



**UNIVERSITI PUTRA MALAYSIA**

***PHYSIOLOGICAL STRESS RESPONSES AND MEAT QUALITY OF  
RABBITS SUBJECTED TO DIFFERENT ROAD TRANSPORTATION  
AND SLAUGHTER METHODS***

**NAKYINSIGE KHADIJAH**

**IPPH 2014 1**



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By

**NAKYINSIGE KHADIJAH**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
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Philosophy**

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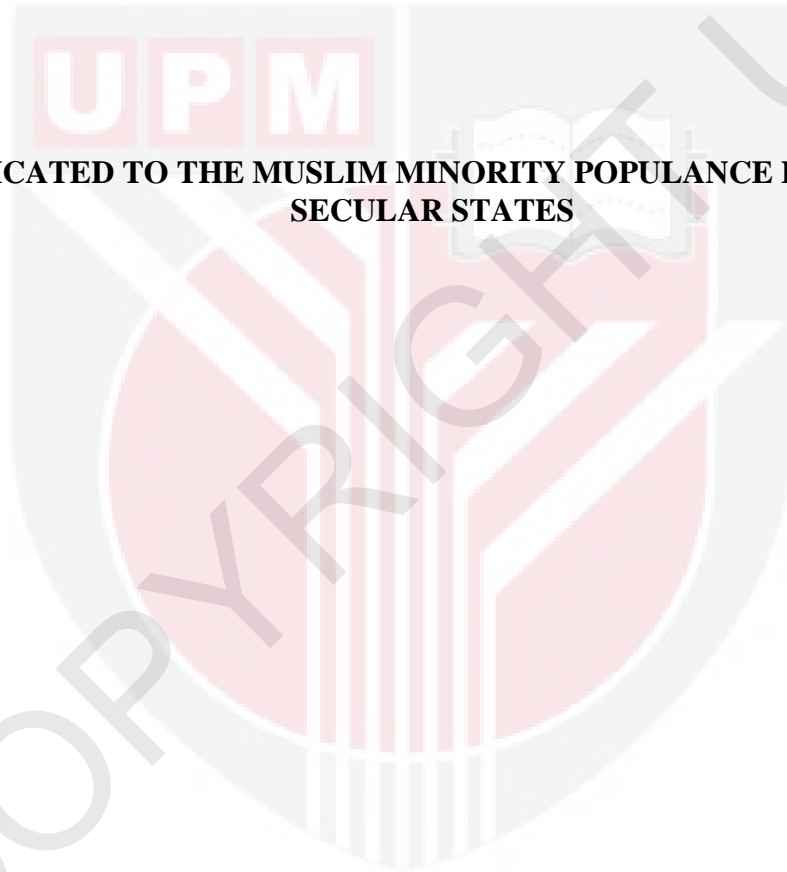
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

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By

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**September 2014**

**Chairman : Awis Qurni bin Sazili, PhD**  
**Institute : Halal Products Research Institute**

This study describes the link between physiological stress responses to transport and slaughter to meat quality using rabbit as a model. The first experiment aimed at determining physiological stress responses and meat quality of rabbits subjected to different transport durations. A control group of ten rabbits remained at the farm and was not transported (T1) while the other two groups of 40 bucks each were transported for either 1 h (T2) or 3 h (T3). Transportation caused a significant rise in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine kinase (CK) activities, packed cell volume, haematocrit, plasma protein, glucose, lactate, corticosterone, live weight loss and the expression of heat shock protein 70 (T3 > T2 > T1, p<0.05). At d 0, the pre-rigor pH (pH<sub>i</sub>) and muscle glycogen were not significantly different among treatments but at days 1 and 7, the pH significantly increased (T3 > T2 > T1, p<0.05) while glycogen significantly reduced (T3 < T2 < T1, p<0.05) with transit time. Transportation reduced lightness (L\*) of the meat (T3 < T2 < T1, p<0.05) but increased the redness (a\*) (T3 > T2 > T1, p<0.05). Shear force values and drip loss were not influenced by transport duration (p>0.05) but cooking loss reduced with transport time (T1 > T2 > T3, p<0.05). No significant correlation was found between stress and meat quality parameters and despite their fluctuation, all stress parameters fell within the normal physiological range for rabbits.

The second experiment aimed at determining physiological stress responses, meat quality, bleeding efficiency and storage stability upon subjecting rabbits to halal slaughter without stunning (HS) and gas stun killing (GK). Eighty male New Zealand White rabbits were divided into two groups of 40 animals and subjected to either HS or GK using 61.4% CO<sub>2</sub>, 20.3% oxygen and 18.3 % nitrogen. Both HS and GK caused a substantial increase in enzyme activities and the concentration of adrenaline, noradrenaline, glucose, lactic acid, calcium and white blood cells. Notably, GK exhibited significantly higher activities of LDH, AST and CK enzymes and the concentrations of adrenaline, noradrenaline and glucose. At d 0 and d 1, the glycogen content of GK and HS was not significantly different.

