



UNIVERSITI PUTRA MALAYSIA

**BIOAVAILABILITY OF AMINO ACIDS OF SOYBEAN MEAL IN
BROILERS SUBJECTED TO HEAT STRESS**

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By

ABDOREZA SOLEIMANI FARJAM

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

August 2006



DEDICATION

To
My father and mother
Agha Bagher & Fatima Khanom



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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Chairman: Associate Professor Azhar Kasim, PhD

Faculty: Agriculture

The effect of high ambient temperature (35 vs 21 °C) and acclimation during 5 and 6 week of age on the bioavailability of protein and amino acids was investigated in broilers. Two experiments were conducted to achieve the above objectives. In both experiments, equal numbers of chickens (90 birds: 45 Male, 45 Female) were assigned to three groups namely 2 weeks heat stress (heat acclimated), 1 week heat stress (non-heat acclimated) and no-heat stress. During the 28-35 days, the 2 weeks heat stress group was challenged to heat stress for 180-min every day. During the 35-43 days, the 1 week heat stress group was challenged to heat stress as well. The no-heat stress group was kept in standard temperature (21°C) as control. Basal diet which contained soybean meal as a sole source of protein was fed to all birds during day 39-41 as adaptation period. Following this, the birds undergone 24 h fasting. Assay diet was then fed to all birds for one hour before starting the heat challenge. For Experiment 1, Assay diet was the same with basal diet without methionine supplementation and for Experiment 2, it was a nitrogen free diet. Feed conversion ratio (FCR) and deep body temperature (T_b) were measured and distal ileum



contents were collected from birds killed by intravenous injection of sodium pentobarbitone through wing vein, immediately after heat challenge. The assay diet for second experiment was a nitrogen-free diet to investigate the endogenous protein and amino acid (AA) output. FCR increased by heat stress ($P < 0.05$). Heat acclimation resulted in 20% improvement in FCR in 2wk heat stressed birds as compared with non-heat acclimated birds (1wk heat stress) at 35-43 days. Tb of both heat stressed groups increased throughout the heat stress period. 2wk heat stressed birds had significantly lower Tb than 1wk heat stressed birds in day 35 and 37 ($P < 0.05$), but there was no difference at day 39 ($P > 0.05$). Crude protein and total amino acids apparent and true digestibility was significantly depressed by heat stress ($P < 0.05$) and this reduction failed to be improved by heat acclimation. Moreover, the reduction was found in all amino acid except for glutamic acid, aspartic acid, methionine, tryptophan, serine, alanine and proline. Acclimation and sex had no effect on total AA availability. Also there was no interaction between sex and heat challenge duration ($P > 0.05$). The mean AA availability of soybean meal observed in the present study was 82% in no-heat stress group and 78% in heat stressed birds. The relatively low values of AA availability in this study may be attributed to the soybean meal quality which was suspected to be underprocessed. One of the striking findings of this study was the low availability value for tyrosine under heat stress condition (11.9% less than no heat stress) which was the lowest among other AA. Analysis of endogenous excretion from the second experiment revealed that glutamic acid, aspartic acid, serine, threonine, leucine and arginine were the predominant endogenous amino acids present in ileal output. In addition, the lowest ileal endogenous AA losses were tryptophan, methionine and cystine. Heat stress markedly elevated total AA content of endogenous flow in both males and females,

but this amplification was more highlighted in female by 77.9% rather than male by 49.7%. It is noteworthy that acclimation had succeeded to significantly ($P < 0.01$) diminish the endogenous AA flows in most amino acids. Although, there were some differences among individual AA in heat stressed and heat acclimated birds, total endogenous AA composition remained unchanged ($P > 0.05$).

In summary, it is concluded that protein and AA availability were suppressed under high ambient temperature by almost 4.1% for crude protein and almost 5.5% for total amino acid. Generally, heat stress reduced AA bioavailability by 5% and heat acclimation which was practised in the present study was unsuccessful to compensate it in both sexes. Total endogenous AA excretion increased under heat stress and it was more severely in females, but it was diminished by heat acclimation.

