UNIVERSITI PUTRA MALAYSIA

EFFECTS OF FEEDING LOW-PROTEIN DIETS FORTIFIED WITH AMINO ACIDS ON BROILER CHICKENS UNDER HIGH ENVIRONMENTAL TEMPERATURES

ELMUTAZ ATTA AWAD MOHAMED

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By

ELMUTAZ ATTA AWAD MOHAMED

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

April 2016

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DEDICATION

This work is dedicated to the soul of my late father
(May Allah rest his soul and forgive him(
My mother who always prays for my success
My brothers for their constant encouragement and support
My wife and son for being my inspiration
My friends and colleagues

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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April 2016

Chairman : Professor Zulkifli B Idrus, PhD
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Feeding amino acids (AA) fortified low-protein diets is of interest to poultry nutritionists because of the ability to reduce feed cost, environmental pollution, and effect of heat stress on birds. However, it is still unclear to what extent the AA fortification can replace the crude protein (CP) without affecting the broiler performance, especially under high environmental temperatures. Hence, this study aimed at determining the effect of using crystalline AA to lower CP in broilers diets on growth performance, carcass characteristics, internal organs weights, blood metabolites, and physiological responses of broilers under high environmental temperatures. Five experiments were conducted; Experiments 1 through 3 were conducted with day-old male broilers under the tropical climate (22-33°C and 70-90% relative humidity) from 1-21 days (starter period).

In Experiment 1, five isocaloric diets were formulated in a gradual CP decline from 22.2% to 16.2% at 1.5% intervals. Weight gains (WG) were suppressed by feeding 16.2 and 17.7% CP. Feeding 16.2% CP reduced serum total protein (TP), uric acid (UA), and increased triglyceride (TG). Heart weights increased but no changes in liver and abdominal fat weights.

Experiment 2, the ability of non-essential amino acids (NEAA) or further essential amino acids (EAA) fortification to overcome the poorer performance in birds fed 16.2% CP was evaluated. Five diets were formulated: (i) 22.2% CP (positive control; PC) (ii) 16.2% CP + all EAA to meet the requirements, negative control (NC) (iii) through (v) were obtained by supplementing NC diet with EAA, NEAA, or EAA+NEAA to equal the AA levels in the PC, respectively. Fortification with EAA alone, only improved FI, whereas, NEAA or EAA + NEAA enhanced the WG and FI.

Experiment 3 was conducted to find out whether all NEAA or a single NEAA was behind the restored performance in experiment 2. Eight diets were formulated: (i) PC; (ii) NC; (iii) NC + glycine (Gly); diets (iv) through (vii) were obtained by supplementing NC diet with individual glutamic acid, proline, alanine, or aspartic acid, respectively; (viii) NC + all NEAA. Among individual NEAA, only Gly had the potential to improve the growth performance of birds.

In Experiment 4, an equal number of male and female broilers were assigned to five diets (PC, NC, and more three diets obtained by supplementing the NC diet with higher Gly concentrations) for a starter and grower (22- 42 days) feeding periods under tropical climate. No significant diet × gender interaction was observed for all the parameters measured. Results for WG and FI showed that birds were more responsive to additional Gly fortification during grower period, where a significant linear response to improving WG and FI was observed with increasing Gly levels. Diet effect was significant for performance parameters, proportional liver and abdominal fat weights, TG and UA, and litter moisture and nitrogen contents. The gender effect was significant for performance parameters, abdominal fat, and litter's moisture and nitrogen.

In Experiment 5, from 1 – 21 days, an equal number of birds were fed either (i) a normal-protein diet or (ii) Gly fortified low-CP diet, under optimum temperatures. On day 22, the total birds from the starter period were redistributed into 12 treatments in a 4 × 3 factorial arrangement {(4 diets: (i) normal-protein during the starter and grower periods, NPNP; (ii) normal-protein during the starter and low-CP diet during the grower period, NPLP; (iii) low protein-CP diet during the starter and normal-protein during the grower, LPNP; (iv) low-CP diets during both periods, LPLP)} × {(3 thermal treatments (i) unheated, constant 23±1°C; (ii) cyclic heated, cyclic 34±1°C for 7 hours; and (iii) constant heated, constant 34±1°C)}. During the starter period, feeding low-CP diet resulted in growth performance equal to those birds fed normal-protein diet. During the grower period, there were significant diet × temperature interactions for growth performance, intestinal morphology and microbial, and triiodothyronine (T_3) parameters. Feeding low-CP diet under constant heat stress resulted in poorer growth performance; lower intestinal *Lactobacillus* and higher *Clostridium*; as well as lower T_3. On contrast, feeding low-CP diet under unheated, and cyclic heat stress resulted in growth performance; intestinal microbial populations and morphology parameters equally well to those fed normal-protein diet as well as higher T_3.

