UNIVERSITI PUTRA MALAYSIA

HEAT SHOCK PROTEIN 70 AND HEAT TOLERANCE IN EARLY-AGE FEED RESTRICTED BROILER CHICKENS

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BY

CHE NORMA MAT TAIB

Thesis Submitted in Fulfilment of the Requirements for the Degree of Master Science in the Faculty of Agriculture Universiti Putra Malaysia

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In the name of Allah, the Beneficial, the Merciful

To

AYAH & BONDA

"In the creation of the heavens and the earth and in the alteration of the night and the day there are indeed signs for people of understanding".

(Al-Qur'an, V:190-191)

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

HEAT SHOCK PROTEIN 70 RESPONSE AND HEAT TOLERANCE IN EARLY-AGE FEED RESTRICTED BROILER CHICKENS

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January 2000

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

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The objectives of this study are to evaluate the effect of various degree of neonatal feed restrictions on heat tolerance later in life, the importance of heat shock protein 70 (HSP 70) in eliciting thermotolerance in broilers and the relationship between heat stress and occurrence of programmed cell death (apoptosis). Broiler chicks that were subjected to 80% feed restriction (F80), 60% feed restriction (F60) and 40% feed restriction (F40) or *ad libitum* feeding from 4 to 6 days of age were exposed to high ambient temperatures (38±1°C) for 2hr/day from 35 to 42 days of age.

Short term feed restriction during the first week of life caused retardation of growth. Although feed restriction reduced initial growth, birds grew more rapidly than those fed *ad libitum* (AL) during refeeding. One day following the imposition of feed limitation, higher levels of HSP 70 expression in the brain tissues and increased heterophil/lympocyte (H/L) ratios were noted among F60 and F40 birds.

Birds subjected to fasting early in life (F60) improved HSP 70 expression, growth, survivability, and reduced H/L ratios compared to those fed AL and F80 in response to the heat treatment. The survivability rate and H/L ratios of F40 chicks were similar to those attained by other feeding regimens (AL and F80). Irrespective of feeding regimen, heat stress resulted in an increase in serum glucose level and appearance of programmed cell death (apoptosis) in the thymus glands. These results suggest that neonatal fasting evokes heat tolerance later in life through enhanced expression of HSP 70. Exposing birds to feed restriction of either lower (F80) or higher (F40) severity do not to have profound influence on subsequent resistance to heat stress later in life.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat untuk Ijazah Master Sains

GERAKBALAS "HEAT SHOCK PROTEIN 70" DAN KETAHANAN TERHADAP TEGASAN HABA PADA SEKATAN MAKANAN DI PERINGKAT AWAL HAYAT AYAM PEDAGING

Oleh

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Januari 2000

Penerusi: **Prof. Madya Dr. Zulkifli Idrus, Ph.D.**

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Eksperimen dijalankan ke atas ayam pedaging bagi menilai kesan beberapa tahap sekatan makanan di peringkat awal hayat terhadap ketahanan sistem badan terhadap tegasan haba yang dikenakan di peringkat akhir hayat, untuk memastikan kepentingan "HSP 70" dalam mengawal tegasan haba pada ayam dan juga untuk menilai hubungan kait diantara tekanan haba dengan pembentukan sel mati terancang ("apoptosis"). Anak ayam diletakkan di bawah pengaruh sekatan makanan iaitu sama ada pengambilan makanan tanpa had (AL), 80% sekatan makanan, 60% sekatan makanan dan 40% sekatan makanan. Sekatan makanan ini dilakukan bermula dari umur 4 hingga 6 hari dan kemudiannya bermula dari umur 35

hingga 42 hari, ayam tersebut kemudian didedahkan pula kepada tegasan haba setingggi 38°±1°C selama 2 jam setiap hari.