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

**BIOAVAILABILITI ASID AMINO MIL KACANG SOYA DALAM AYAM
PEDAGING YANG DIKENAKAN TEGASAN HABA**

Oleh

ABDOREZA SOLEMANI FARJAM

Ogos 2006

Pengerusi : Profesor Madya Azhar Kasim, PhD

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Kesan suhu ambien yang tinggi (35 *lawan* 21°C) dan penyesuaian pada antara peringkat umur 5 dan 6 minggu pada bioavailabiliti protein dan asid amino telah dikaji dalam ayam pedaging. Dua kajian telah dijalankan untuk mencapai objektif di atas. Dalam kedua-dua kajian yang dijalankan, bilangan ayam yang sama (90 ekor ayam: 45 Jantan, 45 Betina) telah ditempatkan kepada tiga kumpulan, iaitu tegasan haba selama 2 minggu (dengan penyesuaian haba), tegasan haba selama 1 minggu (tiada penyesuaian haba) dan tiada tegasan haba. Pada hari 28-35, kumpulan tegasan haba selama 2 minggu didedahkan kepada tegasan haba (180 minit setiap hari). Pada hari 35-43, tegasan haba selama 1 minggu dilakukan. Kumpulan tiada tegasan haba didedahkan kepada suhu biasa (21°C) sepanjang masa dari ini adalah kumpulan kawalan. Makanan asas yang terdiri daripada mil kacang soya sebagai sumber protein diberikan kepada kesemua ayam dari hari 39-41 sebagai penyesuaian terhadap makanan dan kemudian selepas 24 jam rumusan makanan asas diberikan kepada semua ayam selama satu jam dan kemudian didedahkan kepada tegasan

haba. Untuk eksperimen 1, rumusan makanan adalah sama dengan makanan asas tanpa penambahan metionin dan untuk eksperimen 2, ia adalah diet tanpa nitrogen. Nisbah penukaran makanan dan suhu badan dalaman diukur dan kandungan distal ileum dikumpulkan dari ayam yang dibunuh dengan menggunakan suntikan intravenous dengan sodium pentobarbitone selepas pendedahan haba. Rumusan makanan untuk kajian kedua adalah tanpa kandungan nitrogen untuk mengkaji protein dan output asid amino endogen. Nisbah penukaran makanan meningkat berkadar dengan tegasan haba ($P < 0.05$). Penyesuaian haba meningkatkan nisbah penukaran makanan pada tahap 20% dalam tegasan haba selama 2 minggu berbanding dengan ayam yang tiada penyesuaian (tegasan haba selama 1 minggu) pada hari 35-43. Tb pada semua kumpulan meningkat berkadar dengan tempoh tegasan haba. Ayam pedaging yang didedahkan tegasan haba selama 2 minggu dapat merendahkan Tb berbanding dengan ayam yang didedah tegasan haba selama 1 minggu dari hari 35 sehingga 37 ($P < 0.05$), tetapi tidak terdapat perbezaan Tb pada hari 39 ($P > 0.05$). Protein kasar dan kecernaan sebenar dan kecernaan ketara bagi asid amino menurun dengan nyata akibat tegasan haba ($P < 0.05$) dan penurunan tidak dapat dipulihkan dengan penyesuaian haba. Tambahan lagi, pengurangan dijumpai pada semua amino acid kecuali pada asid glutamic, dan asid aspartic, methionine, tryptophan, serine, and proline. Penyesuaian dan jantina tidak memberi kesan kepada kepadatan asid amino. Tidak terdapat interaksi di antara jantina dan tempoh pendedahan tegasan haba ($P < 0.05$). Purata availabiliti asid amino dalam mil kacang soya yang dilihat pada kajian ini adalah 82% dalam kumpulan kawalan dan 78% pada ayam yang didedahkan kepada tegasan haba. Purata nilai availabiliti asid amino yang rendah dalam kajian ini boleh disebabkan oleh kualiti mil kacang soya yang berkemungkinan pemprosesan tidak mencukupi. Satu pertemuan yang ketara dalam