However, at d 7, HS presented significantly greater glycogen than GK. At d 0, HS had significantly lower pH<sub>i</sub> than GK. However, at d 1 and d 7, the statistical significance was absent ( $p>0.05$ ). At d 1, GK showed significantly greater lightness than HS. However, at d 7, the lightness of meat from both HS and GK did not significantly differ. Similarly, redness and enhanced redness of GK and HS did not significantly differ. At both d 1 and 7, the cooking loss for HS was significantly lower than that of GK. At d 1, HS exhibited significantly lower shear force values than GK although at d 7, the values for the two slaughter methods did not differ ( $p>0.05$ ). The HS had presented significantly higher myofibril fragmentation index than the GK. Generally, slaughter caused an intense stress response. However, lack of correlation between stress parameters and meat quality characteristics indicates that the amount of stress might have been below the threshold to negatively affect rabbit meat quality.

While their myoglobin content did not vary ( $p>0.05$ ), HS resulted in significantly higher blood loss and lower residual haemoglobin than the GK. At 5 and 8 days of storage, GK exhibited significantly higher levels of malondialdehyde (lipid oxidation and bacterial counts than HS. Protein oxidation was not affected by slaughter method but increased with aging time through loss of thiols and the degradation of myosin heavy chain and troponin T.

The study was further enriched with a proteomic approach to differentiate meat obtained by HS from that obtained by GK. Adenylate kinase, beta enolase, fructose biphosphate aldolase A, glyceraldehyde 3-phosphate dehydrogenase and creatine kinase M-type were differently expressed thus suggesting their potential as biomarkers for slaughter and stunning methods pending validation and more studies. All in all, the present study has shown that transport and slaughter caused substantial physiological stress responses, and even though these may not necessarily translate into compromising of animal welfare, care must be taken to avoid reaching the threshold to negatively affect meat quality. Additionally, the two slaughter methods did not influence protein oxidation but produced noticeable effects on lipid oxidation and microbial spoilage. Differential expression levels were detected in proteins of relevant metabolic and physiological roles.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah.

**RESPON KETEGASAN FISIOLOGI DAN KUALITI DAGING ARNAB  
YANG TERTAKLUK KEPADA PENGANGKUTAN JALAN DAN  
KAEDAH PENYEMBELIHAN BERBEZA**

Oleh

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Kajian semasa menghuraikan hubungkait di antara tindak balas tegasan secara fisiologi terhadap pengangkutan dan penyembelihan ke atas kualiti daging dengan menggunakan arnab sebagai model. Eksperimen pertama menentukan tindak balas tegasan secara fisiologi dan kualiti daging arnab yang tertakluk kepada jangkamasa penangkutan berbeza. Sekumpulan 10 ekor arnab kawalan tidak tertakluk kepada pengangkutan dan telah dikekalkan di lading, manakala 2 lagi kumpulan yang masing-masing mengandungi 40 ekor arnab telah tertakluk kepada pengangkutan samada selama 1 jam (T2) atau 3 jam (T3). Pengangkutan telah menyebabkan peningkatan signifikan ke atas aktiviti aspartate aminotransferase (AST), alanine aminotransferase (ALT) dan creatine kinase (CK), isipadu sel padat, hematokrit, protein plasma, glukosa, lactate, corticosterone, kehilangan berat hidup dan ekspresi protein heat shock 70 ( $T3 > T2 > T1$ ,  $p < 0.05$ ).

Pada hari ke-0, pH sebelum rigor ( $pHi$ ) dan glikogen otot tidak berbeza di antara kumpulan rawatan. Walau bagaimana pun, pada hari ke-1 dan ke-7, pH meningkat secara signifikan ( $T3 > T2 > T1$ ,  $p < 0.05$ ) manakala glikogen berkurangan secara signifikan ( $T3 < T2 < T1$ ,  $p < 0.05$ ) dengan jangkamasa pengangkutan. Pengangkutan telah mengurangkan nilai kecerahan ( $L^*$ ) ( $T3 < T2 < T1$ ,  $p < 0.05$ ) dan meningkatkan nilai kemerahan ( $a^*$ ) ( $T3 > T2 > T1$ ,  $p < 0.05$ ) pada daging. Daya ricih dan kehilangan titisan tidak dipengaruhi oleh jangkamasa penangkutan ( $p > 0.05$ ) manakala kehilangan memasak berkurangan dengan jangkamasa pengangkutan ( $T1 > T2 > T3$ ,  $p < 0.05$ ). Tiada korelasi signifikan di antara tegasan dan parameter kualiti daging dan walaupun pada paras yang tidak menentu, kesemua parameter tegasan masih lagi terletak di dalam julat fisiologi normal bagi arnab.