Together, it can be concluded that by using EAA, protein in broilers starter diet can be reduced to 19.2% under tropical climate. The addition of extra EAA may not improve the suppression in performance. However, fortification with Gly may improve but not support overall standard growth performance under tropical climate. The Gly fortified low-CP diets can be fed to broilers under acute heat stress without any adverse effects.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PEMAKANAN DIET RENDAH PROTEIN YANG DIPERKAYA DENGAN ASID AMINO TERHADAP AYAM PEDAGING DI PERSEKITARAN SUHU TINGGI

Oleh

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Pemakanan diet rendah protein yang diperkaya asid amino (AA) adalah menarik minat pakar pemakan ayam kerana kemampuannya untuk mengurangkan kos makanan, pencemaran alam sekitar, dan kesan tegasan haba kepada burung. Walau bagaimanapun, ia masih tidak jelas sejauh mana kebolehan AA menggantikan protein mentah (CP) tanpa menjejaskan prestasi ayam pedaging, terutamanya di bawah suhu sekitar yang tinggi. Oleh itu, kajian ini bertujuan menentukan kesan penggunaan kristal AA dalam diet rendah CP terhadap prestasi pertumbuhan, ciri-ciri karkas, berat organ-organ dalaman, metabolit darah, dan tindak balas fisiologi ayam pedaging di bawah suhu ambien yang tinggi. Lima eksperimen telah dijalankan; Eksperimen 1, 2 dan 3 telah dijalankan dengan ayam jantan satu hari dalam iklim tropika (22-33 °C dan 70-90% kelembapan relatif) dari 1–21 hari (fasa pemula).

Dalam Eksperimen 1, lima diet isokalorik telah dirumuskan dan mengandungi CP yang dikurangkan dari 22.2% ke 16.2% dengan selang 1.5%. Penambahan berat (WG) telah disekat dengan memberi makan 16.2 dan 17.7% CP. Pemakanan 16.2% CP mengurangkan jumlah protein (TP), asid urik (UA), dan meningkatkan trigliserida (TG) dalam serum. Berat jantung meningkat tetapi tiada perubahan pada berat hati dan lemak pada bahagian abdomen.

Dalam Eksperimen 2, kebolehan asid amino tidak perlu (NEAA) atau penambahan asid amino perlu (EAA) untuk mengatasi prestasi buruk pada burung yang diberi makan 16.2% CP telah dinilai. Lima diet telah dirumuskan: (i) 22.2% CP (kawalan positif; PC) (ii) 16.2% CP + semua EAA untuk memenuhi keperluan (kawalan negatif; NC) (iii) hingga (v) telah diperolehi dengan menambah diet NC dengan EAA, NEAA, atau EAA + NEAA untuk menyamai tahap AA dalam PC, masing-masing. Penambahan dengan EAA, hanya memperbaiki FI, manakala, NEAA atau EAA + NEAA meningkatkan WG dan FI.

Eksperimen 3 telah dijalankan untuk mengetahui sama ada semua NEAA atau NEAA secara individu yang menyumbang kepada prestasi dalam eksperimen 2. Lapan diet telah dirumuskan: (i) PC; (Ii) NC; (Iii) NC + glycine (Gly); diet (iv) melalui (vii) telah diperoleh dengan penambahan diet NC dengan asid glutamik, proline, alanine, atau asid aspartik secara individu, masing-masing; (Viii) NC + semua NEAA. Antara setiap NEAA, hanya Gly mempunyai potensi untuk meningkatkan prestasi pertumbuhan ayam.

Dalam Eksperimen 4, ayam pedaging jantan dan betina dengan jumlah yang sama telah diberikan lima diet (PC, NC, dan tiga lagi diet diperolehi dengan penambahan diet NC dengan kepekatan Gly lebih tinggi) untuk fasa pemula dan penggemuk (22-42 hari) dan dipelihara di bawah iklim tropika. Tiada interaksi ketara antara diet × jantina untuk semua parameter yang diukur. Keputusan untuk WG dan FI menunjukkan bahawa burung adalah lebih responsif kepada penambahan Gly dalam fasa penggemuk, di mana tindak balas linear yang signifikan diperhatikan apabila peningkatan WG dan FI dengan berlakunya peningkatan tahap Gly. Kesan diet adalah ketara untuk parameter prestasi, berat hati dan lemak di bahagian abdomen, TG dan UA, dan kelembapan sarap dan kandungan nitrogen. Kesan jantina adalah penting untuk parameter prestasi, lemak di bahagian abdomen, dan kelembapan sampah dan nitrogen.

