



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF LOW PROTEIN DIET FORTIFIED WITH LYSINE AND
METHIONINE ON PERFORMANCE OF LAYER HEN***

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METHIONINE ON PERFORMANCE OF LAYER HEN**

By

TENESA A/P MOHAN

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

October 2015

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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

EFFECTS OF LOW PROTEIN DIET FORTIFIED WITH LYSINE AND METHIONINE ON PERFORMANCE OF LAYER HEN

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October 2015

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Protein is one of the important nutrient in diet that needs to be fulfilled for the basic nutrient requirement of animals. Ideal protein diets are based on meeting animals' amino acid requirements for protein accretion and maintenance, while avoiding deficiencies and excesses. The present study was conducted to evaluate the effects of reducing dietary crude protein fortified with lysine and methionine on layer hen performance. Two experiments were conducted in this study. In the first experiment, a total of 144 Hisex Brown aged 16 weeks were randomly assigned to four dietary treatments. The birds were offered 17.5% to 16.5% crude protein supplemented with commercial amino acids (L-Lysine, DL-Methionine) till the birds are 32 weeks old. The amino acids 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 maintained whilst the level of methionine and lysine was manipulated to the high, normal and low levels in the diets. A total of 144 Hisex Brown birds aged 19 weeks were used in this study with 9 treatments till they were of 32 weeks of age. Dietary treatments consisted of 0.77%, 0.97% and 1.77% lysine and 0.42%, 0.46% and 0.50% methionine in 17% crude protein. In the first experiment, higher ($P < 0.05$) egg production, egg mass, Lactic Acid Bacteria microflora and Lactic Acid Bacteria to *Enterobacteriaceae* ratio was observed in 17% CP. The 17% CP had lower ($P < 0.05$) Feed Conversion Ratio and faecal pH compared to other treatment groups. The 17% crude protein diet with amino acid supplementation had a better egg production, increased small intestine villus height, increased liver and spleen weight and promoted growth of beneficial Lactic Acid Bacteria microflora than birds fed with commercial diet. The second experiment concluded that 0.97% lysine and 0.50% methionine levels in the diets had a better Feed Conversion Ratio, Plasma Immunoglobulin G, Plasma Immunoglobulin M, increased Lactic Acid Bacteria microflora, increased Lactic Acid Bacteria to *Enterobacteriaceae* ratio, reduced *Enterobacteriaceae* count and faecal pH. In conclusion, feeding 17% CP with 0.97% lysine and 0.50% methionine to layer hen could be optimal for maximizing production performance, small intestine absorptive capacity and immune response in layer hens.