Eksperimen kedua adalah bertujuan untuk menentukan tindak balas fisiologi kualiti daging, keberkesanan pendarahan dan kestabilan penyimpanan setelah arnab

tertakluk kepada penyembelihan halal tanpa renjatan (HS) dan pembunuhan renjatan gas (GK). Lapan puluh ekor arnab jantan New Zealand White telah dibahagikan kepada dua kumpulan di mana setiap kumpulan mengandungi 40 ekor haiwan yang tertakluk kepada samada rawatan HS atau GK menggunakan 61.4% CO<sub>2</sub>, 20.3% oksigen dan 18.3% nitrogen. Kedua-dua HS dan GK telah menyebabkan peningkatan ketara ke atas aktiviti enzim dan kepekatan adrenalin, noradrenalin, glukosa, asid laktik, kalsium dan sel darah putih. Jelas sekali, GK telah menunjukkan aktiviti enzim LDH, AST dan CK, dan kepekatan adrenalin, noradrenalin dan glukosa yang lebih tinggi ( $p < 0.05$ ). Pada hari ke-0 dan ke-1, kandungan glikogen pada GK dan HS tidak berbeza. Walau bagaimana pun, pada hari ke-7, HS telah menunjukkan paras glikogen yang lebih tinggi berbanding GK. Pada hari ke-0, HS menunjukkan pH yang lebih rendah berbanding GK. Walau bagaimana pun, perbezaan di antara keduanya tidak lagi signifikan pada hari ke-1 dan ke-7 ( $p > 0.05$ ). Pada hari ke-1, GK menunjukkan nilai kecerahan yang lebih tinggi berbanding HS. Walau bagaimanapun, pada hari ke-7, nilai kecerahan daging pada kedua HS dan GK tidak berbeza. Kemerahan dan kemerahan tertingkat pada GK dan HS juga tidak berbeza. Pada hari ke-1 dan ke-7, kehilangan memasak pada HS adalah lebih rendah berbanding GK ( $p < 0.05$ ). Pada hari ke-1, HS mempamerkan nilai daya ricih yang lebih rendah ( $p < 0.05$ ) berbanding GK walaupun, pada hari ke-7, nilai daya ricih bagi kedua kaedah penyembelihan tidak berbeza ( $p > 0.05$ ). Kumpulan HS mempunyai indeks pemecahan myofibril yang lebih tinggi ( $p < 0.05$ ) berbanding GK. Secara amnya, penyembelihan menyebabkan tindakbalas tegasan yang tinggi. Walau bagaimanapun, ketiadaan korelasi di antara parameter tegasan dan nilai kualiti daging menunjukkan bahawa kadar tegasan yang dihadapi adalah masih dibawah paras kritikal yang dapat menyebabkan kesan negative ke atas kualiti daging arnab.

Di dalam keadaan kandungan myoglobin yang tidak berbeza ( $p > 0.05$ ), HS telah menyebabkan kehilangan darah yang lebih tinggi ( $p < 0.05$ ) dan lebih haemoglobin yang lebih rendah ( $p < 0.05$ ) berbanding GK. Pada hari ke-5 dan ke-8 penyimpanan, GK telah menunjukkan paras malondialdehyde (pengoksidaan lemak) dan kiraan bakteria yang lebih tinggi ( $p < 0.05$ ) berbanding HS. Pengoksidaan protein tidak terjejas oleh kaedah penyembelihan tetapi telah menunjukkan peningkatan dengan meningkatnya jangkamasa penuaan melalui kehilangan thio dan pemecahan myosin heavy chain dan troponin T.

Kajian ini telah diperkayakan melalui pendekatan proteomik bagi membezakan daging yang diperolehi melalui kaedah HS berbanding daging yang dihasilkan melalui kaedah GK. Adenyate kinase, beta enolase, fructose biphosphate aldolase A, glyceraldehyd 3-phosphate dehydrogenase dan creatine kinase M-type didapati berbeza dari segi ekspresi dan ini mencadangkan potensi sebagai biomarker untuk kaedah penyembelihan dan renjatan bergantung kepada kajian dan pengenalpastian selanjutnya



Pada keseluruhannya, kajian semasa menunjukkan bahawa pengangkutan dan penyembelihan telah menyebabkan tindakbalas tegasan secara fisiologi. Walaupun, ini tidak semestinya boleh diterjemahkan sebagai kompromi ke atas kebajikan haiwan, namun, langkah berjaga-jaga harus diambil bagi mengelakkan kesan negative ke atas kualiti daging. Tambahan pula, kedua kaedah penyembelihan tidak mempengaruhi pengoksidaan protein tetapi hanya meninggalkan kesan ke atas pengoksidaan lemak dan kerosakan berpunca dari mikrob. Paras ekspresi terbeza telah dikenal pasti di dalam protein yang memainkan peranan metabolik dan fisiologik.



