



UNIVERSITI PUTRA MALAYSIA

***MEAT QUALITY AND MUSCLE PROTEOME OF COMMERCIAL BROILER
CHICKENS SUBJECTED TO PRE SLAUGHTER ELECTRICAL STUNNING
AND GAS KILLING***

SALWANI MD SAAD

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UNIVERSITI PUTRA MALAYSIA
BERILMU BERBAKTI

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By

SALWANI MD SAAD

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

June 2014

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master Science

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June 2014

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Stunning prior to slaughter of animal is desired for i) animal welfare ii) high throughput of processing, and iii) meat quality. However, different stunning procedures may alter the biochemical changes thus the outcome on meat quality differently. This study was conducted to determine the effects of electrical stunning and gas killing prior to slaughter on meat quality and skeletal muscle proteome of commercial broiler chickens. The meat quality parameters examined are pH values, cooking loss, drip loss, tenderness and colour values (L^* , a^* , b^*). Two separate experiments were conducted separately. In Experiment 1, 40 commercial broiler chickens (2.60 ± 0.2 kg) were randomly assigned to electrical stunning (constant voltage of 30 V, 0.2 A, 50 Hz for 5s) and control (without stunning). Immediately after stunning, the broilers were subjected to manual neck cut using a sharp knife. In Experiment 2, 40 broiler chickens (2.60 ± 0.2 kg) were randomly assigned to gas killing (40% carbon dioxide, 30% oxygen and 30% nitrogen) and control (without prior gas killing), following which, the broilers were slaughtered immediately. *Pectoralis major* muscles were sampled for muscle proteome and the remaining were assigned for muscle pH, tenderness, cooking loss, drip loss and colour assessments at 0, 4 and 24 h postmortem. Muscle samples of the electrically stunned broilers presented lower ($p < 0.05$) cooking loss and lightness values (L^*). The gas killing had resulted in lower pH values ($p < 0.05$), lower shear force values ($p < 0.05$), higher cooking loss ($p < 0.05$), higher drip loss ($p < 0.05$) and lower lightness values ($p < 0.05$). The muscle proteome revealed expression changes ($p < 0.05$) of several proteins in both treatments. In electrical stunning, beta enolase, pyruvate kinase muscle isozyme and glycogen phosphorylase were expressed lower as compared to the control. On the contrary, gas killing treatment has significantly increased the expressions of beta enolase and pyruvate kinase, and reduced the expression of creatine kinase. In conclusion, electrical stunning had reduced the glycogen metabolism with improved meat quality. Meanwhile, the gas killing procedure had significantly elevated the glycolysis rate which resulted with poorer meat quality. It seems that the gas killing is more detrimental on meat quality as compared to electrical stunning.

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

**KUALITI DAGING DAN PROTEOM OTOT AYAM PEDAGING KOMERSIL
YANG DIKENAKAN RENJATAN ELEKTRIK DAN PEMBUNUHAN GAS
SEBELUM SEMBELIHAN**

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Renjatan sebelum penyembelihan haiwan sangat diperlukan untuk menjaga kebajikan haiwan dan meningkatkan produktiviti penyembelihan serta menambah baik kualiti daging. Walaubagaimanapun kaedah renjatan yang berbeza akan menyebabkan perubahan biokimia dan seterusnya akan menyebabkan perbezaan kualiti daging yang terhasil. Tujuan kajian ini dijalankan adalah untuk mengenalpasti kesan renjatan elektrik dan kesan pembunuhan gas yang dilakukan sebelum proses penyembelihan manual ke atas kualiti daging dan profil “proteome” otot pada ayam pedaging komersial. Dua eksperimen telah dijalankan secara berasingan. Dalam eksperimen pertama, 40 ekor ayam (2.60 ± 0.2 kg) telah dipilih dan dibahagikan secara rawak kepada dua kumpulan. Kumpulan pertama dikenakan renjatan elektrik (30 V, 0.2 A, 50 Hz selama 5 saat) sebelum penyembelihan dan kumpulan kedua adalah sebagai kawalan (tanpa renjatan). Sebaik sahaja kaedah renjatan dilakukan, semua ayam disembelih secara manual pada bahagian leher. Manakala, dalam eksperimen kedua, 40 ekor ayam (2.60 ± 0.2 kg) juga dibahagikan secara rawak kepada dua kumpulan, dengan kumpulan pertama dikenakan pembunuhan gas (40 % karbon dioksida, 30 % oksigen dan 30 % nitrogen) sebelum penyembelihan dan kumpulan kedua bertindak sebagai kawalan (tanpa pembunuhan gas). Terdapat lima parameter yang dinilai iaitu i) nilai pH ii) nilai tahap kelembutan iii) peratus kehilangan air melalui kaedah memasak iv) peratus “drip loss” dan v) nilai warna daging (kecerahan, kemerahan dan kekuningan), pada sela masa 0, 4 dan 24 jam *post-mortem*. Sampel otot *pectoralis major* ayam dari kumpulan renjatan elektrik menunjukkan kehilangan air melalui kaedah memasak dan nilai kecerahan yang lebih rendah ($p < 0.05$) berbanding dengan kumpulan kawalan. Manakala, sampel otot *pectoralis major* ayam dari kumpulan pembunuhan gas menunjukkan peratus kehilangan air melalui kaedah memasak dan “drip loss” yang tinggi ($p < 0.05$) serta penurunan ($p < 0.05$) nilai pH dan kecerahan pada otot *pectoralis major*. Analisis proteomik mendapati terdapat perubahan pengekspresan ($p < 0.05$) pada beberapa jenis protein dalam otot *pectoralis major* ayam yang dikenakan renjatan elektrik dan pembunuhan gas. Kaedah renjatan elektrik menunjukkan terdapat penurunan pengekspresan protein beta enolase, isozim piruvat kinase dan glikogen fosforilase. Manakala, kaedah pembunuhan gas menunjukkan peningkatan pengekspresan beta enolase dan piruvat kinase serta penurunan pengekspresan kreatina kinase. Semua protein yang terlibat dalam perubahan pengekspresan dari kajian ini diketahui mempunyai peranan dalam metabolisme glikogen yang mana mampu mempengaruhi kualiti daging. Kesimpulannya, hasil kajian ini mendapati bahawa kaedah renjatan elektrik sebelum penyembelihan telah menurunkan kadar metabolisme glikogen dan menyebabkan

