



***MEAT QUALITY, MEAT FATTY ACID COMPOSITION, AND EFFECTS OF
TRANSPORT STRESS ON CROSSBRED PIGS IN MALAYSIA***

MICHELLE FONG WAI CHENG

FPV 2019 20



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By

MICHELLE FONG WAI CHENG

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

July 2019

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DEDICATION

MY FATHER AND MOTHER,

I Love You Forever

MY HUSBAND,

This is for us, and our future

MY SISTERS AND KELVIN,

For all the support and patience you have shown me

MY BEST FRIENDS, S, E & V,

For the times you were there in my ups and downs

MY CG MEMBERS & FRIENDS

For all your prayers and support when I was at my lowest

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

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By

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July 2019

Chairman : Associate Professor Ooi Peck Toung, DVM, PhD
Faculty : Veterinary Medicine

Pork meat quality and local genetic diversity of pigs is relatively unknown in Malaysia since the abolishing of designated breeder farms and centralized suppliers since the Nipah virus outbreak in Malaysia. This is compounded by the presence of unlicensed slaughterhouses, which threatens the quality of pork for the consumer. This study seeks to determine the general pork quality from model farms in Malaysia, as well as the effect of transportation duration on meat quality, stress parameters and HSP70 expression. This study also compares the Warner-Bratzler Shear Force (WBSF) values between the roasted and boiled meat to approximate local consumer preference and laboratory meat quality evaluation method.

Meat quality parameters were evaluated from the *longissimus dorsi* of 10 Duroc crossbreeds to estimate that the local pork quality falls into the category of Red, Soft and Exudative meat (acceptable with mild defects). The fatty acid profile analysis done on the *longissimus dorsi* of 6 Landrace pure breed, 4 Large White pure breed and 10 Duroc crossbreeds showed that the Duroc crossbreeds have the lowest saturated fatty acid content and the highest unsaturated fatty acid to saturated fatty acid ratio compared to pure breeds. However, producers may need to increase the polyunsaturated fatty acid omega 3 (PUFA n-3) levels in Duroc crosses due to high PUFA n-6: n-3 ratio. As for the effects of transportation, 20 Duroc crosses were assigned to long transportation stress (3-hour transportation duration), while 10 Duroc crossbreeds were subjected to short transportation stress (30-minute transportation duration). A transportation duration of 3 hours is correlated with high serum corticosterone levels, down-regulation of heat shock protein 70

(HSP70) expression, higher tendency of muscle lipid oxidation and lower pH_{45minutes} when compared to the short transportation group. This study suggests that the initial stress caused by short transportation durations of 30 minutes up-regulates the expression of HSP70 for cellular repair mechanism. This mechanism is severely impacted after 3 hours of transportation stress, leading to the downregulation of HSP70 and the tendency of increased cellular oxidative potential in the muscle.

In conclusion, the proposed pork quality baseline reference falls within the RSE category, still deemed satisfactory in terms of consumer perception with Duroc cross still demonstrating consumer preferable meat quality and fatty acid composition compared to the other breeds. Results heavily suggests keeping the transportation time limit to within 3 hours to reduce further negative impact on meat quality. These may be achieved contributing to the benefits of the establishments of pig farming areas (PFA) within the country. HSP70 gene expression may be potentially used as a biomarker for drip loss. This study also suggests a pathway where the expression of HSP70 can be associated with changes in meat quality supported by high correlations. This study presents pork quality from Malaysian model pig farms to serve as a guideline to other pork producers, as well as provides insight into how transportation duration impacts ultimate meat quality.