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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

a*	redness
ADK	adenylate kinase
ADP	adenosine diphosphate
ALT	alanine aminotransferase
AMSA	American Meat Scientists Association
ANOVA	analysis of variance
APS	ammonium persulphate
AST	aspartate aminotransferase
ATP	adenosine triphosphate
b*	yellowness
BSA	bovine serum albumin
°C	degrees celcius
Cat #	catalogue number
cfu	colon forming units
CK	creatinine kinase
CO <sub>2</sub>	carbon dioxide
CP	creatine phosphate
cm <sup>2</sup>	square centimeter
d	day
DFD	dark, firm, dry
ddH <sub>2</sub> O	deionized distilled water
DTT	dithiothreitol
EDTA	ethylenediamine tetra-acetic acid
EEG	electroencephalograph
EFSA	European Food Safety Association
FA	fatty acid
FAO	Food and Agricultural Organisation
g	gram
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GK	gas stun kill
h	hour
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
Hb	haemoglobin
HS	halal slaughter
IAA	iodoacetamide
IgG	immunoglobulin G
IMCT	intramuscular connective tissue
kcal	kilocalorie
kg	kilogram
kJ	kilojoule
l	litre
µl	micro litre
L*	lightness
LAB	lactic acid bacteria
LDH	lactate dehydrogenase
LL	<i>Longissimus lumborum</i>
mA	milliampere



Mb	myoglobin
MDA	malondialdehyde
mg	milligram
µg	microgram
MHC	myosin heavy chain
MLC	myosin light chain
min	minute
µm	micrometer
µM	micromole
mM	millimole
ml	millilitre
N <sub>2</sub>	nitrogen
%	percent
O <sub>2</sub>	oxygen
pH <sub>i</sub>	initial pH
pH <sub>u</sub>	ultimate pH
pI	isoelectric point
PUFA	polyunsaturated fatty acid
PSE	pale, soft, exudative
PVDF	polyvinylidene difluoride
ROS	Reactive oxygen species
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
sec	second
TBARS	thiobarbituric acid-reactive substances
TBST	tris buffered saline-tween 20
TCA	trichloroacetic acid
Tris	2 amino-2-(hydroxymethyl)-propane-1,3-diol
USDA	United States Department of Agriculture
VER	visual evoked responses
v/v	volume per volume
WHC	water holding capacity
WHC	World Halal Council
wk	week
w/v	weight per volume



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

**PHYSIOLOGICAL STRESS RESPONSES AND MEAT QUALITY OF RABBITS SUBJECTED TO DIFFERENT ROAD TRANSPORTATION AND SLAUGHTER METHODS**

By

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**September 2014**

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This study describes the link between physiological stress responses to transport and slaughter to meat quality using rabbit as a model. The first experiment aimed at determining physiological stress responses and meat quality of rabbits subjected to different transport durations. A control group of ten rabbits remained at the farm and was not transported (T1) while the other two groups of 40 bucks each were transported for either 1 h (T2) or 3 h (T3). Transportation caused a significant rise in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine kinase (CK) activities, packed cell volume, haematocrit, plasma protein, glucose, lactate, corticosterone, live weight loss and the expression of heat shock protein 70 (T3 > T2 > T1, p<0.05). At d 0, the pre-rigor pH (pH<sub>i</sub>) and muscle glycogen were not significantly different among treatments but at days 1 and 7, the pH significantly increased (T3 > T2 > T1, p<0.05) while glycogen significantly reduced (T3 < T2 < T1, p<0.05) with transit time. Transportation reduced lightness (L\*) of the meat (T3 < T2 < T1, p<0.05) but increased the redness (a\*) (T3 > T2 > T1, p<0.05). Shear force values and drip loss were not influenced by transport duration (p>0.05) but cooking loss reduced with transport time (T1 > T2 > T3, p<0.05). No significant correlation was found between stress and meat quality parameters and despite their fluctuation, all stress parameters fell within the normal physiological range for rabbits.

The second experiment aimed at determining physiological stress responses, meat quality, bleeding efficiency and storage stability upon subjecting rabbits to halal slaughter without stunning (HS) and gas stun killing (GK). Eighty male New Zealand White rabbits were divided into two groups of 40 animals and subjected to either HS or GK using 61.4% CO<sub>2</sub>, 20.3% oxygen and 18.3 % nitrogen. Both HS and GK caused a substantial increase in enzyme activities and the concentration of adrenaline, noradrenaline, glucose, lactic acid, calcium and white blood cells. Notably, GK exhibited significantly higher activities of LDH, AST and CK enzymes and the concentrations of adrenaline, noradrenaline and glucose. At d 0 and d 1, the glycogen content of GK and HS was not significantly different.

However, at d 7, HS presented significantly greater glycogen than GK. At d 0, HS had significantly lower pH<sub>i</sub> than GK. However, at d 1 and d 7, the statistical significance was absent ( $p>0.05$ ). At d 1, GK showed significantly greater lightness than HS. However, at d 7, the lightness of meat from both HS and GK did not significantly differ. Similarly, redness and enhanced redness of GK and HS did not significantly differ. At both d 1 and 7, the cooking loss for HS was significantly lower than that of GK. At d 1, HS exhibited significantly lower shear force values than GK although at d 7, the values for the two slaughter methods did not differ ( $p>0.05$ ). The HS had presented significantly higher myofibril fragmentation index than the GK. Generally, slaughter caused an intense stress response. However, lack of correlation between stress parameters and meat quality characteristics indicates that the amount of stress might have been below the threshold to negatively affect rabbit meat quality.

