INFLUENCE OF DIETARY TRYPTOPHAN TO LARGE NEUTRAL AMINO ACIDS RATIOS ON PERFORMANCE AND WELL-BEING OF BROILER CHICKENS SUBJECTED TO STRESSFUL CONDITIONS

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By

ALHASSAN USMAN BELLO

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

November 2016

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DEDICATION

With deep humility and great appreciation, this thesis is dedicated to

My late father, Mr. Bello Usman
(May Allah forgive him and have Mercy on his soul)
My mother, Mrs. Maimunat Abdullahi, who always support me with her pray for my success
My lovely wife for her constant encouragement, moral supports, and being my inspiration
My lovely sons, Bilal and Khalil for their patience and being my strength
My princess, Juwayriyyah for being my comfort and new strength

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
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November 2016

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Chickens in modern intensive production systems may suffer from multiple stress factors such as heat, handling, and transportation. These stressors are known to be negatively affecting welfare and well-being status of chickens. In search for a practical approach to alleviate the adverse effects attributed to such stressors, two experiments were conducted. The experiments were aimed to use conventional parameters together with novel and advance physiological and biotechnological measurements. In experiment 1, the influence of dietary tryptophan to large neutral amino acids ratios (TRP/LNAA) on performance, serotonin (5-HT), corticosterone (CORT), thyroxine (T_4) and triiodothyronine (T_3) of broiler chickens raised under high environmental temperature from 28-42 d were investigated. A total of 576 day-old male chicks were assigned to two controlled environments. Chicks in half of the chambers were challenged to cyclic high ambient temperature (35±1°C, 8h daily; heated), and the other half were kept at thermoneutral temperature (25±1°C; unheated). Birds were fed using six diets with ascending levels of dietary TRP/LNAA (0.025, 0.030, 0.035, 0.040, 0.045, and 0.050). Performance parameters were measured on a weekly basis. Blood samples were collected at the end of the experiment (42 d). The 5-HT, CORT, T_3 and T_4 were measured in the serum. The results revealed that irrespective of environmental condition, dietary TRP/LNAA had no influence on performance. However, dietary TRP/LNAA level has markedly affected the physiological responses of the birds. Evidently, elevation of TRP/LNAA coincided with reduction in CORT and increase in 5-HT, T_3 and T_4 ($P<0.05$). Increased 5-HT and reduced CORT are clearly indicating a superior welfare and well-being in TRP supplemented birds. In the follow up experiment, the effects of much higher dietary TRP/LNAA (0.050, 0.100, and 0.150%) was tested in broiler chickens receiving different types of pre-slaughter handling followed by transportation. A total of 180 d-old broiler chickens was fed using three dietary TRP/LNAA levels (0.050, 0.100, and 0.150). On day 42, birds were subjected to catching and handling in either short distance (25 meters) or long distance (100 meters) through the house. Blood, breast muscle and cecal samples were collected after 2 h of road transportation from all the birds. The results showed that increasing dietary TRP/LNAA improved FI and meat quality (drip loss and shear force) in broiler chickens and shifted the balance of

pathogenic/non-pathogenic bacteria to a favorable state. Chickens fed higher levels of TRP/LNAA showed superior welfare condition as measured by lower HSP70 and higher 5-HT. Pre-slaughter long handling had minimal adverse effects on broiler chicken welfare condition and meat quality. Putting the data together, elevation of dietary TRP/LNAA is recommended as a practical measure to alleviate the adverse effects of stress on well-being of broiler chickens under stressful conditions.

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

KESAN NISBAH TRIPTOFAN KEPADA AMINO ASID NEUTRAL BESAR DALAM DIET TERHADAP PRESTASI TUMBESARAN DAN KESEJAHTERAAN AYAM PEDAGING YANG DIPELIHARA DALAM KONDISI TEKANAN