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

**KUALITI, KOMPOSISI ASID LEMAK DAN KESAN TEKANAN
PENGANGKUTAN TERHADAP DAGING KHINZIR BAKA KACUKAN DI
MALAYSIA**

Oleh

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Julai 2019

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Kepelbagaian kualiti daging dan baka tempatan khinzir di Malaysia secara relatifnya tidak diketahui sejak ladang pembiakan khusus dan pembekal berpusat dimansuhkan akibat wabak virus Nipah. Keadaan ini menjadi lebih buruk dengan kewujudan rumah penyembelihan yang tidak berlesen, yang mengancam kualiti daging khinzir untuk pengguna. Kajian ini bertujuan untuk menentukan secara umum kualiti daging khinzir dari ladang-ladang contoh di Malaysia, serta kesan tempoh pengangkutan terhadap kualiti daging, parameter tekanan dan penyataan HSP70. Kajian ini juga membandingkan nilai Warner-Bratzler Shear Force (WBSF) antara daging panggang dan rebus mengikut anggaran pilihan pelanggan tempatan dibandingkan dengan kaedah penilaian kualiti daging di makmal.

Parameter kualiti daging dinilai berdasarkan longisimus dorsi baka kacukan 10 untuk mengganggu kualiti daging khinzir tempatan sama ada ia termasuk dalam kategori daging merah dan pucat lembik berair (sedikit kecacatan dapat diterima). Analisis profil asid lemak terhadap longisimus dorsi bagi 6 baka tulen Landrace, 4 baka tulen Large White dan 10 baka kacukan Duroc menunjukkan bahawa baka kacukan Duroc mengandungi asid lemak tepu paling rendah dan asid lemak tak tepu paling tinggi dibandingkan dengan nisbah asid lemak tepu baka tulen. Walau bagaimanapun, pengeluaran mungkin perlu meningkatkan asid lemak poli tak tepu omega 3 (PUFA n-3) bagi baka kacukan Duroc kerana nisbah PUFA n-6: n-3 yang tinggi.

Bagi melihat kesan pengangkutan, 20 kacukan Duroc didedahkan dengan tekanan pengangkutan dalam tempoh yang lama (tempoh pengangkutan selama 3 jam) manakala 10 baka kacukan Duroc didedahkan kepada tekanan pengangkutan yang singkat (tempoh pengangkutan selama 30 minit). Tempoh pengangkutan selama 3 jam dikaitkan dengan tahap kortikosteron serum yang tinggi, pengawalan menurun protein kejutan haba penyataan 70 (HSP70), kecenderungan pengoksidaan lipid otot yang tinggi dan pH^{45minit} yang lebih rendah dibandingkan dengan kumpulan yang didedahkan dengan tempoh pengangkutan yang singkat. Kajian ini menunjukkan bahawa tekanan di peringkat awal yang disebabkan oleh tempoh pengangkutan yang singkat iaitu selama 30 minit mengakibatkan pengawalan menaik penyataan HSP70 untuk mekanisme pembaikan selular. Mekanisme ini terjejas teruk selepas 3 jam tekanan pengangkutan yang membawa kepada pengawalan menurun HSP70 dan kecenderungan peningkatan potensi oksidatif sel otot.

Kesimpulannya, rujukan asas kualiti daging khinzir yang dicadangkan termasuk dalam kategori RSE, masih dianggap memuaskan. Persepsi menunjukkan kualiti daging dan komposisi asid lemak baka kacukan Duroc masih menjadi pilihan pengguna berbanding baka lain. Hasil kajian menunjukkan dengan mengehadkan tempoh pengangkutan tidak melebihi 3 jam dapat mengurangkan kesan negatif selanjutnya terhadap kualiti daging. Jika hal ini dapat dicapai ia dapat memberi faedah kepada kawasan penternakan khinzir (PFA) di negara ini. Penyataan gen HSP70 mungkin berpotensi digunakan sebagai biopenanda bagi kehilangan air. Kajian ini juga menunjukkan satu kaedah yang penyataan HSP70 boleh dikaitkan dengan perubahan pada kualiti daging dengan disokong oleh korelasi yang tinggi. Kajian ini mendapati kualiti daging khinzir dari ladang contoh Malaysia dapat dijadikan panduan kepada pengeluar daging khinzir yang lain, di samping mampu memberikan gambaran tentang impak tempoh pengangkutan terhadap kualiti daging.

