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MODIFYING THE RESPONSE OF MALE BROILER CHICKENS TO HEAT STRESS THROUGH EARLY AGE FEED RESTRICTION AND THERMAL CONDITIONING

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MODIFYING THE RESPONSE OF MALE BROILER CHICKENS TO HEAT STRESS THROUGH EARLY AGE FEED RESTRICTION AND THERMAL CONDITIONING

By

LIEW PIT KANG

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

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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

MODIFYING THE RESPONSE OF MALE BROILER CHICKENS TO HEAT STRESS THROUGH EARLY AGE FEED RESTRICTION AND THERMAL CONDITIONING

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September 2002

Chairman: **Associate Professor Dr. Zulkifli Idrus, Ph.D.**

Faculty: **Agriculture**

Two experiments were conducted to investigate the effects of early age feed restriction and heat conditioning on tolerance to acute and chronic heat stress in male broiler chickens. In both experiments, equal numbers of chickens were subjected to (i) 60% feed restriction on day 4, 5, and 6 (FR), (ii) exposure to 36±1°C and 50-60% relative humidity for 1 hour from day 1 to 21 (HT), (iii) 60% feed restriction on day 4, 5, and 6 and exposure to 36±1°C and 50-60% relative humidity for 1 hour from day 1 to 21 (FRHT). (iv) *ad libitum* feeding and no heat treatment (control). In experiment I, on day 35, all birds were exposed to 39±1°C for 6 hours and 50% relative humidity. Subjecting chicks to FR, HT and FRHT reduced HLR response to the heat challenge. Following heat exposure, the FR and FRHT chick had greater heat shock protein (hsp) 70 density than those of controls. The hsp 70 response of HT birds was not significantly different from the other three groups. The FRHT birds were more hyperthermic than controls during heat challenge. In experiment II, from day 36

– 50, all birds were exposed to 38±1°C and 80 % relative humidity for 2 hours/day. One day following heat exposure (day 37), all birds were administrated intranasally with infectious bursal disease (IBD) vaccine virus. The dosage used was 10x of the recommended level. Subjecting chicks to FRHT improved relative weight gain and resistance to IBD infection and reduced HLR in response to the heat treatment as compared with the control birds. Although there is evidence that FR and HT can improve heat tolerance, the FRHT combination may further enhance the ability of birds to withstand chronic heat stress. The acquired improved heat tolerance resulting from FRHT, FR, and HT could be attributed to enhanced hsp 70 response. The trend of hsp 70 response correlated well with IBD lesion scores, suggesting hsp 70 may play a role in resistance against viral infection. Based on experiment I and II, it can be concluded that the present findings confirmed earlier studies that FR is effective in alleviating the adverse effects of heat stress. Subjecting birds to FRHT can further improve tolerance to chronic but not acute heat stress.

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

MENGUBAH GERAKBALAS AYAM PEDAGING JANTAN TERHADAP TEGASAN HABA MELALUI SEKATAN MAKANAN AWAL HAYAT DAN PENYESUAIAN THERMA

Oleh

LIEW PIT KANG

September 2002

Pengerusi: **Professor Madya Dr. Zulkifli Idrus, Ph.D.**

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Dua eksperimen telah dijalankan untuk menyiasat kesan sekatan makanan awal hayat dan penyesuaian therma terhadap tegasan haba yang akut dan kronik dalam ayam pedaging jantan. Pada kedua-dua eksperimen, bilangan ayam yang sama telah didedahkan kepada (i) 60% sekatan makanan pada hari 4, 5 dan 6 (FR), (ii) 36±1°C dan 50-60% kelembapan relatif selama 1 jam dari hari 1 ke 21 (HT), (iii) 60% sekatan makanan pada hari 4, 5 dan 6 dan 36±1°C dan 50-60% kelembapan relatif selama 1 jam dari hari 1 ke 21 (FRHT), (iv) pemberian makanan secara *ad libitum* dan tidak ada perlakuan haba (kawalan). Dalam eksperimen I, pada hari ke-35, semua ayam telah didedahkan kepada tegasan haba setinggi 39±1°C selama 6 jam dengan 50% kelembapan relatif. Pendedahan ayam kepada FR, HT dan FRHT mengurangkan gerakbalas nisbah heterofil/limfosit (HLR) terhadap tegasan haba. Berikutan tegasan haba, ayam FR dan FRHT mempunyai ketumpatan protein kejutan haba (hsp) 70 yang lebih tinggi daripada ayam kawalan. Penghasilan hsp 70 oleh ayam HT adalah sama dengan ketiga-tiga