While their myoglobin content did not vary ( $p>0.05$ ), HS resulted in significantly higher blood loss and lower residual haemoglobin than the GK. At 5 and 8 days of storage, GK exhibited significantly higher levels of malondialdehyde (lipid oxidation and bacterial counts than HS. Protein oxidation was not affected by slaughter method but increased with aging time through loss of thiols and the degradation of myosin heavy chain and troponin T.

The study was further enriched with a proteomic approach to differentiate meat obtained by HS from that obtained by GK. Adenylate kinase, beta enolase, fructose biphosphate aldolase A, glyceraldehyde 3-phosphate dehydrogenase and creatine kinase M-type were differently expressed thus suggesting their potential as biomarkers for slaughter and stunning methods pending validation and more studies. All in all, the present study has shown that transport and slaughter caused substantial physiological stress responses, and even though these may not necessarily translate into compromising of animal welfare, care must be taken to avoid reaching the threshold to negatively affect meat quality. Additionally, the two slaughter methods did not influence protein oxidation but produced noticeable effects on lipid oxidation and microbial spoilage. Differential expression levels were detected in proteins of relevant metabolic and physiological roles.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah.

**RESPON KETEGASAN FISIOLOGI DAN KUALITI DAGING ARNAB  
YANG TERTAKLUK KEPADA PENGANGKUTAN JALAN DAN  
KAEDAH PENYEMBELIHAN BERBEZA**

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Kajian semasa menghuraikan hubungkait di antara tindak balas tegasan secara fisiologi terhadap pengangkutan dan penyembelihan ke atas kualiti daging dengan menggunakan arnab sebagai model. Eksperimen pertama menentukan tindak balas tegasan secara fisiologi dan kualiti daging arnab yang tertakluk kepada jangkamasa penangkutan berbeza. Sekumpulan 10 ekor arnab kawalan tidak tertakluk kepada pengangkutan dan telah dikekalkan di lading, manakala 2 lagi kumpulan yang masing-masing mengandungi 40 ekor arnab telah tertakluk kepada pengangkutan samada selama 1 jam (T2) atau 3 jam (T3). Pengangkutan telah menyebabkan peningkatan signifikan ke atas aktiviti aspartate aminotransferase (AST), alanine aminotransferase (ALT) dan creatine kinase (CK), isipadu sel padat, hematokrit, protein plasma, glukosa, lactate, corticosterone, kehilangan berat hidup dan ekspresi protein heat shock 70 ( $T3 > T2 > T1$ ,  $p < 0.05$ ).

Pada hari ke-0, pH sebelum rigor ( $pHi$ ) dan glikogen otot tidak berbeza di antara kumpulan rawatan. Walau bagaimana pun, pada hari ke-1 dan ke-7, pH meningkat secara signifikan ( $T3 > T2 > T1$ ,  $p < 0.05$ ) manakala glikogen berkurangan secara signifikan ( $T3 < T2 < T1$ ,  $p < 0.05$ ) dengan jangkamasa pengangkutan. Pengangkutan telah mengurangkan nilai kecerahan ( $L^*$ ) ( $T3 < T2 < T1$ ,  $p < 0.05$ ) dan meningkatkan nilai kemerahan ( $a^*$ ) ( $T3 > T2 > T1$ ,  $p < 0.05$ ) pada daging. Daya ricih dan kehilangan titisan tidak dipengaruhi oleh jangkamasa penangkutan ( $p > 0.05$ ) manakala kehilangan memasak berkurangan dengan jangkamasa pengangkutan ( $T1 > T2 > T3$ ,  $p < 0.05$ ). Tiada korelasi signifikan di antara tegasan dan parameter kualiti daging dan walaupun pada paras yang tidak menentu, kesemua parameter tegasan masih lagi terletak di dalam julat fisiologi normal bagi arnab.

Eksperimen kedua adalah bertujuan untuk menentukan tindak balas fisiologi kualiti daging, keberkesanan pendarahan dan kestabilan penyimpanan setelah arnab