Dalam Eksperimen 5, dari 1-21 hari, ayam dengan jumlah yang sama diberi makan sama ada (i) diet protein normal atau (ii) diet rendah-CP diperkaya Gly, pada suhu optimum. Pada hari ke 22, jumlah burung dari fasa pemula telah diagihkan semula kepada 12 rawatan dalam susunan 4 × 3 faktorial {(4 diet: (i) normal protein semasa fasa pemula dan penggemuk, NPNP; (ii) normal protein pada fasa pemula dan diet rendah-CP pada fasa penggemuk, NPLP; (iii) diet rendah protein-CP pada fasa pemula dan normal protein pada fasa penggemuk, LPNP; (iv) diet rendah CP semasa kedua-dua fasa, LPLP)} × {(3 rawatan haba (i) tidak panas, 23±1°C berterusan; (ii) kitaran panas, kitaran 34±1°C selama 7 jam; dan (iii) panas berterusan, 34±1°C)}. Dalam fasa pemula, diet rendah-CP menyebabkan prestasi pertumbuhan sama dengan ayam diberi makan diet normal-protein. Dalam fasa penggemuk, terdapat interaksi ketara antara diet × suhu terhadap parameter prestasi pertumbuhan, morfologi usus dan mikrob, dan triiodothyronine (T_3). Pemberian makan diet rendah-CP pada tekanan haba menyebabkan prestasi pertumbuhan yang buruk; Lactobacillus usus lebih rendah dan Clostridium yang lebih tinggi; serta T_3 yang lebih rendah. Sebaliknya, pemberian makan diet rendah-CP diperkaya suhu tidak panas dan panas secara kitaran menyebabkan parameter prestasi pertumbuhan; populasi mikrob dan morfologi usus sama baik dengan ayam yang makan diet normal-protein serta T_3 yang lebih tinggi.

Kesimpulan yang boleh dibuat adalah dengan menggunakan protein EAA dalam diet pemula ayam daging boleh dikurangkan kepada 19.2% di bawah iklim tropika. Penambahan EAA tidak meningkatkan prestasi. Walau bagaimanapun, penambahan dengan Gly boleh memperbaiki, tetapi tidak menyokong standard prestasi pertumbuhan di bawah iklim tropika. Diet rendah-CP yang diperkaya Gly boleh diberikan kepada ayam daging di bawah tegasan haba akut tanpa apa-apa kesan buruk.

ACKNOWLEDGEMENTS

First and foremost praise be to Allah, the Beneficent, the most Merciful for enabling me to complete the present studies and thesis, and for often putting so many good people in my way.

I would like to express my sincerest thanks and gratitude to my distinguished major supervisor, Prof. Dr. Zulkifli Idrus for his exceptional guidance, advice, and motivation. I am endlessly thankful not only for his constant support, assistance, and the trust he has put on me but also for his enduring patience especially at the beginning of this study, and all of the time he has given to assuring the high research standards that he has. I would also like to thank my dedicated co-supervisor, Dr. Abdoreza Soleimani Farjam for his guidance, advice, and friendship. He has always been available to discuss every aspect of my research and has been a wonderful mentor from the beginning. I would like to extend my thanks to my respected co-supervisor, Prof. Dr. Loh Teck Chwen for his support, motivation, and encouragement throughout the duration of my study.

I would like to express my very great appreciation to the Sudanese Ministry of Higher Education and the University of Khartoum for the invaluable scholarship. I would also like to extend my appreciation to the Malaysian Ministry of Education for funding my research through the Long-term Research Grant Scheme (LRGS).

Special thanks should be given to Dr. Mohammad Faseleh Jahromi, for his assistance, support, and friendship. My special thanks are extended to Dr. Rony and Assoc. Prof. Dr. Ahmed Mujahid for reviewing Chapters V and VI, respectively, and giving very useful and constructive comments. My sincere thanks also go to my dearest friends Dr. Ahmed Aljuobori, Dr. Mohamed Alshelmani, and Mr. Alhassan Bello, for their assistance, advice, and constant support. I highly appreciate the invaluable assistance of my colleagues Mr./ Mrs./ Ms.: Mohamed Fadlullah, Shahrill, Elizabeth, Fatin, Syikin, Hafizah, Joshua, and Majid, who gave their time to assist me with the farm, sampling, and laboratory works.