Sekatan makanan yang dibuat untuk jangkamasa yang singkat di peringkat awal menyebabkan berlakunya terbantut pertumbuhan berat badan. Meskipun begitu, selepas sekatan tersebut dihentikan, ayam yang mengalami sekatan makanan menunjukkan kadar pertumbuhan berat badan yang lebih baik berbanding dengan ayam yang tidak mengalami sebarang sekatan makanan. Sehari selepas tekanan makanan dijalankan, tahap penghasilan "HSP 70" dalam tisu otak dan nisbah sel heterofil kepada sel limfosit (H/L) dalam darah meningkat bagi ayam yang berada dalam kumpulan F60 dan F40 sahaja. Manakala ayam dari kumpulan F80 masih tidak menunjukkan sebarang perubahan iaitu masih sama dengan ayam kumpulan (AL).

Ayam yang didedahkan kepada 60% sekatan makan di peringkat awal hayat menunjukkan peningkatan penghasilan "HSP 70", kadar pertumbuhan, tahap kehidupan serta mengurangkan nisbah sel H/L jika dibandingkan dengan ayam dari kumpulan lain iaitu (AL dan F80) apabila mereka didedahkan kepada tekanan haba di akhir hayat. Manakala kadar kehidupan dan nisbah sel H/L bagi F40 adalah sama untuk kesemua ayam dari kumpulan lain. Tanpa bersandarkan, kepada tahap sekatan makanan di

peringkat awal hayat, peningkatan suhu badan dari hasil pendedahan kepada suhu panas dapat menaikkan kandungan glukosa dalam darah dan menyebabkan terbentuknya "apoptosis" pada sel kelenjar timus. Kesimpulan dari kajian ini menunjukkan bahawa sekatan makanan di peringkat awal hayat dapat meningkatkan penghasilan "HSP 70". Pendedahan ayam di peringkat awal hayat kepada tahap sekatan makanan yang terlalu mimima (F80) atau terlampau teruk (F40) tidak berjaya menghasilkan ketahanan yang jitu terhadap tegasan haba di peringkat akhir hayat.

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I certify that an Examination Committee met on 3 January, 2000 to conduct the final examination of Che Norma Mat Taib on his Master thesis entitled "Heat Shock Protein 70 and Heat Tolerance in Early-Age Feed Restricted Broiler Chickens" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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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.

(CHE NORMA BT MAT TAIB)

Date: 03 · 03 · 2000

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic Hormone
AL	*Ad libitum*
ATP	Adenosine Triphosphate
BSA	Bovine Serum Albumin
CRF	Corticotrophin-Releasing Factor
CP	Crude Protein
E	Epinephrine
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme Linked Immunosorbent Assays
FCR	Feed Conversion Ratio
FR	Feed Restriction
hr	Hour
HPA	Hypothalamic-Pituitary-Adrenal
HSP	Heat Shock Protein
HSC	70 kDa Heat Shock Cognate Protein
IgG	Immunoglobulin G
kcal	Kilocalorie
kDa	Kilodalton

kg	Kilogram
LH	Luteinizing Hormone
LHRH	Luteinizing Hormone Releasing Hormone
ME	Metabolisable Energy
min	Minute
mRNA	Messenger Ribonucleic Acid
MT	Mesotocin
NE	Norepinepherine
PBS	Phosphate Buffer Saline
pH	Pulssan Hydrogen (Hydrogen-ion Concentration)
PVDF	Polyvinylidene Diflouride Membrane
PVN	Paraventricular Nucleus of the Hypothalamus
RNA	Ribonucleic Acid
r-T_3	Reverse Triidothyronine
SDS-PAGE	Sodium Dodecyl -Polyacrylamide Gel
T_3	Triidothyronine
T_4	Thyroxine
TEM	Transmission Electron Microscope
Tris-HCL	Tris (Hydroxymethyl aminomethane)
V	Volt

µ	Micron
µg	Microgram
µl	Microliter

CHAPTER I

INTRODUCTION

The combined efforts of man and nature have brought tremendous changes in the performance of commercial broiler chickens. Over the period of time, the age to slaughter and the amount of feed required to produce a given quality of meat have been more than halved. However, fast growing broilers are more susceptible to various environmental insults, particularly heat stress. Intense selection for rapid growth rate in commercial meat-type chickens results in concomitant increase in metabolic heat production while heat dissipation capacity is not affected (Sandercock *et al.*, 1995).