tertakluk kepada penyembelihan halal tanpa renjatan (HS) dan pembunuhan renjatan gas (GK). Lapan puluh ekor arnab jantan New Zealand White telah dibahagikan kepada dua kumpulan di mana setiap kumpulan mengandungi 40 ekor haiwan yang tertakluk kepada samada rawatan HS atau GK menggunakan 61.4% CO<sub>2</sub>, 20.3% oksigen dan 18.3% nitrogen. Kedua-dua HS dan GK telah menyebabkan peningkatan ketara ke atas aktiviti enzim dan kepekatan adrenalin, noradrenalin, glukosa, asid laktik, kalsium dan sel darah putih. Jelas sekali, GK telah menunjukkan aktiviti enzim LDH, AST dan CK, dan kepekatan adrenalin, noradrenalin dan glukosa yang lebih tinggi ( $p < 0.05$ ). Pada hari ke-0 dan ke-1, kandungan glikogen pada GK dan HS tidak berbeza. Walau bagaimanapun, pada hari ke-7, HS telah menunjukkan paras glikogen yang lebih tinggi berbanding GK. Pada hari ke-0, HS menunjukkan pH yang lebih rendah berbanding GK. Walau bagaimanapun, perbezaan di antara keduanya tidak lagi signifikan pada hari ke-1 dan ke-7 ( $p > 0.05$ ). Pada hari ke-1, GK menunjukkan nilai kecerahan yang lebih tinggi berbanding HS. Walau bagaimanapun, pada hari ke-7, nilai kecerahan daging pada kedua HS dan GK tidak berbeza. Kemerahan dan kemerahan tertingkat pada GK dan HS juga tidak berbeza. Pada hari ke-1 dan ke-7, kehilangan memasak pada HS adalah lebih rendah berbanding GK ( $p < 0.05$ ). Pada hari ke-1, HS mempamerkan nilai daya ricih yang lebih rendah ( $p < 0.05$ ) berbanding GK walaupun, pada hari ke-7, nilai daya ricih bagi kedua kaedah penyembelihan tidak berbeza ( $p > 0.05$ ). Kumpulan HS mempunyai indeks pemecahan myofibril yang lebih tinggi ( $p < 0.05$ ) berbanding GK. Secara amnya, penyembelihan menyebabkan tindakbalas tegasan yang tinggi. Walau bagaimanapun, ketiadaan korelasi di antara parameter tegasan dan nilai kualiti daging menunjukkan bahawa kadar tegasan yang dihadapi adalah masih dibawah paras kritikal yang dapat menyebabkan kesan negative ke atas kualiti daging arnab.

Di dalam keadaan kandungan myoglobin yang tidak berbeza ( $p > 0.05$ ), HS telah menyebabkan kehilangan darah yang lebih tinggi ( $p < 0.05$ ) dan lebih haemoglobin yang lebih rendah ( $p < 0.05$ ) berbanding GK. Pada hari ke-5 dan ke-8 penyimpanan, GK telah menunjukkan paras malondialdehyde (pengoksidaan lemak) dan kiraan bakteria yang lebih tinggi ( $p < 0.05$ ) berbanding HS. Pengoksidaan protein tidak terjejas oleh kaedah penyembelihan tetapi telah menunjukkan peningkatan dengan meningkatnya jangkamasa penuaan melalui kehilangan thio dan pemecahan myosin heavy chain dan troponin T.

Kajian ini telah diperkayakan melalui pendekatan proteomik bagi membezakan daging yang diperolehi melalui kaedah HS berbanding daging yang dihasilkan melalui kaedah GK. Adenyate kinase, beta enolase, fructose biphosphate aldolase A, glyceraldehyd 3-phosphate dehydrogenase dan creatine kinase M-type didapati berbeza dari segi ekspresi dan ini mencadangkan potensi sebagai biomarker untuk kaedah penyembelihan dan renjatan bergantung kepada kajian dan pengenalpastian selanjutnya

Pada keseluruhannya, kajian semasa menunjukkan bahawa pengangkutan dan penyembelihan telah menyebabkan tindakbalas tegasan secara fisiologi. Walaupun, ini tidak semestinya boleh diterjemahkan sebagai kompromi ke atas kebajikan haiwan, namun, langkah berjaga-jaga harus diambil bagi mengelakkan kesan negative ke atas kualiti daging. Tambahan pula, kedua kaedah penyembelihan tidak mempengaruhi pengoksidaan protein tetapi hanya meninggalkan kesan ke atas pengoksidaan lemak dan kerosakan berpunca dari mikrob. Paras ekspresi terbeza telah dikenal pasti di dalam protein yang memainkan peranan metabolik dan fisiologik.



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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

a*	redness
ADK	adenylate kinase
ADP	adenosine diphosphate
ALT	alanine aminotransferase
AMSA	American Meat Scientists Association
ANOVA	analysis of variance
APS	ammonium persulphate
AST	aspartate aminotransferase
ATP	adenosine triphosphate
b*	yellowness
BSA	bovine serum albumin
°C	degrees celcius
Cat #	catalogue number
cfu	colon forming units
CK	creatinine kinase
CO <sub>2</sub>	carbon dioxide
CP	creatine phosphate
cm <sup>2</sup>	square centimeter
d	day
DFD	dark, firm, dry
ddH <sub>2</sub> O	deionized distilled water
DTT	dithiothreitol
EDTA	ethylenediamine tetra-acetic acid
EEG	electroencephalograph
EFSA	European Food Safety Association
FA	fatty acid
FAO	Food and Agricultural Organisation
g	gram
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GK	gas stun kill
h	hour
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
Hb	haemoglobin
HS	halal slaughter
IAA	iodoacetamide
IgG	immunoglobulin G
IMCT	intramuscular connective tissue
kcal	kilocalorie
kg	kilogram
kJ	kilojoule
l	litre
µl	micro litre
L*	lightness
LAB	lactic acid bacteria
LDH	lactate dehydrogenase
LL	<i>Longissimus lumborum</i>
mA	milliampere