I wish to express my great thanks to the staff of Poultry Research Unit, Faculty of Agriculture (Mr. Azam and Mr. Khairul); and the Animal Research Centre, Institute of Tropical Agriculture (Mr. Farhan, Mr. Eddy, and Mr. Hujan), for their endless assistance during my farm work. Also, I would like to thank the staff and postgraduate colleagues and friends of Animal Production Laboratory, Institute of Tropical Agriculture for all the help, patience, and incredible support throughout the entire course of my study. I wish to thank several people for their contribution to this study: Mr. Saiful, a lab technician in Serology laboratory, Faculty of Veterinary Medicine; Mr. Fadzlee, a lab technician in Radioisotope laboratory, Faculty of Medicine and Health Sciences; and Mr. Yap, a lab technician in Theriogenology laboratory, Faculty of Veterinary Medicine, for their invaluable technical assistance.

Most importantly, no words are ever enough to express my everlasting gratitude, appreciation and thanks to my beloved and wonderful family for being the light in my life. My mother, late father, and brothers without their warm love, care, sincere prayers and support, it would have been impossible for me to reach this level. My wife and son inspired me to keep on when I thought no longer could. I feel extremely grateful to have such a wonderful family, and I deeply appreciate their unlimited patience and incredible love for which no words can describe.

I certify that a Thesis Examination Committee has met on 27 April 2016 to conduct the final examination of Elmutaz Atta Awad Mohamed on his thesis entitled "Effects of Feeding Low-Protein Diets Fortified with Amino Acids on Broiler Chickens under High Environmental Temperatures" in accordance with the Universities and University Colleges 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 Doctor of Philosophy.

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

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LIST OF ABBREVIATIONS

AA	Amino Acids
AABA	L-α-Amino-n-Butyric Acid
ACTH	Adrenocorticotrophic hormone
AGP	Serum α-1-acid glycoprotein Concentration
Ala	Alanine
Alb	Serum Albumin
APPs	Acute Phase Proteins
Arg	Arginine
Asp	Aspartic Acid
ANOVA	Analysis of Variance
AOAC	Association Official Agricultural Chemists
Ca	Calcium
CFU	Colony Forming Unit
CH_3CN	Acetonitrile
$CH_3COONa.3H_2O$	Sodium acetate trihydrate
Cl^-	Chloride ion
cm	Centimeter
CP	Crude Protein
CPN	Serum Ceruloplasmin Concentration
CORT	Serum Corticosterone Concentration
CRD	Completely Randomized Design
Cys	Cysteine
d	Day
DEP	Dietary Electrolyte Balance
DM	Dry Matter
DNA	Deoxyribonucleic acid
EAA	Essential Amino Acid
FCR	Feed Conversion Ratio
FI	Feed intake
IU	International Unit
g	Gram
GLM	General Linear Models
Glu	Glutamic Acid

Gly	Glycine
h	Hour
HCl	Hydrochloride acid
HCOOH	Formic acid
His	Histidine
HPA	Hypothalamic-Pituitary-Adrenal
HPLC	High Performance Liquid Chromatography
HS	Heat stress
H_2O_2	Hydrogen Peroxide
H_2SO_4	Sulphuric acid
H_3PO_4	Phosphoric acid
Ile	Isoleucine
K^+	Potassium ion
Kcal/kg	Kilocalorie per Kilogram
L	Liter
Leu	Leucine
$LiOH.H_2O$	Lithium hydroxide monohydrate
LVI	Limited volume insert
Lys	Lysine
m	Meter
ME	Metabolizable Energy
Meth	Methionine
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimetre
mM	Mille mole
N	Nitrogen
NaOH	Sodium hydroxide
NC	Negative control
NEAA	Non-Essential Amino acids
NH_3	Ammonia
nmole	Nanomole
nm	Nanometer
NRC	National Research Council

OVT	Serum Ovotransferrin Concentration
P	Phosphorus
PC	Positive control
PCR	Polymerase chain reaction
PER	Protein Efficiency Ratio
pH	Hydrogen ion concentration
Phe	Phenylalanine
pmole	Picomole
Pro	Proline
RIA	Radioimmunoassay
r-T_3	Reverse triiodothyronine
rpm	Revolutions Per Minute
SAS	Statistical Analysis System
Sec	Second
SEM	Standard Error of Mean
Ser	Serine
TEA	Triethlamine
TG	Serum Triglyceride
THI	Temperature and humidity index
Thr	Threonine
TMA	Trimethylamine
TP	Serum Total Protein
Trp	Tryptophan
Tyr	Tyrosine
T_3	Triiodothyronine
T_4	Tetraiodothyronine
UA	Serum Uric Acid
Val	Valine
WG	Weight gain
µg	Microgram
µL	Microliter
µm	Micrometer
µmole	Micromole
ºC	Degree Celsius
v.	Versus