peningkatan kualiti daging. Manakala kaedah pembunuhan gas telah meningkatkan kadar metabolisme glikogen yang telah mungkin penyebab kepada kerendahan kualiti daging. Maka, hasil kajian mendapati kaedah pembunuhan gas sebelum penyembelihan telah menyebabkan penurunan kualiti daging berbanding kaedah renjatan elektrik.



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I certify that a Thesis Examination Committee has met on 20 June 2014 to conduct the final examination of Salwani Md Saad on her thesis entitled “Meat Quality and Muscle Proteome of Commercial Broiler Chickens Subjected to Pre-Slaughter Electrical Stunning and Gas Killing” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

%	percentage
g	gram
kg	Kilogram
km	Kilometer
µg	microgram
ml	mililiter
w/v	weight per volume
v/v	volume per volume
kDa	kilo Daltons
M	molar
mM	milimolar
µl	microlitre
µm	micron
°C	degree Celcius
Cm	centimeter
nm	nanometre
mA	miliAmpere
OD	optical density
V	Volts
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
TEMED	tetramethylethylenediamine
EDTA	ethylenediamine tetra acetic acid
APS	Ammonium persulphate
NaCl	sodium chloride
BSA	bovine serum albumin
TCA	trichloroacetic acid
Tris	2 amino-2-(hydroxymethyl)-propane-1,3-diol
Tris-HCl	Tris in hydrocholic acid
TBS	tris buffered saline
HCl	hydrochloric acid
CHAPS	3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate
SDS	Sodium dodecyl sulfata
p	Probability
H	Hour/hours
Pm	Postmortem
min	Minute
S	Second
IEF	Isoelectric focussing
TGS	Tris, glycine saline
MALDI-TOF/TOF	Matrix assisted lased desorption ionization-time of flight
ACN	Acetonitrile

IAA	Iodoacetamide
DTT	Dithiothreitol
Vh	volt hour
pHu	Ultimate pH
FA	Formic acid
ES	Electrical stunning
US	Unstunned control
GK	Gas kiliing
CNT	Control without prior gas stunning
rpm	revolutions per minute (unit
APS	ammonium persulfate
TEMED	N,N,N',N'-Tetramethylethane-1,2-diamine





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CHAPTER 1

INTRODUCTION

There is no doubt that chicken meat has a great demand both locally and globally because of its versatility, relative affordability and high nutritional content (Arshad *et al.*, 2007). This has therefore resulted in a steady increase in the production of chicken over the last few years in order to meet the increasing demand for the meat products. The poultry industry has thus incorporated technologies for higher productivity. Noteworthy, more important than to scale up production, procedures such as pre slaughter stunning have also been imposed so as to eliminate the animals' sensibility towards unnecessary pain and distress throughout the slaughtering process (Coenan *et al.*, 2009). This has led to the gazette of animal's rights acts in several countries to protect and ensure animals being treated humanely throughout the entire production; from farm to plate (Grandin, 1998). However, livestock slaughtering is a concern of many stakeholders that therefore requires major changes on policy making and law enforcement especially regarding issues of animal welfare, hygiene and safety as well as workers welfare (Nakyinsige *et al.*, 2013; Bergeaud-Blackler, 2007). Any procedure at slaughter is tailed with concerns of animal welfare, meat quality and demographic preferences. Meat quality is affected by various factors before and after slaughter. Thus, regardless of demographic preferences and/or cultural, religious and regulatory requirements, any method of killing should not compromise the quality of the meat as well as the welfare of animals.