Mb	myoglobin
MDA	malondialdehyde
mg	milligram
µg	microgram
MHC	myosin heavy chain
MLC	myosin light chain
min	minute
µm	micrometer
µM	micromole
mM	millimole
ml	millilitre
N <sub>2</sub>	nitrogen
%	percent
O <sub>2</sub>	oxygen
pH <sub>i</sub>	initial pH
pH <sub>u</sub>	ultimate pH
pI	isoelectric point
PUFA	polyunsaturated fatty acid
PSE	pale, soft, exudative
PVDF	polyvinylidene difluoride
ROS	Reactive oxygen species
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
sec	second
TBARS	thiobarbituric acid-reactive substances
TBST	tris buffered saline-tween 20
TCA	trichloroacetic acid
Tris	2 amino-2-(hydroxymethyl)-propane-1,3-diol
USDA	United States Department of Agriculture
VER	visual evoked responses
v/v	volume per volume
WHC	water holding capacity
WHC	World Halal Council
wk	week
w/v	weight per volume

# CHAPTER 1

## GENERAL INTRODUCTION

Meat has been a significant constituent of the human diet since pre-historical times. Its high content of biologically valuable nutrients and processing use led to its being ranked in the group of the most common food items (Adams and Moss, 2002). Meat is not only a major source of proteins in the human diet but it is also a medium rich in social meaning owing to its association with cultural habits and rituals, both secular and religious (Fiddes, 1992). Due to its high digestibility, medium-low juiciness, little coarseness, odour and flavour, low-calories, lowest fatty feeling in the mouth and tenderness, rabbit meat is gaining preference by many meat consumers. A sensory map made by Rødbotten et al. (2004), comparing meat from 15 commercial animal species, placed rabbit meat amongst the most tender. Compared to red meats, rabbit meat has lower calories (on average 618 kJ/100 g fresh meat), fat (on average 6.8 g/100 g fresh meat), and cholesterol content (on average 53 mg/100 g fresh meat), iron (1.34 mg/100g), sodium (47mg/100g) and energy (119 kcal<sup>2</sup>) (Cavani et al., 2009). Rabbit meat fits well the contemporary consumer demand for a low-fat meat with a high degree of unsaturated fatty acids and low cholesterol and sodium levels (Cavani et al., 2009; Hernandez and Gondret, 2006). In fact, it can also have favorable effects on human health including a balanced n-6 to n-3 polyunsaturated FA (PUFA) ratio, conjugated linoleic acid, vitamins and antioxidants (Cavani et al., 2009; Hernandez, 2008; Barroeta, 2006). However, despite its high digestibility, taste, low-calories and high recommendation over other meats by nutritionists, rabbit meat is still considered a niche product (Cavani and Petracchi, 2004).

This explains why meat processing industries in most part of the world, Europe inclusive are gradually expanding and improving the availability of rabbit meat in a multiplicity of processed ready- meat in order to meet the demands of consumers (Dallezotte, 2002). Large rabbit industry integration has become more important and the development of rabbit meat production has forced processing plants to improve their slaughter capacities through use of high-speed and more automated slaughter lines (Cavani and Petracchi, 2004).

Religion is one of the most influential factors determining choice and subsequent selection or purchase of foods, particularly, meat. In fact, of all foods groups, meat is the most strictly regulated with regard to religion. Due to the sheer number of Muslims globally and their determination to uphold the tenets of their religion, it has become evident that the market for meat from halal slaughtered animals is an important proportion of the global production and supply (Anil, 2012a). Unlike other consumers whose decision to purchase meat is often guided by such sensory traits as colour,

tenderness, juiciness and flavour or aroma and the perception of safety or healthiness (Raes et al., 2003; Verbeke and Viane, 1999), Muslims consider the halal status of the meat before any other factor. Halal slaughter is legally recognized as the appropriate method for killing animals intended for meat consumption by Muslims. The method follows prescriptions and practices described in the Holy Quran and hadith (traditions of Prophet Muhammad peace be upon him).

The *halalan-thoyyiban* concept not only includes acceptable slaughter methods but is rather a holistic approach, which involves all of the processes between the farm and the fork, and also includes meat quality traits and sensory characteristics, safety and shelf life as well as perceptions about the conditions of the animal in relation to animal welfare. Even though their duration is quite short, transport and slaughter are important critical points in the meat production chain (Grandin, 2007) with potential risks (Buil et al., 2004) and their mismanagement or poor handling can ruin efforts made by producers during the longer growing and fattening phases (Villarroel et al., 2001). Transport and such related events preceding animal slaughtering are critical for not only animal welfare, but also for meat quality and safety. As demonstrated by Mitchell et al. (1988), the stress induced by slaughter may be different from that induced by handling and transport. In 1989, the World Veterinary Association specified five freedoms applicable to all animal species. These rights were revised by the Farm Animal Welfare Council (FAWC) in terms of 5 freedoms, namely, freedom from thirst, hunger or malnutrition, freedom from discomfort, freedom from pain, injury and disease, freedom to carry out natural behaviour and freedom from fear and anxiety (Buil et al., 2004). These five freedoms need to be respected in the entire production chain. In Europe, welfare regulations, guidelines and recommendations for rabbits have been documented by the European Food Safety Authority for transportation (EFSA, 2004, 2011) and slaughter (EFSA, 2006a, b).

