



**UNIVERSITI PUTRA MALAYSIA**

***MEAT QUALITY ATTRIBUTES AND PHYSIOLOGICAL, METABOLIC  
AND ELECTROENCEPHALOGRAPHIC RESPONSES TO SLAUGHTER  
POSITION AND KNIFE SHARPNESS IN CATTLE***

**IMLAN JURHAMID COLUMBRES**

**IPTSM 2020 11**



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AND ELECTROENCEPHALOGRAPHIC RESPONSES TO SLAUGHTER  
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By

**IMLAN JURHAMID COLUMBRES**

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

**June 2019**

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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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**IMLAN JURHAMID COLUMBRES**

**June 2019**

**Chairman : Professor Awis Qurni Sazili, PhD**  
**Institute : Tropical Agriculture and Food Security**

This study was aimed at determining the effects of slaughtering methods on the welfare, physiological response and carcass and meat quality of cattle. To achieve this objective, the following experiments were conducted.

In the first experiment, electroencephalographic changes, blood biochemistry and meat quality characteristics following upright and lateral recumbent slaughter position were assessed. Twenty Brahman cows were divided into two groups of 10 animals each and subjected to either upright slaughter position (UP) or lateral recumbent slaughter position (LP). Based on the EEG results, the changes in brain electrical activities were significantly different between animals slaughtered in an upright position (UP) and animals slaughtered in a lateral recumbent position (LP). Moreover, the results demonstrated that upright slaughter position caused hyperglycemia, lactic acidemia, and an increase in the levels of catecholamines and activities of liver enzymes. Slaughter positions affected electroencephalographic, physiological stress and blood biochemical responses in cattle.

The second experiment compared the sharpness of the knife used in slaughter of cattle on physiological stress response, encephalographic changes and meat quality. Twenty Brahman cows were divided into two groups of 10 animals each and subjected to either sharp knife (SK) or less sharp knife (LSK) used in slaughter. Analysis of the sticking blood revealed that all variables ( $p < 0.0001$ ) were higher than their values in blood samples taken at pre-slaughter and post-slaughter. Following slaughter, the LSK animals had higher changes of electrical activity of the brain than that of pre-slaughter. Animals slaughtered in less sharp knife group (LSK) exhibited higher ( $p < 0.0001$ ) lactate, shear force, sarcomere length and myofibrillar fragmentation index than sharp

knife group. The carbonyl and thiol contents determination revealed that the protein oxidation increased with aging time but was not affected by the slaughter knife. The catecholamines, glucose and liver enzymes were lower in animals slaughtered with a sharper knife. The present findings indicate that less sharp knife caused substantial physiological stress responses which compromises the animal welfare and meat quality. Likewise, the EEG profiles indicated that animals may have endured lesser amount of pain when being slaughtered using a sharp knife. Sharpness of the knife is very important factor to minimize the pain during slaughter.



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

**PERHUBUNGAN KUALITI DAGING DAN FISIOLOGI, METABOLIK DAN ELEKTROENSEFALOGRAF BERGERAK BALAS TERHADAP KEDUDUKAN PENYEMBELIHAN DAN KETAJAMAN PISAU PADA LEMBU**

Oleh

**IMLAN JURHAMID COLUMBRES**

**Jun 2019**

**Pengerusi : Profesor Awis Qurni Sazili, PhD**  
**Institut : Pertanian Tropika dan Sekuriti Makanan**

Kajian ini bermatlamat untuk menentukan kesan kaedah penyembelihan terhadap kebajikan, tindak balas fisiologi dan kualiti karkas dan daging lembu. Bagi mencapai objektif ini, beberapa eksperimen telah dijalankan.

Di dalam eksperimen pertama, perubahan elektroensefalograf, sifat biokimia darah dan ciri-ciri kualiti daging berdasarkan kedudukan penyembelihan secara menegak dan mengiring telah dinilai. Dua puluh ekor lembu Brahman telah dibahagikan kepada dua kumpulan iaitu sebanyak 10 ekor haiwan setiap kumpulan dan tertakluk kepada posisi sembelihan secara menegak (UP) atau mengiring (LP). Berdasarkan keputusan EEG, perubahan dalam aktiviti elektrik otak berbeza secara signifikan diantara haiwan yang disembelih di posisi menegak (UP) dan haiwan yang disembelih dalam posisi mengiring (LP). Tambahan pula, keputusan ini menunjukkan bahawa kedudukan penyembelihan secara menegak menyebabkan hiperglisemia, *lactic acidemia* dan peningkatan tahap *catecholamines* dan aktiviti enzim hati. Posisi penyembelihan mempengaruhi terapi elektroensefalograf, tekanan fisiologi dan tindak balas biokimia darah lembu.