The water bath head only electrical stunning is commonly practiced in medium and large scale poultry abattoirs. It involves hanging chickens upside-down on a moving shackle leading toward a water tank that is constantly supplied with a sufficient amount of electrical current. The effect of electrical stunning involving only the head region is generally reversible (Vogel *et al.*, 2011). However, it is subjective in defining sufficient current that causes unconsciousness which lasts long enough for the slaughtering process and death to ensue. This is because; the effectiveness of electrical stunning varies depending on biological and external factors. Such biological factors include size of the animal, weight, sex and breed, whilst the electrical inputs (amount of currents and voltage, waveform, frequency and duration) are some of the external factors (Kranen *et al.*, 2000). Those factors will affect the amount of current received by the individual animal during stunning which may cause insufficient or over stunning (Turcsan *et al.*, 2003). Therefore, to meet the halal requirements which accept only stunning with reversible effects, a specific range of amperage is imposed for use in production of halal chicken meat (MS 1500; 2009, Department of Standards Malaysia). To date, the significance of such regime in relation to broilers meat quality has not been studied.

The manipulation of atmospheric air in order to set back consciousness before slaughter has become a popular option in poultry processing. This newer alternative has a purpose to render the bird unconscious with minimal impact on meat quality. Common problems such as blood spots and hemorrhages derived from the shackling procedure in electrical stunning are avoidable through induction of gas while the chickens are still in crates. Gas stunning/killing is claimed to be more humane as it releases consciousness rapidly by euthanasia (Forslid, 1987; Boogaard *et al.*, 1985). However, the application is tangled with concerns over the use of gases such as carbon dioxide which is unpleasant and painful because of its acidity property (Lambooj *et al.*, 1999) or the use of inert gases

which are probable for causing a sense of breathlessness and hyperventilation (Raj and Gregory, 1995). Additionally, the effectiveness of gas stunning/killing varies depending on gas inputs (gas type, mixture, concentration) and biological status of animal (size and health status). The application of gas stunning is generally irreversible. However, the span of consciousness is relatively short which make them impractical to sustain unconsciousness long enough for slaughtering process and until death occurs (Mohan Raj and Gregory, 1990). In Malaysia, there is hardly any study on the use of gas stunning in broilers. Furthermore, until now, the National Fatwa Council of Malaysia has yet to decide on the practicability of the use of gas stunning/killing in halal slaughter because of the limitation of scientific facts. Thus, this study was conducted in an attempt to investigate the effects of irreversible gas stunning (gas killing) on broilers meat quality.

Meat quality is developed during the postmortem phase through different factors both ante mortem and postmortem via different reactions in metabolic pathways. The ante mortem factors include slaughter processes such as pre slaughter stunning and handling. This is why the procedure of slaughtering is probably the most important procedure of transforming a livestock into pieces fit for human consumption (Nakyinsige *et al.*, 2013). For example, the current received in electrical stunning will suppress the transmission of impulses (Heath *et al.*, 1994) causing brain failure and shutting the pain reactivity as measured in somatosensory evoked potential (SEP) and electroencephalogram (EEG) assessments (Gregory and Wolton, 1990). It further causes relaxation of muscles preventing violent struggling and wing flapping. Lee *et al.* (1979) reported that delayed onset of rigor by the application of electrical stunning might be the cause for improvement of meat tenderness at 24 h postmortem. On the other hand, the anesthetic effect of gas stunning is responsible for the increasing rate of glycogen metabolism (Savenjie *et al.*, 2002).

Proteomics is a powerful tool to understand the development of meat quality traits at the molecular level. Protein expression is dynamic where a little changes in external in isoelectric properties, weight and intensity of proteins can be viewed using this approach. Therefore, due to its potential, 2D approach has been used in meat science related factors may affect the proteome profile (Bakry *et al.*, 2011). The 2D gel electrophoresis allows a complex mixture of proteins to be individually mapped on a gel. This approach was informative in revealing mechanisms of postmortem proteolytic activity (Koohmaraie *et al.*, 1991), and protein expression in association with meat quality traits (Hollung *et al.*, 2007; Bendixen, 2005; Lametsch *et al.*, 2003). However, to the best of our knowledge, there are limited resources on the effect of electrical stunning and gas killing on muscle proteome in broilers. Hence, this study was conducted to investigate the effect electrical stunning and gas killing on *Pectoralis major* muscle proteome and to associate it with meat quality.

Hypotheses

The application of electrical stunning and gas killing prior to slaughter will improve selected meat quality traits of muscle pH, shear force value, drip and cooking loss and color values (L^* , a^* and b^*) Furthermore, the application of electrical stunning and gas killing will reduce the expression of some glycolytic enzymes in the skeletal muscle proteome in commercial broiler chickens.

Objectives

This study was aimed to achieve the following objectives:

1. To determine the effects of electrical stunning on meat quality and skeletal muscle proteome of commercial broiler chickens
2. To determine the effects of gas killing on meat quality and skeletal muscle proteome of commercial broiler chickens



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