kajian ini ialah nilai availabiliti yang rendah pada tyrosine pada keadaan tegasan haba (11.9% lebih rendah dari kawalan) di mana adalah yang terendah di antara asid amino lain. Analisis perkumuhan endogen dari kajian kedua menunjukkan asid glutamic, asid aspartic, serine, threonine, leucine dan arginine adalah asid amino endogen yang unggul dalam output ileal. Tambahan lagi, kehilangan endogen ileal terendah adalah pada tryptophan, methionine and cystine. Pendedahan kepada tegasan haba telah meningkatkan aliran asid amino endogen dalam kedua-dua ayam jantan dan betina, tetapi amplifikasi adalah tinggi pada betina iaitu 77.9, berbanding dengan jantan iaitu 49.7%. Penyesuaian telah berjaya melenyapkan aliran asid amino endogen pada semua asid amino. Walaupun terdapat beberapa perbezaan komposisi di antara asid amino endogen secara individu semasa tegasan haba dan selepas penyesuaian haba, pada keseluruhannya, komposisi asid amino tidak berubah ($P>0.05$).

Secara ringkasannya, ia boleh disimpulkan bahawa availabiliti protein dan asid amino dikurangkan pada suhu ambien yang tinggi pada tahap hampir 4.1% untuk protein kasar dan hampir 5.5% bagi jumlah asid amino. Secara amnya, tegasan haba mengurangkan availabiliti asid amino pada tahap 5% dan penyesuaian haba di dalam kajian ini tida berjaya memulihkan dalam kedua-dua jantina. Secara keseluruhannya, rembesan asid amino endogen meningkat semasa tegasan haba dan diperlihatkan lebih tinggi pada betina, tetapi menyusut dengan penyesuaian haba.

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LIST OF ABBREVIATIONS

AA	Amino Acids
AABA	L- α -Amino-n-Butyric Acid
ACTH	Adrenocorticotrophic Hormone
AIA	Acid Insoluble Ash
ANOVA	Analysis of Variance
Arg:Lys	Arginine:Lysine
Ca	Calcium
CP	Crude Protein
°C	Degree Celsius
DCP	Di Calcium Phosphate
DM	Dry Matter
EAA	Endogenous Amino Acid
EE	Ether Extract
ELISA	Enzyme-Linked Immunosorbent Assays
FCR	Feed Conversion Ratio
FI	Feed Intake
g	Gram
GIT	Gastro Intestinal Tract
GLM	General Linear Models
h	Hour
HPLC	High Performance Liquid Chromatography
ISR	Internal Standard Ratio
K	Potassium



KOH	Potassium Hydroxide
BW	Body Weight
ME	Metabolizable Energy
mg	Milligram
ml	Milliliter
µg	Microgram
µl	Microlitre
mm	Millimetre
N	Nitrogen
NRC	National Research Council
P	Phosphorus
PDI	Protein Dispersibility Index
rpm	Revolutions Per Minute
RH	Relative Humidity
RWG	Relative Weight Gain
SAS	Statistical Analysis System
SEM	Standard Error of the Mean
T ₃	3,3',5-Triiodothyronine
T ₄	Tetraiodothyronine
T _a	Ambient Temperature
T _b	Deep Body Temperature
wk	Week

CHAPTER 1

INTRODUCTION

Animal and particularly broiler production in developing nations such as Asia, Africa and South America will continue to maintain its strong growth rate. However, production performance in these regions remain relatively inferior compared to performance obtained in temperate countries in North America and Western Europe. Although, many factors are obviously involved, the combination of high temperature and relative humidity resulting in heat stress remains one of the major challenges to improved production efficiencies in these warm regions. While heat stress is only an occasional challenge during the summer months in temperate countries, it is a constant problem in many tropical countries.

High ambient temperature reduces growth rate, feed consumption, and survival in broiler chickens, resulting decrease profitability (Dale and Fuller, 1980; Teeter *et al.*, 1985; Deaton *et al.*, 1986). Moreover, broilers raised under heat stress have increased abdominal fat (Kubena *et al.*, 1972; McNaughton and Reece, 1984) and decreased carcass protein (Geraert *et al.*, 1996; Tankson *et al.*, 2001). The heat-related problems associated with growing broilers in warm weather boils down to the sensitivity of the fast-growing commercial broilers to high temperature during the growing-finishing period. The high susceptibility of commercial broilers to heat stress can be attributed in part to the inferior development of their cardiovascular and respiratory system in comparison to their rapid growth potential (Yahav, 2000).



