UNIVERSITI PUTRA MALAYSIA

PHYSIOLOGICAL RESPONSES, FEAR-RELATED BEHAVIOUR AND MEAT QUALITY OF BROILER CHICKENS SUBJECTED TO TRANSPORTATION AND OTHER STRESSORS

ABDULAZIZ AHMED AL-AQIL

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BY

ABDULAZIZ AHMED AL-AQIL

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

March 2009

DEDICATION

With appreciation and respect this thesis is dedicated to my beloved parents, my wife, sons, daughter, brothers and sisters.

Abstract of thesis presented to the Senate of Universisti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman: Professor Zulkifli Idrus, PhD

Faculty: Agriculture

Five experiments were conducted to investigate the effects of pre-slaughter process and other stressors on adrenocortical (CORT) reaction, heterophil and lymphocyte ratios (HLR), fear-related behaviour (TI), meat quality, heat shock protein (hsp) 70 expression, performance, mortality and some blood parameters in broiler chickens.

In Experiment I, 200 day-old broiler chickens (Cobb x Cobb) showing short or long tonic immobility responses were classified as low-fear (STI) or high-fear (LTI) responders, respectively. On day 41, they were subjected to either crating or heat challenge ($34\pm1^{o}C$) for 3 h and its effect on plasma corticosterone concentration, heterophil/lymphocyte ratios and heat shock protein 70 expression in brain tissue were determined. Crating and heat exposure elevated heterophil/lymphocyte ratios in both STI and LTI birds. Circulating corticosterone, however, was higher in LTI than STI birds following crating and heat challenge. Although differences between fear

responder groups for hsp 70 were negligible prior to heat challenge, following 3 h of heat exposure, the response was greater for the LTI than the STI group. Both STI and LTI showed similar increases in hsp 70 following crating.

In Experiment II, 432 day-old broiler chicks (Cobb x Cobb) were housed either in an (i) environmentally controlled house (CH) which was maintained at 23±1 ºC from day 21 onwards or, (ii) conventional open-sided house (OH) with cyclic temperatures (minimum, 24 ºC; maximum, 34 ºC). Equal number of chicks of each housing system was subjected to either *ad libitum* feeding (AL) or 60% feed restriction on d 4, 5 and 6 (FR). On day 42, heterophil to lymphocyte ratios (HLR) were significantly lower in OH birds fed AL than those of CH. The CH birds had greater body weights, higher feed intake and better FCR than those of OH. Raising birds under OH, as measured by CORT, was more stressful than CH. The lower CORT in FR birds compared to their AL counterparts suggests improved heat tolerance in the former. Within the AL group, the OH chicks had lower HLR than CH and this could be associated with the more rapid growth rate in the latter. Raising birds in OH, where the birds were exposed to a wide variety of stimuli, shortened TI duration. The FR birds had shorter TI duration than their AL counterparts.

In Experiment III, two trials were conducted to investigate the effects of housing system and early age feed restriction on CORT, HLR, heat shock protein (hsp) 70 expression and some blood parameters in response to either day (11:00 h) or night (20:00 h) transportation. Chicks were raised either in an (i) environmentally controlled chamber (CH) which was maintained at 23±1 ºC from day 21, or (ii) conventional open-sided house (OH) with cyclic temperatures (minimum, 24 ºC;

maximum, 34 ºC). Equal number of chicks for each housing system was subjected to either ad libitum feeding (AL) or 60% feed restriction on d 4, 5 and 6(FR). On day 42, all the birds from each housing system-feeding regimen subgroup were subjected to road transport either at 11:00 h (Trial 1) or 20:00 h (Trial 2) for 6 hours (h) in open truck. Irrespective of time, transportation resulted in a marked elevation in HLR and CORT, The HLR and CORT data suggested that the magnitude of stress attributed to transportation increased with transit time. The chickens failed to habituate to stress following 6 h of transit. The OH chickens, as measured by HLR and CORT, were less distressed than their CH counterparts following crating and transportation. The improved ability of OH to cope with stress of transportation could be attributed to the greater hsp 70 response. Irrespective of time, FR dampened HLR reaction to transportation. For both day and night transportation, hsp 70 expression increased with transit time. Only day transportation had a consistent effect on serum levels of cholesterol, glucose and electrolytes. Chickens transported during the day had higher serum levels of sodium, chloride, glucose and cholesterol with transit time.

In Experiment IV, two trials were conducted to investigate the effects of housing system and early age feed restriction on measurements of TI duration, meat quality and some blood parameters. Chicks raised either in an (i) environmentally controlled chamber (CH) which was maintained at 23±1 ºC from day 21 old, or (ii) conventional open-sided house (OH) with cyclic temperatures (minimum, 24 ºC; maximum, 34 ºC). Equal number of chicks from each housing system was subjected to either ad libitum feeding (AL) or 60% feed restriction on d 4, 5 and 6(FR). On day 42, all the birds from each housing system-feeding regimen were subgrouped to road transport either at 11:00 h (Trial 1) or 20:00 h (Trial 2) for 6 h in an open truck.