Oleh

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Ayam yang dipelihara dalam sistem ternakan intensif moden sering terdedah kepada pelbagai sumber tekanan seperti haba panas, pengendalian dan pengangkutan. Umum mengetahui, kesemua punca tekanan ini memberi kesan negatif kepada keadaan kebajikan dan kesejahteraan ayam. Dua eksperimen telah dijalankan untuk mengkaji kaedah yang praktikal bagi mengurangkan implikasi tekanan ke atas ayam pedaging. Kajian ini mensasarkan penggunaan parameter konvensional bersama dengan kaedah-kaedah fisiologi dan bioteknologi yang maju dan terkini. Eksperimen 1 dijalankan untuk melihat kesan nisbah triptofan kepada amino asid neutral besar (TRP/LNAA) dalam diet terhadap prestasi tumbesaran, serotonin (5-HT), kortikosteron (CORT), tiroksin (T4), dan triiodotironin (T3) pada ayam pedaging yang dipelihara dalam suhu persekitaran tinggi selama 28 – 42 hari. Sebanyak 576 ekor anak ayam berumur 1 hari telah dibahagikan kepada dua ruang persekitaran terkawal secara sama rata, iaitu suhu ambien tinggi secara kitaran (35±1°C, 8 jam sehari) dan suhu termoneutral (25±1°C). Ayam seterusnya telah dibahagikan kepada enam kumpulan rawatan diet yang mempunyai tahap TRP/LNAA dalam diet yang berbeza (0.025, 0.030, 0.035, 0.040, 0.045, and 0.050). Prestasi tumbesaran ayam dinilai secara berkala setiap minggu. Sampel darah diambil di penghujung eksperimen (42 hari) manakal nilai 5-HT, CORT,T3 dan T4 ditentukan melalui sampel serum yang diambil. Dapatan kajian menunjukkan kadar TRP/LNAA dalam diet tidak memberikan sebarang impak terhadap prestasi tumbesaran, sama ada dalam suhu persekitaran yang tinggi atau normal. Akan tetapi, kadar TRP/LNAA dalam diet telah dilihat memberikan kesan terhadap respon fisiologi ayam apabila peningkatan TRP/LNAA dalam diet telah menurunkan nilai CORT dan meningkatkan nilai 5-HT, T3 dan T4 ($P<0.05$). Peningkatan nilai 5-HT dan penurunan CORT menunjukkan ayam yang diberi suplemen TRP berada pada tahap kebajikan dan kesejahteraan yang lebih baik. Dalam eksperimen yang berikutnya, nisbah TRP/LNAA yang lebih tinggi dalam diet diberi kepada ayam pedaging dan diuji kesannya pada respon terhadap jenis pengelolaan pra-penyembelihan yang berbeza dan pengangkutan. Sejumlah 180 ekor anak ayam berumur 1 hari dibahagikan kepada tiga kumpulan diet yang mengandungi kadar nisbah TRP/LNAA yang berbeza (0.050, 0.100, dan 0.150).

Pada hari 42, ayam ditangkap dan dipindahkan pada dua jarak yang berbeza (25 atau 100 meter). Sampel darah, otot dada dan sekal diambil selepas 2 jam tempoh pengangkutan di jalan raya. Hasil kajian menunjukkan peningkatan nisbah TRP/LNAA dalam diet telah meningkatkan pengambilan makanan dan kualiti daging (kehilangan jus dan daya mengoyak) ayam serta mengimbangkan populasi bakteria patogenik/bukan patogenik ke tahap yang lebih baik. Ayam yang mendapat kadar nisbah TRP/LNAA yang lebih tinggi dalam diet mempunyai tahap kebajikan yang lebih baik apabila nilai HSP70 menurun dan 5-HT meningkat. Selain itu, tempoh pengelolaan pra-penyembelihan yang panjang merekodkan impak buruk yang minimal terhadap kondisi kebajikan dan kualiti daging ayam. Secara konklusi, hasil kajian ini mengesyorkan peningkatan nisbah TRP/LNAA dalam diet merupakan langkah praktikal bagi menangani impak kondisi stress terhadap kesejahteraan dan kebajikan ayam pedaging.

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I certify that a Thesis Examination Committee has met 11 November 2016 to conduct the final examination of Alhassan Usman Bello on his thesis entitled "Influence Of Dietary Tryptophan to Large Neutral Amino Acids Ratios on Performance and Well-Being of Broiler Chickens Subjected to Stressful Conditions" 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 Master of Science.