kumpulan yang lain. Suhu badan ayam FRHT adalah lebih tinggi daripada ayam kawalan semasa tegasan haba. Dalam eksperimen II, dari hari 36-50, semua ayam telah didedahkan kepada 36±1°C dan 80% kelembapan relatif selama 2 jam/hari. Sehari selapas tegasan haba (hari 37), semua ayam dicabaran dengan menggunakan virus vaksin penyakit berjangkit bursal (IBD). Pendedahan ayam kepada FRHT meningkatkan penambahan berat badan dan ketahanan terhadap cabaran IBD, dan mengurangkan peningkatan HLR akibat tegasan haba berbanding dengan ayam kawalan. Walaupun ada bukti menunjukkan bahawa FR dan HT boleh meningkatkan tahanan terhadap tegasan haba, kombinasi FRHT boleh meningkatkan lagi keupayan ayam untuk menahan suhu persekitaran yang tinggi. Peningkatan toleransi terhadap suhu yang diperolehi daripada FRHT, FR, and HT mungkin ialah hasil daripada peningkatan gerakbalas hsp 70. Trend untuk penghasilan hsp 70 berkait rapat dengan skor lesi IBD, mencadangkan bahawa hsp 70 mungkin terlibat dalam ketahanan terhadap jangkitan virus. Berdasarkan kepada eksperimen I dan II, kesimpulan yang boleh dibuat ialah keputusan eksperimen megesahkan kerja-kerja yang lebih awal iaitu FR adalah berkesan untuk meringankan kesan-kesan buruk akibat tegasan haba. Pendedahan ayam kepada FRHT meningkatkan lagi toleransi terhadap tegasan haba kronik tetapi bukan akut.

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I certify that an Examination Committee met on 20th September 2002 to conduct the final examination of Liew Pit Kang on his Master thesis entitled "Modifying the Response of Male Broiler Chickens to Heat Stress Through Early Early Age Feed Restriction and Thermal Conditioning" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia Regulations (Higher Degree) 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follow:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that this thesis has not been previously or concurrently submitted for any other degree at UPM or any institutions.

LIEW PIT KANG.

LIEW PIT KANG

Date: 30/9/2002

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone
ANOVA	Analysis of Variance
APS	Ammonium Persulfate
ATP	Adenosine Triphosphate
ATPase	Adenosine Triphosphatase
BCIP/NBT	5-Bromo-4-Chloro-3-Indolyl-Phasphate/Nitroblue Tetrazolium
BSA	Bovine Serum Albumin
CP	Crude Protein
CRF	Corticotrophin-Releasing Factor
ddH_2O	Deionised Distilled Water
ELISA	Enzyme-linked Immunosorbent Assays
DNA	Deoxyribonucleic Acid
EDTA	Ethylene-Diamine-Tetraacetic Acid
FCR	Feed Conversion Ratio
FR	60% Feed Restriction on day 4, 5, and 6
FRHT	Combination of FR and HT
g	Gravity
GaINAcT	N-acetylgalactosaminyl transferase
GAS	General Adaptation Syndrome
GLM	General Linear Models
H	Heterophil
HE	Haematoxylin and Eosin

HLR	Heterophil Lymphocyte Ratio
HPA	Hypothalamic-Pituitary-Adrenal
hsp	Heat Shock Protein
HT	Exposed to 36±1°C and 50-60% relative humidity for 1 hour from day 1 to 21
IBD	Infectious Bursal Disease
IDV	Integrated Density Value
IGF-I	Plasma Insulin-like Growth Factor-I
KCal	KiloCalorie
kDa	KiloDalton
kg	Kilogram
L	Lymphocyte
ME	Metabolisable Energy
µ	Micron
µg	Microgram
µl	Microlitre
mA	Mili-Ampere
ml	Millilitre
mm	Millimetre
mRNA	Messenger Ribonucleic Acid
nm	Nanometer
NCM	Negative Control Mean
ND	Newcastle Disease
PCM	Positive Control Mean

PVDF	Polyvinylidene Diflouride
rpm	Revolutions Per Minute
RH	Relative Humidity
R_f	Relative Mobility
SAS	Statistical Analysis System
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
S/P	Sample to Positive Ratio
T_3	3,3',5-Triiodothyronine
T_4	Tetraiodothyronine
TEMED	N, N, N', N'-Tetramethyleethylenediamine
TTBS	Tween-20-Tris-Buffered saline
V	Volt
Wk	Week(s)

CHAPTER I

INTRODUCTION

Climatic conditions have always been critical in poultry production. High temperatures in combination with high humidity create an undesirable environment that may cause extreme thermal stress in poultry. This harsh environment is not uncommon in tropical countries where the temperature and relative humidity are high. Although birds can survive within wide range of temperature, temperature above the comfort zone will certainly compromise the actual genetic potential of the birds. This is mainly due to elicitation of abnormal physiological, hormonal, and behavioural responses under the thermal stress condition. Furthermore, intense selection for high growing rate, increase stocking density, and green house effects definitely will direct or indirectly exacerbate the thermal stress conditions. It is a tremendous challenge to poultry producers to minimize the losses attributed to the hot and humid conditions.