For both day and night transportation, TI durations increased with transit time. The OH birds which were exposed to a wider variety of stimuli, as measured by TI duration, were less fearful than their CH counterparts. Birds subjected to FR and transported during day had shorter TI duration than those fed AL. Irrespective of transportation time, the muscle glycogen content of broilers reduced markedly following transportation. Higher muscle glycogen content was noticed in the OH birds compared to CH following both day and night transportation. Muscle pH declined with transit time in birds transported during the day. Similar response was not noticed among birds transported at night. Following 2 h of transportation, OH birds had lower muscle pH but the reverse was observed after 6 h of transit. Both day and night transportation reduced serum lactic acid levels. Among the AL birds, the day time transportation lowered serum levels of lactic acid but converse was noted following night transportation. While serum creatine kinase (CK) activity was not affected by duration of transportation, birds transported at night showed lower serum levels of CK with transit time.

In Experiment V, the influence of pleasant and unpleasant experiences with human beings, and their combinations, on heat shock protein (hsp) 70 expression, and stress and fear responses in response to road transportation and disease resistance were studied in 750 day-old female broiler chicks. The pleasant treatment involved individual handling and gentle stroking for 30 sec daily from day 1 to 28 (PL). The unpleasant treatment involved individual handling, suspension by both legs, and exposure to noise (97 decibels) for 30 sec daily from day 1 to 28 (UNPL). The combination treatment involved exposure to either PL (day 1 to 14) and subsequently UNPL (day 15 to 28) or UNPL (day 1 to 14) and subsequently PL (day 15 to 28). On

day 42, 60 birds per treatment were road transported for 3 h. Transportation resulted in higher HLR and CORT, and longer TI duration. The HLR of the PL birds was significantly lower than other groups. The CORT of PL and UNPL birds were not significantly different but lower than the other groups. Both PL-UNPL and UNPL-PL failed to attenuate HLR and CORT reactions following transportation. Although transportation had negligible effect on serum CK levels, the enzyme levels in PL birds were significantly lower than their control, PL-UNPL and UNPL counterparts. The PL and PL-UNPL birds were less fearful, as indicated by shorter TI duration. Irrespective of human treatment group, the amount of hsp 70 in the brain tissue was significantly higher following transportation. The hsp 70 reaction was significantly greater in the PL birds as compared to other groups. As measured by bursa to body weight ratio and bursal histological lesion score, human contact treatment had no consistent effect on resistance to infectious bursal disease.

It can be concluded that subjecting birds to pre-slaughter processes which include handling by humans, crating and transportation elicited both stress and fear reactions, and changes in meat quality and hsp 70 expression. Subjecting birds to early age feed restriction, pleasant human contact and raising birds in conventional open-sided housing system can improve the ability of birds to cope with environmental insults.

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

REAKSI FISIOLOGI DAN KELAKUAN, DAN KUALITI DAGING PADA AYAM YANG DISUBJEKKAN PADA PROSES PRA-PENYEMBELIHAN DAN LAIN-LAIN TEKANAN

Oleh

ABDULAZIZ AHMED AL-AQIL

March 2009

Pengerusi: **Professor Zulkifli Idrus, PhD**

Fakulti: **Pertanian**

Lima eksperimen dijalankan untuk mengkaji kesan proses pra penyembelihan dan lain-lain tekanan ke atas reaksi adrenokortikal (CORT), nisbah heterofil limfosit (HLR), tempoh *tonic immobility* (TI), kualiti daging, protein kejutan haba (hsp) 70, pencapaian, kadar kematian dan beberapa parameter darah dalam ayam pedaging.

Dalam eksperimen I, 200 ekor anak ayam pedaging berumur sehari (Cobb x Cobb) yang menunjukkan reaksi *tonic immobility* pendek atau panjang masing-masing diklasifikasikan sebagai responden ketakutan rendah (STI) atau ketakutan tinggi (LTI). Pada hari ke 41, ayam-ayam tersebut disubjekkan kepada kurungan dalam raga atau tegasan haba (34±1^{o}C) selama 3 jam bagi menentukan kesan ke atas aras kortikosteron plasma, nisbah heterofil limfosit dan ekspresi hsp 70 dalam otak. Kurungan dalam raga dan pendedahan kepada tegasan haba meningkatkan nisbah heterofil / limfosit kedua-dua ayam STI dan LTI. Walau bagaimanapun, aras kortikosteron lebih tinggi pada ayam LTI berbanding STI selepas dikurung dan didedahkan kepada haba. Walaupun perbezaan antara kumpulan responden ketakutan untuk hsp 70 adalah kecil sebelum pendedahan pada kepanasan, kumpulan LTI

menunjukkan reaksi lebih tinggi berbanding STI selepas 3 jam pendedahan. Kedua-dua STI dan LTI menunjukkan peningkatan yang sama dalam hsp 70 selepas dikurung.