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

KESAN DIET RENDAH PROTEIN DENGAN PENAMBAHAN LYSINE DAN METHIONINE KEPADA AYAM PENELUR

Oleh

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Protein adalah salah satu nutrien yang penting dalam diet yang perlu dipenuhi bagi keperluan asas haiwan. Konsep protein ideal adalah berdasarkan kepada memenuhi keperluan asid amino haiwan untuk pertambahan protein dan penyelenggaraan, sambil mengelakkan kekurangan dan lebihan. Kajian ini telah dijalankan untuk menilai kesan mengurangkan protein kasar di dalam diet dan diperkaya dengan lysine dan methionine terhadap prestasi ayam penelur. Dua eksperimen telah dijalankan dalam kajian ini. Dalam eksperimen pertama, sebanyak 144 Hisex Brown yang berusia 16 minggu telah dibahagikan secara rawak kepada empat diet. Ayam telah diberi 17.5% sehingga 16.5% protein kasar ditambah dengan asid amino komersial (L-Lysine dan DL-Methionine). Asid amino di dalam diet yang berbeza telah diselaraskan ke tahap yang sama. Daripada lanjutan daripada eksperimen pertama, eksperimen kedua dijalankan. Tahap optimum diet protein kasar dikekalkan iaitu 17% manakala tahap lysine dan methionine telah dimanipulasi ke tahap yang tinggi, normal dan rendah dalam diet. Sebanyak 144 ayam Hisex Brown berumur 19 minggu telah digunakan dalam kajian ini dengan 9 diet sehingga mereka adalah 32 minggu. Rawatan diet terdiri daripada 0.77%, 0.97% dan 1.77% lysine dan 0.42%, 0.46% dan 0.50% methionine. Dalam eksperimen pertama, 17% protein kasar menunjukkan peningkatan ($P < 0.05$) penghasilan telur, mass telur, mikroflora Bakteria Laktik Asid, Bakteria Laktik Asid nisbah Enterobakteria. Kumpulan yang mengandungi 17% protein kasar menunjukkan Nisbah penukaran makanan dan pH tinja yang rendah ($P < 0.05$) berbanding kumpulan rawatan lain. Kumpulan yang mengandungi 17% protein kasar dengan tambahan asid amino mempunyai pengeluaran yang lebih baik telur, peningkatan tinggi villus dalam morfologi usus, penambahan berat hati dan limpa dan mikroflora Bakteria Laktik Asid bermanfaat meningkat berbanding makanan diet komersial. Eksperimen kedua memberi kesimpulan bahawa 0.97% lysine dan 0.50% methionine dalam diet mempunyai nisbah penukaran makanan yang lebih baik, nilai lebih tinggi bagi Plasma Immunoglobulin G, Plasma Immunoglobulin M, meningkatkan mikroflora Bakteria Laktik Asid, meningkat Bakteria Laktik Asid nisbah Enterobakteria dan mengurangkan kiraan Enterobakteria dan pH tinja. Kesimpulannya, memberi makanan 17% CP dengan 0.97% lysine dan 0.50% methionine untuk ayam penelur boleh memberikan keputusan yang optimum untuk memaksimumkan prestasi pengeluaran, kapasiti penyerapan usus kecil dan tindak balas imun dalam ayam penelur.

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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 of Science. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	ii
ACKNOWLEDGEMENTS	iii
APPROVAL	iv
DECLARATION	vi
LIST OF TABLES	xi
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xiv
 CHAPTER	
 1. INTRODUCTION	 1
 2. LITERATURE REVIEW	
2.1. Protein in Poultry Diets	3
2.2. Amino Acids	4
2.3. Ideal Amino Acid Profile	6
2.4. Low Protein Diets	9
2.4.1. Advantages of Reducing Dietary Crude Protein	9
2.4.2. Effects of Low Crude Protein Diet with Amino Acid Supplementation on Production and Egg Quality	10
2.4.3. Effects of Low Crude Protein Diet with Amino Acid Supplementation on Intestinal Morphology and Bacterial Ecology	11
2.4.4. Effects of Low Crude Protein Diet with Amino Acid Supplementation on Immunity	13
 3. GENERAL MATERIALS AND METHODS	
3.1. Feeding Trial	15
3.1.1. Animal Housing System and Management	16
3.1.2. Data Collections and Samplings	16
3.2. Proximate Analysis	16
3.2.1. Dry Matter	16
3.2.2. Ash	16
3.2.3. Crude Protein	17
3.2.4. Ether extract (EE)	17
3.2.5. Crude Fiber	17
3.2.6. Gross Energy	18
3.3. Amino Acid Analysis	18
3.3.1. Preparation of Samples for Amino Acid Analysis	18
3.3.1.1. Acid Hydrolysis	19
3.3.1.2. Performic Acid Oxidation	19
3.3.1.3. Alkaline Hydrolysis	19
3.4. Data Sampling and Collection	19
3.4.1. Production Performance	19
3.4.2. Egg Quality	20