ACKNOWLEDGEMENTS

I would like to sincerely acknowledge the support and guidance I received from my supervisors especially the chairperson, Assoc Prof Dr Ooi Peck Toung, first for giving me the opportunity to work together with his swine team and for showing me his patience, guidance and support, mentally and financially, throughout the period of my study. To other members of my supervisory committee, Assoc Prof Dr Goh Yong Meng and Assoc Prof Dr Awis Qurni Sazili, I humbly appreciate your diligent efforts, positive suggestions and valuable observations which have guided me on my academic endeavours.

I would like to acknowledge the contribution of the staff of various laboratory, such as the Virology Laboratory and Physiology Laboratory of the Faculty of Veterinary Medicine, the Meat Science Laboratory of the Faculty of Agriculture, Institute of Tropical Agriculture and Food Security (ITAFoS).

These are various people whom I would like to extend a personal note of thanks:

Dr Suriya Kumari Ramiah, Dr Mahdi Ebrahimi, Dr Azad Behnan Sabow, Dr Ubedullah Kaka, Dr Mohd, Dr Seetha Jaganathan King, Dr Jasbir Singh, Elaine Loh Yee Ling, Wanpuech Parnsen, Dr Wasu Kasimani, Dr Lee Jin Wee, Mdm Veronica, Dr Rachel Fong Wai Jing, Dr Rufina Lee Leng Jie, Dr Tan Yi Wei, Dr Yeoh Wei Yang, Dr Moo Kar Shen, Dr Daniel Mohan, Dr Lee Chee Yien, Vynter Low Suet Ee, Dr Goh Chee Yeen, Dr Woon Ning Xian, Dr Tee Chiou Yan, Dr Chow Guo Hao, Peter, and Dr Lew Hong Chuan.
Thank you for contributing to the completion of my project.

I am indebted to my family, especially Dr, Rachel Fong Wai Jing and husband, for their full support during the entire course of my endeavour. Their unique patience, motivation and understanding were the foundation of my completion. I sincerely appreciate all the support that they have given me, through constant encouragement and finance support.

My special gratitude to my husband, Dr. Kenneth Fung Hon Ngen, who has given me unconditional love, understanding and assurance that ensures the successful completion of my study, without which this may not be possible. This is for us, and for our future.

I also want to submit my sincere gratitude to my best friends, Shirley Mohanakumar, and Erin Yong Min Sze, always being there for me in my ups

and downs. They have seen me through the struggling periods of my life and have always stuck by me.

Last but not least, I would also like to thank my friends, especially Venice Wan Wei Ynn, who have given me immense inspiration and motivation throughout my study.



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

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LIST OF ABBREVIATIONS

DFD	Dark, Firm and Dry
DL	Drip loss
FAME	Fatty acid methyl esters
FAO	Food and Agriculture Organization
FID	Flame Ionization Detector
GAP	Good Animal Husbandry Practice
GMP	Good Management Practices
HACCP	Hazard Assessment Critical Control Point
HSP	Heat shock proteins
HSP70	Heat shock protein 70
ICC	Intra-class correlation coefficient
LD	<i>Longissimus dorsi</i>
LOA	Limits of Agreement
LSD	Least significant difference
LSQ	Lenden-Speck Quotient
MDA	Malondialdehyde
MUFA	Monounsaturated fatty acids
MyGAP	Malaysian Good Agricultural Practices
NPPC	National Pork Producers Council
PCR	Polymerase Chain Reaction
PFA	Pig Farming Area
PCR-RFLP	Polymerase Chain Reaction – Restriction Fragment Length Polymorphism
PSE	Pale, Soft and Exudative
PSS	Porcine Stress Syndrome
PUFA	Polyunsaturated fatty acids
RSE	Reddish-pink, Soft and Exudative
RYR1	Ryanodine Receptor-1
SALT	Livestock Farm Accreditation Scheme
SD	Standard Deviation
SEM	Standard Error of Means
SFA	Saturated fatty acids
SOP	Standard Operating Procedures
SPSS	Statistical Package for Social Sciences
TBARS	Thiobarbituric Acid Reacting Substances
UFA	Unsaturated fatty acids
VHM	Veterinary Health Mark
WBSF	Warner-Bratzler Shear Force
WHC	Water holding capacity