In addition, their rapid growth rate is supported by high feed intake; thus, as they grow, metabolic heat production increases but their heat dissipation capacity does not (Teeter, 1994). Some diets have been developed to reduce heat increment and thus improved growth performance at high ambient temperatures. For example, the digestion and absorption of dietary fat produces less heat increment than dietary proteins and carbohydrates (Shannon and Brown, 1969). Other examples are diets with high crude protein levels at the expense of energy. Heat increment is much larger when protein is a source of energy than if carbohydrate or fat is the source of energy. Heat increment for protein is much greater when the animal's ambient temperature is high than when it is low (Musharaf and Latshaw, 1999).

Chickens are able to maintain constant deep body temperature (T_b) within a thermoneutral zone through various mechanisms. Teeter *et al.*, (1992) defined 24 °C as being optimum thermoneutral temperature for broilers. T_b has been documented to be a useful and reliable indicator of heat stress in poultry (Teeter, *et al.*, 1992). The T_b of unstressed chickens normally varies between 41.0°C and 41.5°C (Lacey, 1999). Van Kampen *et al.*, (1979) found that the body temperature of domestic fowl is practically constant up to an ambient temperature of 27°C, but rises with ambient temperature above this. In heat-stressed animals, blood flow to the upper respiratory tract and other organs that are active in heat dissipation increases at the expense of capillary blood flow to the digestive system (Wolfenson, 1986). Blood flow is decreased more at the proventriculus than at the jejunum and ileum. This would reduce proteolytic enzymatic activities occurring in the upper part of the digestive tract and affects protein digestion (Bottje and Harrison, 1986; 1987). Conversely, feed passage rate was slower at a high temperature (Wilson *et al.*, 1980), which

might enhance nutrient absorption. These apparently opposite changes might explain the absence of temperature effect on ME digestibility, whereas digestibility of protein and other nutrients were reduced under high ambient temperature (Koelkebeck *et al.*, 1998). In an earlier study by Zuprizal *et al.*, (1993), they found that the true digestibilities of 12 amino acids were generally depressed in two rapeseed and two soybean meal diets when fed to broilers subjected to an increasing ambient temperature exposure from 21 to 32 °C. Furthermore, Wallis and Balnave (1984b) found that the influence of environmental temperature on amino acid ileal digestibility was sex-related, with high temperature causing decrease amino acids digestibility in female but not male broilers.

It is well documented that an individual's ability to respond to a particular environmental stress is not fixed, but can be enhanced by prolonged exposure to heat stress. Zulkifli and Siegel (1995) stated that heat acquisition at an early age alleviates various stress response of birds later in life other than improved heat tolerance. In other word, thermoregulatory system and behavioral thermoregulation of birds will be matured during first 2-3 weeks of life (Dawson and Whittow, 2000). Therefore, early age heat acquisition at 3-5 days of age can be a practical procedure that creates two mutual effects during 6th week of age: increased growth rate and increased thermotolerance (Yahav and Hurwitz, 1996; Arjona *et al.*, 1988; Yahav and McMurtry, 2001). Similar results were obtained with acclimation at older age with various heat challenges (May, *et al.*, 1987; Lott, 1991; Teeter, *et al.*, 1992).

Acclimation is a more extensive adaptation than thermal conditioning, which involves hypothalamic thermoregulatory threshold changes that enable chickens, within certain limits, to cope with acute exposure to unanticipated high ambient

temperature (Yahav and McMurtry, 2001). Thus, it seems that acclimation must be brought by increasing heat loss and decreasing heat production. Indeed, acclimated chickens developed enlarged combs and wattles, contained less fat and their feather cover was less than that of controls (Van Kampen, 1981*b*).

Concerning sex effect, Meltzer (1983) showed that for a body weight of 1 kg, the resting metabolic rate of the female broiler is higher by about 6% than that of the Leghorn pullet, and lower by about 24% than that of the male broiler. Besides, female birds are always fatter than males because female hormones stimulate fat deposition. From a practical point of view, these differences limit the age in which females can be marketed (Rondelli *et al.*, 2003).

To date, to the best of our knowledge no study has been done to compare the effect of induced heat acclimation on protein and amino acid availability and its variation in male and female chicken. Besides, there is no publication on endogenous excretion rate under heat stress condition. Accordingly, the aim of this dissertation was to study how commercial male and female broilers respond to heat stress and heat acclimation with regard to soybean meal amino acids ileal digestibility and productive parameter.