3.4.3.	Faecal pH, Faecal Lactic Acid Bacteria (LAB) and <i>Enterobacteriaceae</i> (ENT) count	20
3.4.4.	Small Intestine Morphometry	20
3.4.5.	Spleen and Liver Weight	22
3.4.6.	Plasma Immunoglobulin Profile	22
4.	EFFECTS OF FEEDING DIFFERENT LEVELS OF LOW CRUDE PROTEIN DIETS WITH AMINO ACID SUPPLEMENTATION ON LAYER HEN PERFORMANCE	
4.1.	Introduction	23
4.2.	Materials and Methods	23
4.2.1.	Birds and Experimental Design	23
4.2.2.	Data and Sample Collection	26
4.2.3.	Data Analysis	26
4.3.	Results	26
4.3.1.	Production Performance	26
4.3.2.	Egg Quality	27
4.3.3.	Small Intestine Morphometry	28
4.3.4.	Spleen and Liver Weight	29
4.3.5.	Faecal pH and Faecal Microflora Count	29
4.3.6.	Plasma Immunoglobulin	30
4.4.	Discussion	31
4.4.1.	Production Performance	31
4.4.2.	Egg Quality	32
4.4.3.	Small Intestine Morphometry	33
4.4.4.	Spleen and Liver Size	34
4.4.5.	Faecal pH and Faecal Microflora Count	35
4.4.6.	Plasma Immunoglobulin	35
4.4.7.	Cost Benefit Analysis	36
4.5.	Conclusion	36
5.	EFFECTS OF FEEDING LOW CRUDE PROTEIN DIETS WITH DIFFERENT LEVELS OF LYSINE AND METHIONINE ON LAYER HEN PERFORMANCE	
5.1.	Introduction	37
5.2.	Materials and Methods	37
5.2.1.	Birds and Experimental Design	37
5.2.2.	Data and Sample Collection	40
5.2.3.	Data Analysis	40
5.3.	Results	40
5.3.1.	Production Performance	40
5.3.2.	Egg Quality	43
5.3.3.	Small Intestine Morphometry	46
5.3.4.	Spleen and Liver Weight	49
5.3.5.	Faecal pH and Faecal Microflora Count	52
5.3.6.	Plasma Immunoglobulin	55
5.4.	Discussion	58
5.4.1.	Production Performance	58
5.4.2.	Egg Quality	59
5.4.3.	Small Intestine Morphometry	60
5.4.4.	Spleen and Liver Weight	61

5.4.5.	Faecal pH and Faecal Microflora Count	61
5.4.6.	Plasma Immunoglobulin	62
5.4.7.	Cost Benefit Analysis	63
5.5.	Conclusion	63
6.	GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATION	
6.1.	General Discussion	64
6.2.	Conclusion	65
6.3.	Recommendations	65
	REFERENCES	66
	BIODATA OF STUDENT	83
	LIST OF PUBLICATIONS	84

LIST OF TABLES

Table	Page
2.1 Ideal Amino Acid Profile for Layer Hens Based on Previous Study	7
3.1 Hisex Brown Nutrition Management Guide according to Layer Hen Age	15
4.1 Ingredients and Composition of Experimental Diets	25
4.2 Production Performance of Layer Hens at Different Levels of Crude Protein Diets with Amino Acid Supplementation	27
4.3 Egg Quality of Layer Hens at Different Levels of Crude Protein Diets with Amino Acid Supplementation	27
4.4 Villus Height and Crypt Depth of Layer Hens at Different Levels of Crude Protein Diets with Amino Acid Supplementation	28
4.5 Spleen and Liver Weight of Layer Hens at Different Levels of Crude Protein Diets with Amino Acid Supplementation	29
4.6 Faecal pH and Faecal LAB and ENT Count of Layer Hens at Different Levels of Crude Protein Diets with Amino Acid Supplementation	30
4.7 Plasma IgG and IgM of Layer Hens at Different Levels of Crude Protein Diets with Amino Acid Supplementation	30
5.1 Ingredients and Composition of Experimental Diets	38
5.2 Production Performance as Affected by Lysine and Methionine Factor in Low Crude Protein Diets Fed to Layer Hens	41
5.3 Production Performance of Layer Hens Supplemented with Different Levels of Lysine and Methionine in Low Crude Protein Diets	42
5.4 Egg Quality as Affected by Lysine and Methionine Factor in Low Crude Protein Diets Fed to Layer Hens	44
5.5 Egg Quality of Layer Hens Supplemented with Different Levels of Lysine and Methionine in Low Crude Protein Diets	45
5.6 Small Intestine Morphometry as Affected by Lysine and Methionine Factor in Low Crude Protein Diets Fed to Layer Hens	47
5.7 Small Intestine Morphometry of Layer Hens Supplemented with Different Levels of Lysine and Methionine in Low Crude Protein Diets	48