CHAPTER 1

INTRODUCTION

Malaysia is one of the developing countries which is largely self-sufficient in pork, where with the local production supplying up to 95% of the domestic consumption. However, the culling of 1.1 million pigs during the Nipah virus outbreak in 1998 severely crippled the swine industry. Currently, Malaysia has approximately 772 farms and around 0.17 million sows in the production line, pushing the ex-farm value of the swine industry to an estimated 2.2 billion ringgit (King et al., 2017). Malaysia has a pork consuming population of around 30 % out of 32 million, at per capita consumption of about 9kg annually.

Since the Nipah virus outbreak, the swine industry was devastated (Hosono, et al., 2006). The breeder farms, which were established since 1926 and monitored by the Malaysian government, were abolished (Singh & Fong, 2014). Hence, there was no constant source which supplies cross-bred breeder animals to local farmers in Malaysia. From there onwards, the genetic diversity of breeder herds was managed by each farm individually. Some larger farms resort to import breeder stock from countries like USA, Canada, Denmark and many more (DVS, 2017), while others buy breeder animals from these larger farms, or resort to inbreeding (Singh & Fong, 2014).

For nearly two decades since the outbreak of Nipah, the swine industry in Malaysia runs on individual farms' self-developed swine herd breeding programme, where the advantage lies with large private sectors with their own research and development facilities. Therefore, information on the current standard of commercial pork quality is highly variable and remains unknown to the public. With consumer preferences changing towards the selection of good quality pork for consumption, there is a need to establish a reference for the operators in the industry to gauge the performance of their animals and the pork quality produced. This is also important to increase the competitiveness between producers to continuously improve the pork quality available to consumers.

There are many slaughterhouses distributed all around Malaysia, where some are licenced or owned by the local government, while others are yet to be licensed (Sinclair, et al., 2019; Abdullahi, et al., 2016a; Kong, L., 2011; Cruz, A.F., 2006, Mak, K.W., 2005). Slaughterhouses for pigs and livestock animals are not mixed as according to the Halal standard and procedure within the country and are therefore managed independently. The official slaughtering figures per year is about 1.5 million pigs per year, with only 9 pig slaughterhouses are government owned or licenced by the authorities and only 2 slaughterhouses have attained the Veterinary Health Mark (VHM) certification (DVS, 2017). The Veterinary Health Mark (VHM) Certification Scheme was introduced to provide proper tracking system of agricultural produces from farm to table. Government owned and licenced abattoirs are obligated by the law to implement food safety