For the last several decades, extensive studies have been conducted to combat the dire consequences of thermal stress. These approaches included dietary supplementation of vitamins and electrolytes (Blalock *et al.*, 1984; Kafri and Cherry, 1984; Pardue *et al.*, 1984; Eden and Campbell, 1985; Teerter *et al.*, 1985; Eden, 1986; El-Boushy 1988; Njoku and Nwazota, 1989; Ait-Boulahsen *et al.*, 1989; 1993, 1995; McKee *et al.*, 1997), short-term fasting (McCormick *et al.*, 1980a;b; Preston, 1987; Mench, 1992; Zulkifli and Fauzi, 1996), genetic manipulation (Bohren *et al.*, 1981; El-Gendy and Washbun 1989; 1992; Yamada and Tanaka, 1992)

and provision of high fat and low protein diet (Ramlah and Sarinah 1992). Work by Arjona *et al.* (1988; 1990), and Yahav and Hurwitz (1996) demonstrated that exposing five day-old broiler chicks to controlled elevated temperatures enhanced their tolerance to heat stress later in life. An animal does not always have to be preconditioned to the same stressor for habituation to take place. Zulkifli *et al.* (1994a;b) demonstrated that chicks subjected to 60% feed restriction at an early age, were more resistant to marble spleen disease, had better weight gain and lower heterophil to lymphocytes ratios as juveniles than those fed *ad libitum* throughout the experiment in response to high ambient temperatures. The authors suggested that such a husbandry procedure might be more realistic under field situations than prestressing with heat. There is also, the question of whether severity of neonatal stimulation may influence degree of tolerance later in life. To the best of my knowledge, the relationship between severity of early-age stressful experience and tolerance to subsequent insults has not been documented.

Despite the findings that thermal tolerance later in life could be elicited through neonatal fasting, the mechanism involved in the phenomenon is unknown. Zulkifli *et al.* (1994b; 1995) found that neonatal stress without concurrent increases in the synthesis and liberation of glucocorticoid might not help an animal in responding subsequent stressors. However, there is the question of what corticosterone-elicited physiological changes occurred following neonatal stimulation. The

acquired improved thermotolerance by prior exposure to controlled thermal stressors in poultry has been associated with improved heat-shock protein (HSP) response (Eden *et al.*, 1992; Wang, 1992; Wang and Eden, 1993). When prokaryotic and eukaryotic cells are exposed to a variety of physiological stresses such as a non lethal temperature (40-43°C) and heavy metal, the synthesis of most proteins is suppressed but a small number of highly conserved proteins are rapidly synthesized. This reaction is referred to as a "stress response" or "heat shock response" and the induced proteins are called stress proteins or heat shock proteins (HSPs) (Craig, 1985; Lindquist, 1986; Lindquist and Craig, 1988; Morimoto *et al.*, 1990; Craig and Gross, 1991; Nover, 1991). Wang and Eden (1993) suggested that the synthesis rate of the HSP was very low at the control temperature. When the incubation temperature was raised to a level above body temperature (T_b), the HSP synthesis rate accelerated dramatically and reached a maximal rate at 45°C for 30 minutes. The response of cells or whole organism to heat shock is extremely rapid but transient, and it involves the redistribution of preformed HSPs within the cell, as well as immediate translation of preformed messenger RNA (mRNA) into HSPs, immediate transcription of genes encoding heat shock proteins and cessation of transcription or translation of other genes or mRNA (Yost *et al.*, 1990). It is widely accepted that one of the most important functions of HSP is to protect organisms from toxic effects of heating (Barbe *et al.*, 1988). In a heat shocked cell, the HSP may bind to heat sensitive proteins and protect them from degradation, or may prevent damaged for