5.8	Spleen and Liver Weight as Affected by Lysine and Methionine Factor in Low Crude Protein Diets Fed to Layer Hens	50
5.9	Spleen and Liver Weight of Layer Hens Supplemented with Different Levels of Lysine and Methionine in Low Crude Protein Diets	51
5.10	Faecal pH and Faecal Microflora Count as Affected by Lysine and Methionine Factor in Low Crude Protein Diets Fed to Layer Hens	53
5.11	Faecal pH and Faecal Microflora Count of Layer Hens Supplemented with Different Levels of Lysine and Methionine in Low Crude Protein Diets	54
5.12	Plasma IgG and IgM as Affected by Lysine and Methionine Factor in Low Crude Protein Diets Fed to Layer Hens	56
5.13	Plasma IgG and IgM of Layer Hens Supplemented with Different Levels of Lysine and Methionine in Low Crude Protein Diets	57

LIST OF FIGURES

Figure		Page
3.1	Villus Height Measurement	21
3.2	Crypt Depth Measurement	22



LIST OF ABBREVIATIONS

°C	Degree Celsius
μL	Microliter
μm	Micrometer
AA	Amino Acid
ACN	Acetonitrile
ANOVA	Analysis Of Variance
AOAC	Association of Analytical Communities
BW	Body Weight
CD	Crypt Depth
CFU	Colony forming units
cm	Centimeter
CP	Crude Protein
dH ₂ O	Deionized water
DM	Dry Matter
EAA	Essential Amino Acid
EDTA	Ethylene Diaminetetraacetic Acid
EE	Ether Extract
ENT	<i>Enterobacteriaceae</i>
FCR	Feed Conversion Ratio
FI	Feed Intake
FMOC	Fluorenylmethoxycarbonyl chloride
g	Gram
H	Hydrogen Atom
H ₂ O ₂	Hydrogen Peroxide
H ₂ SO ₄	Hydrogen Sulfuric Acid
HBr	Hydrogen Bromide
HCL	Hydrochloric Acid
HPLC	High Pressure Liquid Chromatography
IBD	Infectious Bursal Disease
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Kcal	Kilo Calorie
LAB	Lactic Acid Bacteria
LBWG	Live Body Weight Gain
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
MRS – agar	Lactobacillus – Agar De Man, Rogosa and Shape
MRS	Man Rogosa Sharpe
N	Nitrogen
Na ₂ HPO ₄	Di-Sodium Hydrogen Phosphate
NaOH	Sodium hydroxide

NEAA
-NH₂
NH₃
nm
OPA
P
R
rpm
SAS
SBM
SEM
TiO₂
TSAA
UPM
UPP
v/v
VH
w/v

Non- Essential Amino Acid
Amino Group
Ammonia
nanometer
O - phthaldialdehyde
Significant level
Variable Group
Revolutions per minute
Statistical analysis system
Soybean Meal
Standard Error Means
Titanium Oxide
Total Sulfur Amino Acid
University Putra Malaysia
Proteasome pathway
Volume Versus Volume
Villi Height
Weight Versus Volume

CHAPTER 1

INTRODUCTION

Over the past years, worldwide poultry industry has developed significantly to fulfill and accommodate the ever increasing demands for poultry products. In developing countries, poultry production is one of the rapidly growing animal protein supply (Adeyemo *et al.*, 2012). Despite its rapid progress, there still lies several concerns in the poultry industry, for example production, growth reduction and increase in feed costs. Increase in feed cost will subsequently lead to increase in poultry products prices and eventually followed by increase of other food prices (MacDonald, 2008).