Dalam eksperimen II, 432 ekor anak ayam berumur sehari (Cobb x Cobb) di tempatkan samada dalam (i) rumah persekitaran terkawal (CH) yang suhunya dikekalkan 23±1^{o}C dari hari ke 21 dan seterusnya, atau (ii) rumah konvensional (OH) dengan suhu kitaran (minimum, 24^{o}C; maximum,34^{o}C). Anak ayam dari setiap sistem perumahan disubjekkan pada pemberian makanan secara *ad libitum* (AL) atau 60% sekatan makanan pada hari ke 4, 5 dan 6 (FR). Ayam CH mempunyai berat badan dan pengambilan makanan yang lebih tinggi, kadap pertukaran makanan yang lebih baik dari kumpulan OH. Memelihara ayam di bawah OH, seperti yang diukur dengan CORT, lebih tertekan berbanding CH. Nilai CORT yang lebih rendah dalam ayam FR berbanding ayam AL mencadangkan toleransi haba pada ayam FR. Dalam kumpulan AL, anak ayam OH mempunyai HLR yang lebih rendah berbanding CH dan ini mungkin dihubungkaitkan dengan kadar tumbesaran yang lebih cepat pada ayam CH. Memelihara anak ayam dalam OH, dimana ia terdedah kepada pelbagai ransangan memendekkan jangkamasa TI. Ayam FR mempunyai jangkamasa TI lebih pendek berbanding AL.

Dalam eksperimen III, dua ujian dijalankan untuk mengkaji kesan sistem perumahan dan kadar sekatan makanan awal hayat ke atas CORT, HLR, hsp 70 dan beberapa parameter darah pada ayam yang diangkut pada waktu siang (11:00 h) atau malam (20:00 h). Anak ayam dibesarkan samada dalam (i) rumah persekitaran terkawal (CH) di mana suhunya dikekalkan 23±1^{o}C dari hari ke 21 dan seterusnya, atau (ii) rumah konvensional (OH) dengan suhu kitaran (minimum, 24^{o}C; maksimum,34^{o}C). Anak ayam disubjekkan pada pemberian makanan secara *ad libitum* (AL) atau 60%

sekatan makanan pada hari ke 4, 5 dan 6 (FR). Pada hari ke 42, semua ayam dari setiap subkumpulan sistem perumahan-kaedah pemberian makanan disubjekkan kepada pengangkutan darat samada pada 11:00 h (Ujian 1) atau 20:00 h (Ujian 2) selama 6 jam dalam trak terbuka. Tanpa mengira masa pergerakan, pengangkutan menyebabkan peningkatan HLR dan CORT. Data HLR dan CORT menunjukkan secara tidak langsung bahawa magnitud tekanan disebabkan oleh pengangkutan meningkat dengan tempoh pengangkutan. Ayam gagal membiasakan diri pada tekanan selepas 6 jam pengangkutan. Ayam OH yang diukur melalui HLR dan CORT adalah kurang tertekan berbanding ayam CH yang setara dengannya selepas di kurung dalam raga dan diangkut. Peningkatan keupayaan OH untuk menghadapi tekanan pengangkutan mungkin disebabkan tindak balas hsp 70 yang lebih tinggi. Tanpa mengambil kira faktor masa, FR mengurangkan reaksi HLR pada pengangkutan. Untuk kedua-dua pengangkutan siang dan malam, ekspresi hsp 70 meningkat dengan masa pengangkutan . Hanya pergerakan siang mempunyai kesan konsisten ke atas paras serum kolesterol, glukos dan elektrolit. Ayam yang diangkut pada waktu siang mempunyai bacaan lebih tinggi pada paras serum natrium, klorida, glukos dan kolesterol dengan pergerakan masa.

Dalam eksperiment IV, dua ujian dijalankan untuk mengkaji tentang kesan sistem perumahan dan sekatan makanan awal hayat ke atas pengukuran jangkamasa TI, kualiti daging dan beberapa parameter darah. Anak ayam dibesarkan samada dalam (i) rumah persekitaran terkawal (CH) di mana suhunya dikekalkan 23±1^{o}C dari hari ke 21 dan seterusnya, atau (ii) rumah konvensional (OH) dengan suhu kitaran (minimum, 24^{o}C; maksimum,34^{o}C). Anak ayam disubjekkan pada pemberian makanan secara *ad libitum* (AL) atau 60% sekatan makanan pada hari ke 4, 5 dan 6 (FR). Pada hari ke 42, semua ayam dari setiap subkumpulan sistem perumahan-