Extensive studies have been done in the past decades to combat the adverse effects of thermal stress and to improve the performance of poultry under such conditions. As it is expensive to cool animal buildings, several methods have been examined to alleviate the effects of high environment temperatures on poultry performance and well being. These approaches included genetic manipulation (Bohren *et al.*, 1982; El-Gendy and Washburn, 1989), dietary supplementation of anti-stress agents such as vitamins and electrolytes

(Pardue and Thaxton, 1982; 1986; Kafri and Cherry, 1984; Pardue *et al*., 1985a; b; Gross, 1988; Ferket and Qureshi, 1992; Koelkebeck *et al*., 1993; Ait-Boulahsen *et al*., 1995; Mckee and Harrison, 1995; Bollengier-Lee, *et al*., 1998; Chen and Balnave, 2001; Puthpongsiriporn *et al*., 2001), manipulation of poultry diet such as provision of high fat and low protein diet (Ramlah and Sarinah, 1992), and short term fasting (McCormick *et al*., 1980a; b; c; Preston, 1987; Zulkifli and Fauzi, 1996).

Habituation through acclimatization is essential and vital in helping an animal to survive in an environment in which it has to live. Early age stressful experiences may have long-lasting beneficial effects in modifying tolerance to thermal stress in poultry. Work by Arjona *et al*. (1988), and Yahav and Hurwitz (1996), demonstrated that birds preconditioned to heat stress early in life were better able to withstand high ambient temperature later in life. Acclimation to heat stress may also occur by preconditioning to non-temperature stressor. Bowen and Washburn (1984) acclimated chickens at 21 to 24 days of age by repeated handling. Work by Zulkifli *et al*. (1994a; b; 2000) reported that neonatal feed restriction, as indicated by growth, disease resistance, humoral immunity, survivability and leucocytic count, enhanced heat tolerance at juvenile stage. At a molecular level, thermotolerance by exposure to controlled thermal stress or mild feed restriction early in life has been associated with enhanced heat shock protein (hsp) 70 expression (Wang and Edens, 1993; 1994; 1998; Zulkifli *et al*., 2002).

It is well documented that when prokaryotic and eukaryotic cells are exposed to a variety of physiological stressors, the synthesis of most proteins is suppressed but a small number of proteins known as hsp are rapidly synthesized (Tissieres, 1974; Ashburner and Bonner, 1979; Lindquist, 1986). The synthesis of hsp is temperature- and time-dependent. The amount of hsp synthesized depends upon the severity of the hyperthermia or non-thermal stressor. The synthesis rate accelerates dramatically when the incubation temperature was raised to a level above body temperature and tends to diminish after the temperature reaches critical level at 45°C (Wang and Eden, 1993). Generally, hsp can be classified into 3 families by molecular weight and functions. These proteins have molecular weight of approximately 90, 70 and 27 kiloDalton (kDa) and referred to as hsp 90, hsp 70 and hsp 27, respectively (Itoh and Tashima, 1991, Lindquist, 1986). There is another group of hsp called ubiquitin. The molecular weight is between 7 to 8 kDa. Ubiquitin has recently found to be heat inducible in both yeast and chickens (Lindquist, 1986). During thermal stress, heat shock factor is interacts with heat shock elements, which is a conserved DNA sequence, to produce hsp. It also binds to other regions of genome, suppressing translation and transcription of other genes. Hsp produced will bind to heat sensitive site, for example nucleus, during thermal stress. Besides, it may bind to cellular protein that have been denatured by the heat and prevent their catastrophic precipitation. Of the many hsp, hsp 70 is one of the most studied. Hsp 70 is evolutionarily conserved and appears to correlate best with heat resistance, either transient or permanent (Li and Werb, 1982, Lindquist, 1986, Craig and Gross, 1991). Hsp 70 acts as a molecular chaperone by binding to

newly synthesized proteins and prevent them from aggregating and precipitating before they are properly folded, transported and incorporated into complexes of organelles during stress (Yahav and Hurwitz, 1996). There is strong evidence that a causal relationship exists between the enhanced synthesis of hsp and cell survival under specific stress (Gloria, 1983). Barbe *et al.* (1988) reported that hsp is to protect organisms from detrimental effects of heating. In the complexity of a homeotherm animal, there is a positive correlation between thermotolerance and concentration of hsp 70 (Givisiez *et al.*, 1999).