It is well known that man has been slaughtering animals for food since pre-historic times. All this while, hardly any attention was given to minimising the pain and suffering which animals may feel during the slaughter process. It was in the last part of the twentieth century, precisely the last 150 or so years that the Western society found it ethically appropriate to attempt to reduce animals' pain and suffering during slaughter (Zivotofsky and Strous, 2012). An array of methods collectively known as *humanely developed* accomplish this. In 1958, the Humane Slaughter law was passed by the United States Department of Agriculture (USDA), section two of which requires all USDA inspected animals to be stunned before exsanguination despite giving exemption to religious slaughter. In Europe, pre-slaughter stunning was made compulsory according to EU Council Directive 93/119 (European Union, 1993) and lately the European Community Council Regulation No 1099/2009. Consequently, religious slaughter without stunning has been banned in several EU countries including Denmark, Netherlands, Norway, Sweden and



Switzerland. Nevertheless, significant numbers of Muslims and Jews demand meat products from animals killed using methods that are compliant with their religious requirements. The overwhelming increase in Muslim populations in European countries and meat exports into Muslim countries has made the provision of meat obtained by halal slaughter necessary not only ethically but also economically.

The meat industry on the other hand, aims at improving quality in order to satisfy customer demand yet maintaining good animal welfare. As new technologies are swiftly being accepted and absorbed, consumers seek meat products of increased functional and nutritional properties and improved sensory quality yet combined with a traditional and wholesome image in addition to guaranteed safety and extended shelf life but yet less processing (Nychas et al., 2008). The industry aims at achieving customer acceptability through the development and control of processes in order to produce wholesome products with high quality and safety (Castro-Giráldez, 2010). The transformation of livestock into meat (slaughter) is a chain of events that begins with handling and loading on the farm, then, transport to the market, pens and/or slaughterhouse, off-loading plus holding and finally slaughter. The obligation in the conversion of slaughter animals into consumable products and/or useful by-products is to slaughter animals in a humane manner as well as to process carcasses in not only hygienic but also efficient ways (FAO, 2001). This has made the study of the effect of transport and slaughter method on physiological stress responses (animal welfare indicators) and meat quality very important for both legislators and meat processors. This study therefore sets out to assess the effect of transport duration as well as gas stun killing and halal slaughtering on animal welfare and meat quality of rabbits.

Despite the claim that gas stunning is more humane as it releases consciousness rapidly by euthanasia (Forslid, 1987), its application is tangled with concerns over the use of gases such as carbon dioxide which is unpleasant and painful due to its acidic property (Lambooij et. al., 1999) or the use of inert gases which are probable for causing a sense of breathlessness and hyperventilation (Raj and Gregory, 1995). The application of gas stunning is generally irreversible and this contradicts the Islamic requirement of animals being alive at the time of slaughter. In Malaysia, the use of gas stunning is not acceptable in the production of halal meat. In fact, the procedure was omitted from the Malaysian standard, its first revision (MS1500:2004) and the second revision (MS1500:2009). To date, the National Fatwa Council of Malaysia has yet to decide on the practicability of the use of gas stunning in halal slaughter due to limitation of scientific facts. Furthermore, during its 11th Annual General Meeting held in Istanbul, Turkey on 8<sup>th</sup> September 2013, World Halal Council (WHC), which is an umbrella body of Halal Certifiers worldwide decided that gas stunning before slaughter is unacceptable (WHC, 2013). Hypothesizing that gas stun killing is superior in interms of animal welfare but comparable to halal slaughter without stunning interms of meat quality, this study was conducted in an attempt to compare the effects of halal slaughter with out stunning and gas stun killing on animal welfare, meat quality,

bleeding efficiency and storage stability as well as to identify peptide biomarkers for differentiating the two methods using rabbit as a model.

### **Main Objective**

- ¾ To determine physiological stress responses and meat quality of rabbits subjected to road transportation and different slaughter methods.

### **Specific Objectives**

- ¾ To determine physiological stress responses and meat quality of New Zealand White rabbits subjected to different transport durations under hot, humid tropical conditions.
- ¾ To determine physiological stress responses and carcass or meat quality of New Zealand White rabbits subjected to halal slaughtering with out stunning or gas stun killing.
- ¾ To evaluate the influence of halal slaughter with out stunning and gas stun killing on bleeding efficiency and storage stability of rabbit meat.
- ¾ To identify biomarker differences between meats from non-stunned and gas stunned animals.

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