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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 Supervisory Committee were as follows:

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Name of Member of Supervisory Committee: Associate Professor Dr. Goh Yong Meng

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LIST OF ABBREVIATIONS

AA	Amino Acids
ACTH	Adrenocorticotropic Hormone
AADC	Aromatic Acid Decarboxylase
ANOVA	Analysis of Variance
AOAC	Association Official Agricultural Chemists
BBB	Blood Brain Barrier
BW	Body Weight
CIE	Commission Internationale de l'Eclairage
CRF	Corticotrophin Releasing Factor
cm	Centimeter
CP	Crude Protein
CNS	Central Nervous System
CO	Carbon Monoxide
CORT	Corticosterone
CRD	Completely Randomized Design
DCP	Di Calcium Phosphate
DFD	Dark Firm Dry
DM	Dry Matter
DNA	Deoxyribonucleic acid
DOA	Dead on Arrival
EAA	Endogenous Amino Acid
EIA	Enzyme-Immunoassay
ELISA	Enzyme-Linked Immunosorbent
ENS	Enteric Nervous System
FCR	Feed Conversion Ratio
FI	Feed Intake
IU	International Unit
g	Gram
g	Unit for measuring centrifugation force
GE	Gross Energy
GIT	Gastrointestinal Tract
GLM	General Linear Model
h	Hour
HAT	High Ambient Temperature
H/L	Heterophil/Lymphocyte ratio
HPA axis	Hypothalamus Pituitary Adrenal axis
HSP70	Heat Shock Protein 70
Kcal.	Kilocalorie
KYNA	Kynurenic Acid
LAB	Lactic Acid Bacteria
LNAA	Large Neutral Amino Acids
M.E.	Metabolizable Energy
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimetre
mM	Mille mole
mRNA	Messenger Ribonucleic Acid

NAD	Nacotinamide Adenine Dinucleotide
NCBI	National Center for Biotechnology Information
NH	Negative Handling
ng	Nanogram
nm	Nanometer
nmol	Nanomoles
NMDA	N-Methyl D-Aspartate
NO	Nitric oxide
NRC	National Research Council
NSP	Non Starch Polysaccharide
^{o}C	Degree Celsius
PCR	Polymerase Chain Reaction
PH	Positive Handling
pH	Hydrogen Ion Concentration
pmol	Picomole
PSE	Pale Soft and Exudative
PVN	Paraventricular Nucleus of Hypothalamus
QA	Quinolinic Acid
RH	Relative humidity
RIA	Radioimmunoassay
rpm	Revolutions Per Minute
rRNA	Ribosomal RNA
SAS	Statistical Analysis System
s.c.	Subcutaneously
SCN	Suprachiasmatic Nuclei
SCN-SNS-Adrenal	SCN Sympathetic Nervous System Adrenal
SEM	Standard Error of Mean
TC	Total Count
TDO	Tryptophan 2,3-Dioxygenase
TI	Tonic Immobility
TMA	Trimethylamine
T_3	3,3',5-Triiodothyronine
T_4	Tetraiodothyronine
TSH	Thyroid Stimulating Hormone
TRH	Tryptophan Hydroxylase
TRP	Tryptophan
WG	Weight Gain
µg	Microgram
µl	Microlitre
µmol	Micromoles
v.	Versus
5-HT	Serotonin
5-HTP	5-hydroxytryptophan
5-HIAA	5-Hydroxy-Indol Acetic Acid
5-HIAL	5-hydroxyindole Acetaldehyde
5-HTOL	5-hydroxytryptophol