Mother nature is unpredictable. Scientific ways to ameliorate the adverse effects of heat stress must have long-lasting impact that protects the birds from undesirable external environment changes. Short term fasting prior to thermal stress is less practical under field condition mainly due to unpredictable weather. Continue dietary supplementation of anti-stress agents such as vitamins and electrolytes is costly. In the search of other solutions, habituation gives an alternative way. Although early age heat conditioning (Wang and Edens, 1998) and neonatal feed restriction (Zulkifli *et al.*, 1994a, b; 2000, 2002) can improve the production of hsp 70, it is unknown whether the combination of these two techniques will further improve heat tolerance in broiler chickens. To the best of my knowledge, the only documented study on the effect of early age heat conditioning and fasting was on heat tolerance in broiler by Yahav and Plavnik (1999). They concluded that thermal conditioning has advantages over feed restriction and the combination of both. On a cautionary note, however, the feed restriction regimen practiced by the authors was more severe than those of

Zulkifli *et al.* (2000). Interestingly, Zulkifli *et al.* (2000) found that the magnitude of stress perceived early in life was critical in determining the levels of tolerance to a subsequent stressor in broiler chickens. Optimum level of stress early in life may result beneficial long-lasting physiological alterations.

Most research on hsp in poultry has emphasized its association with performance, body temperature and survivability (Givisiez *et al.*, 1999, Wang and Eden, 1993, Yahav *et al.*, 1997). There is a paucity of information on the relationship among hsp activity and disease resistance under stressed conditions in poultry. Zulkifli *et al.* (1994a) reported early feed restriction improved resistance to marble spleen disease in heat stress birds. Works by Zulkifli *et al.* (2000, 2002) suggest a possible positive relationship between ability to produce hsp 70 and viral disease resistance in poultry.

The objectives of the studies were

1. To evaluate the effects of early age feed restriction, thermal conditioning and their combinations on heat stress in male broiler chickens.
2. To determine hsp 70 response in heat-stressed male boiler chickens.
3. To ascertain the relationship between hsp 70 and resistance to infectious bursal disease in heat-stressed broiler chickens.

CHAPTER II

LITERATURE REVIEW

Introduction and Theory of Stress

Stress is very abstract. Just like we say ' A flower is beautiful', but 'beauty' itself is abstract. We will never see 'stress' but we can always feel it because 'stress' cannot and should not be avoided. The definition of stress is imprecise and very subjective during the early stage of research. Even until now, the definition of stress in dictionary still unclear. According to The Concise Oxford Dictionary (1995) stress is 'a demand on physical or mental energy, and distress is cause by stress'. Baillière's Comprehensive Veterinary Dictionary (1988) defined stress as 'the sum of the biological reactions to any adverse stimulus, physical, mental or emotional, internal or external, that tends to disturb the homeostasis of an organism, and should these reaction be inappropriate, they may led to disease states'. Scientifically, a precise definition to describe the physiological responses by unfolding the mechanism of stress underneath is essential.

During the first half of the twentieth century, scientists had gradually formulated the concept of stress and elucidated the overall response mechanisms. Walter B. Cannon, a Harvard physiologist who called the power to maintain

constancy in livings *homeostasis* discovered the 'fight and flight response'. On the other hand, Hans A. Selye, the grandmaster of stress research who defined 'stress' as the non-specific response of the body to any demand, proposed the 'General Adaptation Syndrome' (GAS). 'Fight and flight response' is a coordinated result of increased secretion of neurogenic amines (catecholamines), notably, adrenaline by adrenal medulla and noradrenaline by the postganglionic sympathetic nerve ending. It is a short-term response that creates the optimum situation for the survival of the animal by fighting the adversary or fleeing from it. Whereas, the GAS is a longer-term adjustment of the animal to environment change. It was first published in *Nature* in 1936 and subsequent description of the details in 1937. Generally, a stress agent, called stressor, will elicit GAS as a result of disturbances of the *milieu intérieur* of the body. It involves the classical pathway, which is hypothalamic-pituitary-adrenal (HPA) axis, and whole syndrome evolves in time through the three stages: (1) the alarm reaction, (2) the stage of resistance, and (3) the stage of exhaustion. Each stage is indicated by mobilization of the anterior pituitary-adrenal axis, involution of the thymio-lymphatic system, and the appearance of peptic ulcer, accordingly (Selye, 1976).

With 'fight and flight response' and GAS, it becomes possible to describe the state of stress in physiological terms, and to approach the underlying mechanism experimentally. These changes in biological concept of stress have become the bases for a vast number of studies of stress in human beings and animals which including poultry. Baylè (1967), as cited by Siegel (1995), indicated that stress hormone in birds is primarily corticosterone. Ever since,