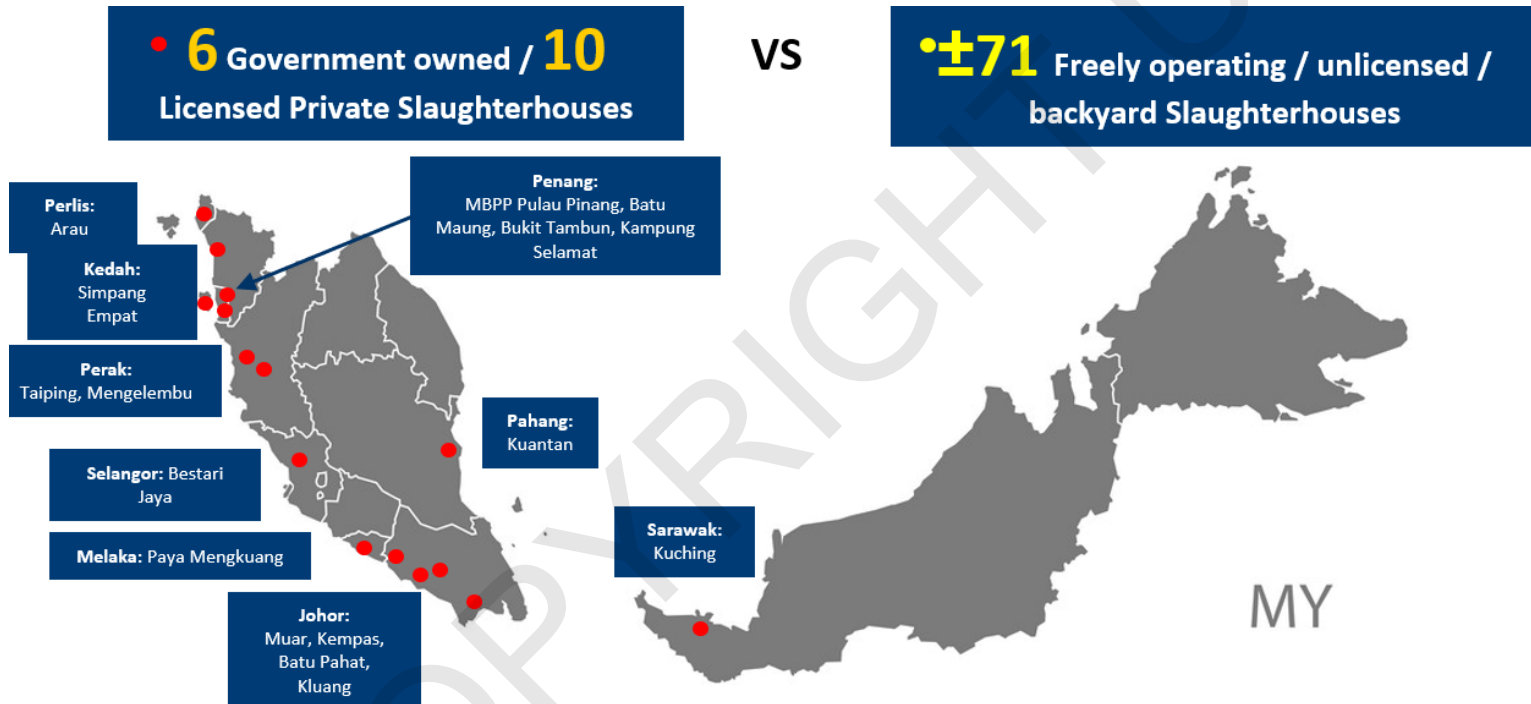


Figure 1.1: Distribution of Slaughterhouses in Malaysia (Adapted from Jabatan Perkhidmatan Veterinar Malaysia, 2019).

programs such as HACCP (Hazard Assessment Critical Control Point), as part of their certification requirements. These are also meat inspections and surveillance inspection programs done by representatives from the government, to provide consumers the certainty that the produce is safe for consumption, being free of zoonotic diseases, hazardous contaminations, unhealthy drug residues and microbial counts. It also ensures that animals are being slaughtered in a humane manner with minimal stress, giving them a competitive edge in terms of meat quality. This will be able to boost consumer's confidence towards the produces from these accredited abattoirs, and also allow these produces to have easier penetration into foreign markets. Slaughterhouses which falls short of those requirements are unable to obtain their licence.

Majority of the individually operating slaughterhouses in the country have not yet been licensed or are still in the processed of being registered with the Department of Veterinary Services, Malaysia (DVS). Slaughterhouses that have not been accredited their license may or may not adhere to these standards, and thus, compromising not only the quality of the meat, but also the health of the consumers.

Due to close proximity of the unlicensed slaughterhouses to its surrounding farms, most of these unlicensed slaughterhouses tend to be located less than one hour away to the pig farms. Government licenced abattoirs are located relatively further away, up to 3 or more hours journey from the farm. Due to the cost, distance and the reduction in transportation time, many farmers may favour to send pigs to the surrounding unlicensed registered slaughterhouses for slaughter. This proves to be of big concern in terms of meat quality control, traceability, disease control and public health monitoring in the country. In general, farmers are of the opinion that while licensed slaughterhouses provide better services, the transportation duration takes to travel from the farm to the slaughterhouse is high and will cause unnecessary stress to the pigs, especially under high ambient temperature in a tropical country (personal communication). Therefore, there is a need to study the effects of transportation duration and its effects on stress and meat quality in tropical environments, where ambient temperature is constantly high, such as in Malaysia.

