.

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