CHAPTER 1

GENERAL INTRODUCTION

Poultry production, among other agricultural industries, has expanded significantly in the last few decades. In the tropics, demand for poultry meat and eggs is expected to be much higher as a consequence of the expected increase in human population. However, as of late, the prices of corn and soybean meal, which are the main ingredients in most poultry diets, have sharply increased due to higher international poultry product demand and competition with other industries such as biofuel production. Therefore, the increasing prices of imported feedstuffs and the hot temperatures throughout the year will affect the potential growth of the poultry industry in many tropical countries. Competitive feeding strategies such as using cheaper local or imported feed ingredients or dilution of dietary crude protein (CP) by supplementation of synthetic amino acids (AA) can decrease the production cost. Besides, lowering CP has a particular advantage for producers in the tropical region. It is well-established that heat increment of CP is highest compared to fat and carbohydrate (Musharaf and Latshaw, 1999). Thus, lowering CP can potentially alleviate heat stress (HS) adverse effects on poultry performance (Waldroup, 1982; Zaman et al., 2008). Therefore, poultry nutritionists are continuously optimizing formulations by supplementing various types and concentrations of AA to low-CP diets. The application of low-CP, AA-supplemented diets may also reduce environmental pollution by decreasing nitrogen (N) excretion.

Broiler chickens require a precise and balanced amount of essential amino acids (EAA) and nitrogen to synthesize protein and non-essential amino acids (NEAA), rather than CP per se (NRC, 1994). The early use of synthetic feed-grade EAA such as methionine and lysine (the first and second limiting amino acids in corn-soybean diets) contributed to the reduction of CP and savings in cost. In last decade, work by Corzo et al. (2005); Dean et al. (2006) and Ospina-Rojas et al. (2014) suggested that when low-CP diet supplemented with a mixture of NEAA, it enhanced the growth performance of birds. Some other authors even reported similar enhancement by fortification with only single NEAA such as glycine and glutamic acid in both broilers and layers (Dean et al., 2006; Bezerra et al., 2015). However, all these studies have been conducted under temperate conditions, and no data is available specific to the hot and humid condition. It is reported that high environmental temperature may affect the nutrient requirement of birds and CP and AA digestibility of diet (Zuprizal et al., 1993; Ojano-Dirain and Waldroup, 2002b; Soleimani et al., 2010). Heat stress is known reduce growth performance and extend the time to reach to the marketing weight (Teeter and Belay, 1996; Hai et al., 2000; Ahmad and Sarwar, 2005).

The exact mechanism underlying the impaired growth performance in birds fed with excessively low CP diets is not clear yet. Furthermore, adding the environmental temperature factor to the puzzle may complicate the solution. To date, there is no agreement on the extent of reducing CP level by AA supplementation in that growth performance remains unchanged. Thus, current studies designed to elucidate the extent of feeding low-CP, AA-supplemented diets to broiler chickens reared under high environmental temperatures.

The specific objectives were:

(i) To determine the effect of varying low-protein diets on growth performance, selected serum metabolites, weight of organs, and abdominal fat of broiler chickens during starter period (1 – 21 d), under the hot and humid tropical condition.
(ii) To evaluate the growth performance, selected serum metabolites, organs weight, breast yield, and abdominal fat of broiler chickens fed low-protein diets fortified with varying levels of essential or non-essential amino acids under the hot and humid tropical condition.
(iii) To examine the influence of individual non-essential amino acids fortification of low-protein diets on growth performance, selected serum metabolites, organ weight, breast yield, and abdominal fat in broiler chickens under the hot and humid tropical condition.
(iv) To investigate the influence of different levels of glycine fortification to low-protein diets on growth performance, carcass yield, the relative weight of organs and abdominal fat, selected serum metabolites, and litter characteristics of male and female broiler chickens under the hot and humid tropical condition.
(v) To evaluate the effect of feeding glycine fortified low-protein diet on growth performance, intestinal morphology and microbial population, physiological stress indicators, and triiodothyronine in broiler chickens reared under cyclic or constant high environmental temperatures.

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