Transportation duration to slaughter houses is one of the major pre-slaughter stresses contributing to the ultimate pork quality, and transportation stress has been shown to result in poor meat quality (Pérez et al., 2002). Acute pre-slaughter transportation was reported to trigger the adrenocortical response, increase serum corticosteroid levels, deplete the glycogen storage in the muscle and lower the ultimate pH values (Hambrecht et al., 2004; Huff-Lonergan et al., 2002; L. Rey-Salgueiro et al., 2018). There are many factors influencing stress during transportation, which will be further described in Literature view. Therefore, it is important to provide a control for these factors across experimental groups when evaluating stress during transportation. Ambient temperature in tropical environment can also be a big factor influencing transportation stress. This can be equated to summer weather, where it has been shown that it has twice the incidence of pale, soft and exudative (PSE) meat as

compared to in winter (Guardia *et al.*, 2004). The occurrence of PSE meat has been associated with acute pre-slaughter stressful conditions, such as transportation immediately before slaughter and also the RYR1 gene mutation (Gajana *et al.*, 2013; Guardia *et al.*, 2004).

Heat shock proteins (HSPs) are proteins that are produced when animals are subjected to stress and have been proven to play a critical role to protect cells the initial insults and provide resistance to subsequent similar insult (Li *et al.*, 2007). It acts as a molecular chaperon for intracellular transportation of essential proteins and prevent the aggregation of damaged proteins that will lead to further cell damage during stress (Chirico, Waters, & Blobel, 1988). HSP 70 is a pro-inflammatory stimulant, where it facilitates the infiltration of immune cells to the injured sites (Kovalchin *et al.*, 2006), without which there is a risk of necrosis or apoptosis of the cells. However, there seem to be conflicting results on levels of HSP70 expressed in different tissues (Yu *et al.*, 2007; Zhang *et al.*, 2012), as HSP70 controls different stress-related functions in those tissues during transportation. Most studies were done at an ambient temperature of 15°C to 25°C in temperate countries, while in Malaysia, temperatures and high humidity is high, and may even go up to 40°C on a hot sunny afternoon. The extent at which this may affect the expression of HSP70 in the pigs in Malaysia during transportation should be investigated. Moreover, there may also be a difference between the expression of HSP70 in different breeds.

Therefore, hypothesis statements were formed for the current study to address these issues in the swine industry in Malaysia with the following objectives.

1. To determine the WBSF values between the average values of roasted and boiled pork meat and its reliability of measurements.
2. To obtain the average commercial pork meat qualities of Duroc cross, as a baseline reference data for important pork quality parameters in Malaysia and compare the commercial meat quality parameters and fatty acid profile of Landrace, Large White and Duroc crossbreds.
3. To determine the effects of long and short transportation duration in Malaysian tropical terrains on serum corticosterone levels, glycogen levels, oxidative potential, meat quality characteristics and HSP70 mRNA expression in RYR1 mutation free Duroc crosses in tropical ambient temperature applicable in local slaughtering conditions
4. To compare effects of breeds (Landrace, Large White and Duroc crosses), on serum corticosterone levels, HSP70 mRNA expression, lipid oxidation and meat quality during short transportation.
5. To determine the correlation between transportation duration, breeds, HSP 70 mRNA expression, serum corticosterone, glycogen, thiobarbituric acid-reacting substances (TBARS) and meat quality

It is hypothesized that:

1. The Warner-Bratzler Shear Force (WBSF) values between the roasted and boiled pork measurement can be used interchangeably
2. The commercial pork meat quality and fatty acid profile for the breeds available in Malaysia is comparable to international standards
3. Transportation duration of 30minutes and 3 hours produces acceptable changes in meat quality indicating that the transportation from farm to licensed slaughterhouse will not affect the production good meat quality
4. Transportation duration in different breeds available in Malaysia produces different response to stress, HSP70 mRNA expression and meat quality



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