CHAPTER 1

GENERAL INTRODUCTION

Growing chicken as meat source turned to industrial scale a century ago. Most of the pedigree lines for current broiler strains has been selected and developed since then. The basis for such development was entirely performance oriented with the goal of cheaper, faster and higher weight gain (Crawford, 1993). The customers also only look for the meat and its quality. Both side of producers and consumers has overlooked the drawback of such approach to chicken itself. Chickens in intensive production systems suffered from multiple stresses throughput their life including metabolic and non-metabolic diseases, heat, crowding, catching, handling, crating, and transportation (Mitchell et al., 1992; Scanes et al., 2004; Ondrašovicová et al., 2008; Lara and Rostagno, 2013). Gradually, customer's awareness increased and they demand authorities to observe and implement animal welfare and well-being standards (Smith and Gunn, 1981). In this scenario, scientists and producers developed various solution to enhance such welfare standards. Among the common stressors in tropical area, heat stress and handling and transportation are the main problems. The solutions to these two problems are installation of roof sprinklers, using close-house systems, provision of cool drinking water, feeding at cooler times of the day, increasing air velocity, house insulation, reducing the stocking density, night transportation, early age acclimatization and using feed additives and nutritional manipulations (Lott, 1991; Yahav and Hurwitz, 1996; Furlan et al., 2000; Yahav et al., 2004; Zulkifli et al., 2009; Jiang et al., 2015). Nutritional intervention receive big attention and extensive studies has been carried out on supplementation of vitamin C, A, and E and selenium (Zulkifli et al., 2000; Sahin et al., 2002; Attia et al., 2009), propolis (Mahmoud et al., 2016), probiotic (Song et al., 2014), oleuropein (Sarica et al., 2015), cello-oligosaccharide (Song et al., 2013), lycopene (Sahin et al., 2016; Lee et al., 2016) and zinc (Sahin et al., 2003, 2009) and optimization of dietary electrolyte balance (Gous and Morris, 2005; Ahmad and Sarwar, 2006), energy to protein balance (Furlan et al., 2004; Zulkifli et al., 2007; Campos et al., 2015) and dietary arginine/lysine (Mejia et al., 2012; Zhu et al., 2014). However, there are no studies available to report on the usage on tryptophan to alleviate the adverse of heat or handling stress in chicken. Tryptophan (TRP) is the fourth limiting amino acid in diet and beside the protein synthesis, it has multiple functional roles in the body homeostasis. Tryptophan is metabolized via three pathways producing serotonin (5-HT) and melatonin (antioxidant), indole and its derivate and niacin (kunurenine pathway) (Fukuwatari and Shibata, 2013). Tryptophan-induced serotonergic activity in the brain has been associated with regulation of several behavioral and physiological processes. These include moodiness, feed intake, stress response, sleeping patterns, and aggressiveness (Lacy et al., 1982; Leathwood, 1987; Baranyiova, 1991; Shea et al., 1990, 1991; Adeola and Ball, 1992; Newberry and Blair, 1993; Huether et al., 1999; Markus et al., 2000; Le Floc'h et al., 2007). The beneficial effects of TRP in regulation of feed intake and behavior in piglets is well established (Le Floc'h et al., 2007; Black et al., 2009; Suryawan et al., 2009). It is noteworthy to mention that most of the earlier studies on TRP feeding neglected the fact that TRP uptake and utilization depend partly on the level of large neutral amino acids (LNAA). The LNAA are consisted of valine, leucine, isoleucine, tyrosine, and phenylalanine and compete for the same receptor in the blood brain barrier to enter the central nervous system (Fernstrom, 2013). Therefore, the current study designed to investigate the influence of dietary TRP/LNAA levels on performance, hormonal response and well-being of broiler chickens subjected to two type of stresses namely heat stress and handling and transportation stress.

The specific objectives were:

(i) To investigate the influence of dietary TRP/LNAA on performance, 5-HT, corticosterone, thyroxine (T_4) and triiodothyronine (T_3) of broiler chickens raised under high environmental temperature from 28 to 42 d.

(ii) To determine the effects of dietary TRP/LNAA and handling on performance, behavior, 5-HT, heat shock protein 70 (HSP70), microbial population, and meat quality of broiler chicken subjected to road transportation.

Accordingly, in the second experiment, finishing broiler chickens fed with TRP/LNAA of 0.50-0.150 and subjected to two type of handling (short and long) and transportation for 2 h. Result showed that FI has increased significantly by dietary TRP/LNAA elevation together with meat quality (drip loss and shear force). Interestingly provision of TRP in this experiment functioned as a prebiotic and *Bifidobacterium* at the cost of *E. coli*, *Clostridium*, *Enterobacter*, and *Campylobacter*. This is the first report of such effect and may put the ground for the first amino acid prebiotic in poultry nutrition. Feed grade TRP is commercially available and easily can be used by any farmers. Special importance of such application is reducing the prevalence of food-borne pathogens in fresh poultry meat and enhancing food safety standards. Handling as practiced in this study had minimal effect on welfare and well-being aspects of broiler chicken. The protocol the current study followed was to walk with birds in hand for 25 or 100 meters. This was to accommodate the maximum possible distance that worker may pace in a broiler house during the catching and crating for transportation to abattoir. Although shortening the handling distance has been recommended by welfare standards, it seems that it does not have significant effect on bird's well-being and welfare.

In conclusion, the present experiments suggested that supplementing dietary TRP in order to enhance broiler performance require further investigations to be optimized. However, higher levels of TRP/LNAA (0.100 and above) may improve broiler's well-being and welfare as measured by higher 5-HT and lower CORT and HSP70. Furthermore, broilers fed with diets containing 0.100 and above of TRP/LNAA have superior meat quality and less cecal pathogenic/non-pathogenic bacteria. Further study is recommended to establish the interplay between microbiota and dietary TRP/LNAA and its consequences for the bird's physiology, behavior and performance. It is also recommended to test the response in another avian species to changes in dietary TRP/LNAA.

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BIODATA OF STUDENT

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