Nutritionists and poultry producers have shown immense interest in lowering protein level in poultry rations as this will have several benefits. Firstly, there will be diet costs reduction. Production cost can be reduced with lower crude protein diet, as a big SRUWLRQRIWKHFRVWOLHVLQIXOILQJWKHELUGVDPQLQRDFLGUHTXLUHPHQWV7R by environmental reasons; by reducing crude protein in the diet, nitrogen emission could be reduced. Other than that, there would be reduction in excess amino acids that are not used by the birds (Perry *et al.*, 2004). Maintaining growth and profitability, producing high quality products and decreasing production cost are important in the poultry industry (Eits *et al.*, 2005; Corzo *et al.*, 2004).

Similarly, Kamran *et al.* (2004) pointed out that reducing feed cost for optimum economic return proved to be major concern for the modern poultry as feed represents approximately 70% of total production cost. Diets can be formulated on a crude protein (CP) basis if several protein supplying feed ingredients such as corn, soybean meal, and meat and bone meal are used. Laying hens have a physiological requirement for protein and amino acids for body and egg proteins synthesis (Bregendahl and Roberts, 2006).

Ideal protein concept was established in order to meet the requirement of the bird and at the same time to maximize production and profitability. This can be achieved by lowering CP in diets combined with inclusion of limiting amino acid to their required levels. The goal is to provide ideal levels of essential amino acids to optimize hen performance while minimizing excess amino acid provided by dietary CP (Novak *et al.*, 2006). This feeding strategy is becoming increasingly popular with a variety of synthetic amino acid becoming more available and affordable (Keshavarz and Austic, 2004; Meluzzi *et al.*, 2001).

In laying hens fed corn soybean diets, methionine and lysine are usually the first and the next limiting amino acids. Specifically for laying hens, methionine is considered the first limiting amino acid in low protein corn soybean meal diet. However, there is still controversy about this subject in the literature (Abdel-Maksoud *et al.*, 2010). Numerous studies have reported that the efficiency of protein utilization is increased by supplementation of methionine and lysine (Alagawany *et al.*, 2014; Burley *et al.*, 2013; Waldroup *et al.*, 2005; Novak *et al.*, 2004).

Formulating diets based on an ideal protein concept with supplementation of amino

acid has been done in many studies in different animal species such as laying hens, broilers, pigs and turkeys (Namroud *et al.*, 2008; Roberts *et al.*, 2007ab; Keshavarz and Austic, 2004). Burley *et al.* (2013) highlighted a number of university scale studies regarding feeding laying hen on ideal amino acid basis. Burley *et al.* (2013) observed that this feeding strategy can maintain most egg production parameters for various durations of time and production phase and at the same time increase producer revenue by lowering feed costs. The promising results of these studies have led to the question of whether reduced CP in laying hen diets, supplemented with essential amino acid, has effect

The objectives of this study are to investigate the effect of feeding low CP diet with different levels of lysine and methionine on production performance, egg quality, faecal Lactic acid bacteria (LAB) and *Enterobacteriaceae* (ENT) count, gut morphometric and immune response of layer hen. Thus the specific objectives of this study are:

1. To determine the production performance, egg quality, LAB and ENT intestinal microflora, villus height and crypt depth, spleen and liver weight and IgG and IgM levels as a result of low CP diet.
2. To determine the effect of different levels of lysine and methionine levels in low CP diet on production performance, egg quality, LAB and ENT intestinal microflora, villus height and crypt depth, spleen and liver weight and IgG and IgM levels.

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