kaedah pemberian makanan disubjekkan kepada pengangkutan darat samada pada 11:00 h (Ujian 1) atau 20:00 h (Ujian 2) selama 6 jam dalam trak terbuka. Pada hari ke 42, semua ayam dari setiap subkumpulan sistem perumahan-kaedah pemberian makanan disubjekkan kepada pengangkutan darat samada pada 11:00 h (Ujian 1) atau 20:00 h (Ujian 2) selama 6 jam dalam trak terbuka. Untuk kedua-dua pengangkutan siang dan malam, jangkamasa TI meningkat dengan tempoh pengangkutan. Ayam yang disubjekkan kepada FR dan pengangkutan siang hari mempunyai jangkama TI lebih pendek berbanding yang diberi pemakanan AL. Tanpa mengira masa pengangkutan, kandungan glikogen otot menurun dengan nyata selepas pengangkutan. Kandungan glikogen lebih tinggi pada ayam OH berbanding CH selepas kedua-dua pengangkutan siang dan malam. pH otot menurun dengan tempoh pengangkutan pada ayam yang diangkut pada waktu siang. Tindak balas yang serupa tidak didapati pada ayam yang diangkut pada waktu malam. Selepas 2 jam pengangkutan, ayam OH mempunyai pH otot lebih rendah tetapi keadaan sebaliknya selepas 6 jam pengangkutan. Kedua-dua pengangkutan siang dan malam menurunkan paras serum laktik. Di kalangan ayam AL, pengangkutan siang merendahkan paras serum laktik tetapi sebaliknya didapati selepas pengangkutan malam. Sementara itu, aktiviti serum kreatin kinase (CK) tidak dipengaruhi oleh jangkamasa pengangkutan. Ayam yang diangkut pada waktu malam menunjukkan paras serum CK lebih rendah dengan masa pergerakan.

Dalam eksperimen V, pengaruh pengalaman selesa atau tidak selesa dengan manusia ke atas ekspresi hsp 70, gerak balas takanan dan ketakutan akibat pengangkutan darat dan ketahanan penyakit diuji kepada 750 ekor anak ayam betina berumur sehari. Perlakuan selesa melibatkan memegang secara individu dan membelai selama 30 saat sehari dari hari pertama ke hari 28 (PL). Perlakuan tidak selesa melibatkan

memegang secara individu, digantung dengan kedua belah kaki, dan didedahkan kepada kebisingan (97 desibel) selama 30 saat sehari dari hari pertama ke hari 28 (UNPL). Kombinasi perlakuan melibatkan pendedahan kepada samada PL (hari 1 hingga 14) dan diikuti UNPL (hari 15 hingga 28) atau UNPL (hari 1 hingga 14) dan diikuti PL (hari 15 hingga 28). Pada hari ke 42, 60 ekor ayam setiap perlakuan melalui proses pengangkutan darat selama 3 jam. Pengangkutan menyebabkan peningkatan HLR, CORT, dan jangkamasa TI lebih panjang. HLR ayam PL lebih rendah berbanding perlakuan lain. CORT bagi PL dan UNPL tidak banyak berbeza tetapi lebih rendah berbanding perlakuan lain. Kedua-dua PL- UNPL dan UNP-PL gagal mengurangkan reaksi HLR dan CORT selepas pengangkutan. Walaupun pengangkutan mempunyai kesan yang sangat kecil ke atas paras serum CK, paras enzim dalam ayam PL sangat rendah berbanding kumpulan PL-UNPL dan UNPL. Ayam PL dan PL-UNPL kurang takut seperti yang ditunjukkan oleh jangkamasa TI yang lebih pendek. Tanpa mengambil kira kumpulan perlakuan manusia, kuantiti hsp 70 dalam tisu otak sangat tinggi selepas pengangkutan. Reaksi hsp 70 sangat tinggi dalam ayam PL berbanding kumpulan lain. Seperti yang diukur oleh bursa kepada nisbah berat badan dan skor lesi histologi bursa, perlakuan sentuhan manusia tidak memberi kesan yang konsisten ke atas ketahanan kepada penyakit jangkitan bursa.

Sebagai kesimpulan, mendedahkan ayam pada proses pra-sembelih yang melibatkan tangkapan oleh manusia, dimasukan dalam sangkar dan pengangkutan mengakibatkan gerakbalas tegasan, ketakutan dan perubahan kauliti dagaing dan ekspressi hsp 70. Keadaan pemeliharaan dan kualiti kontak manusia member kesan besar ke atas gerakbalas ayam pada pengangkutan.

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This thesis submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy.
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Date: 14 May 2009

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 declared that this thesis has not been previously or concurrently submitted for any other degree at UPM or any other institutions.

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ABDULLAZIZ AHMED AL-AQIL

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