Eksperimen kedua membandingkan ketajaman pisau yang digunakan dalam penyembelihan ternakan terhadap tindak balas tekanan fisiologi, perubahan ensefalograf dan kualiti daging. Dua puluh ekor lembu Brahman telah dibahagikan kepada dua kumpulan dengan 10 ekor haiwan setiap kumpulan dan tertakluk kepada pisau tajam (SK) atau pisau kurang tajam (LSK) yang digunakan dalam proses penyembelihan. Analisis terhadap pelekatan darah mendedahkan bahawa semua pembolehubah ( $p < .0001$ ) lebih tinggi berbanding nilai sampel darah yang diambil

sebelum penyembelihan dan selepas penyembelihan. Berikutan penyembelihan, haiwan LSK mempunyai perubahan aktiviti elektrik otak yang lebih tinggi berbanding sebelum penyembelihan. Kumpulan haiwan yang disembelih dengan pisau kurang tajam (LSK) menunjukkan lebih tinggi ( $p < 0.0001$ ) laktat, daya ricih, panjang *sarcomere* dan indeks pemecahan *myofibrillar* berbanding kumpulan pisau tajam. Penentuan kandungan karbonil dan thiol mendedahkan bahawa pengoksidaan protein meningkat dengan masa proses penuaan tetapi tidak terjejas oleh ketajaman pisau penyembelihan. Kandungan *catecholamines*, glukosa dan enzim hati lebih rendah pada haiwan yang disembelih dengan pisau yang lebih tajam. Hasil kajian ini menunjukkan bahawa pisau yang kurang tajam menyebabkan tindak balas tekanan fisiologi dimana menjejaskan kebajikan haiwan dan kualiti daging. Begitu juga, profil EEG menunjukkan bahawa haiwan mungkin mengalami kesakitan lebih rendah apabila disembelih dengan menggunakan pisau yang tajam. Ketajaman pisau merupakan faktor yang sangat penting bagi mengurangkan kesakitan semasa penyembelihan.

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**Awis Qurni Sazili, PhD**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Zulkifli Idrus, PhD**

Professor Dato'  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Goh Yong Meng, PhD**

Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

---

**ZALILAH MOHD SHARIFF, PhD**

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Name of Chairman  
of Supervisory  
Committee: Professor Dr. Awis Qurni Sazili

Signature: \_\_\_\_\_  
Name of Member  
of Supervisory  
Committee: Professor Dato' Dr. Zulkifli Idrus

Signature: \_\_\_\_\_  
Name of Member  
of Supervisory  
Committee: Professor Dr. Goh Yong Meng

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## LIST OF ABBREVIATIONS

a*	Redness
APS	Ammonium Persulfate
AS	slaughter following minimal anesthesia
ATP	Adenosine Triphosphate
b*	Yellowness
BK	Blunt knife
BSA	Bovine Serum Albumin
CaCl <sub>2</sub> •2H <sub>2</sub> O	Calcium Chloride dihydrate
cfu	colony forming units
CK	Creatine Kinase
cm	Centimeter
CNS	Central Nervous Systems
d	Day
DAB	3,3-diaminobenzidine
ddH <sub>2</sub> O	Deionized Distilled Water
DFD	dark, firm and dry
DTNP	2, 2-dithiobis (5-nitropyridine)
EC	European Community Council Regulations
EDTA	Ethylene Diamine Tetraacetic Acid
EEG	Electroencephalogram
EFSA	European Food Safety Association
F50	median frequency
FAO	Food and Agricultural Organization
g	Gram

h	Hour
Hb	Hemoglobin
H <sub>2</sub> O <sub>2</sub>	Hydrogen Peroxide
HO•	Hydroxyl Radicals
HPA	Hypothalamic Pituitary Adrenal
HS	Halal Slaughter
HSA	Humane Slaughter Association
K <sub>3</sub> Fe (CN) <sub>6</sub>	Potassium Ferricyanide
KCl	Potassium Chloride
kg	Kilogram
KH <sub>2</sub> PO <sub>4</sub>	Monopotassium Phosphate
l	Liter
L*	Lightness
LDH	Lactate Dehydrogenase
LL	Longissimus lumborum
LP	Lateral position
LW	body weight pre-slaughter
M	Mole
MAC	minimum alveolar concentration
Mb	Myoglobin
MDA	Malondialdehyde
MFI	Myofibrillar Fragmentation Index
MgCl <sub>2</sub>	Magnesium Chloride
MgCl <sub>2</sub> •6H <sub>2</sub> O	Magnesium Chloride Hexahydrate
min	Minute
µg	Micro gram

$\mu\text{l}$	Microliter
$\mu\text{l}$	Micro liter
$\mu\text{m}$	Micrometer
$\mu\text{M}$	Micromole
$\mu\text{mole}$	Micromole
ml	Milliliter
mmole	Millimole
ms	Millisecond
$\mu\text{V}$	Microvolt
MTT	Thiazolyl Blue Tetrazolium Bromide
$\text{Na}_2\text{HPO}_4$	Disodium Phosphate
$\text{NaCl}$	Sodium Chloride
NAD	$\beta$ -nicotinamide adenine dinucleotide
ng	Nanogram
nm	Nanometer
nmol	Nanomole
$^{\circ}\text{C}$	degree Celsius
OD	optical density
OIE	World Organization for Animal Health
%	Percent
pH0	pre-rigor pH
pH45min	45 min postmortem pH
pHu	ultimate pH
PSE	pale, soft and exudative
Ptot	total power
RMS	root mean square

ROS	reactive oxygen species
s	Second
SK	Sharp knife
ST	Semitendinosus
SWS	slaughter without stunning
TBARS	Thiobarbituric Acid-Reactive Substances
TCA	Trichloroacetic Acid
TEMED	Tetramethylethylenediamine
Tris	2-amino-2-(hydroxymethyl)-propane-1,3-diol
US	Upright slaughter
USDA	United States Department of Agriculture
v/v	volume per volume
VER	Visual Evoked Responses
w/v	weight per volume
WHC	Water Holding Capacity



## CHAPTER 1

### GENERAL INTRODUCTION

Meat is a good source of high quality animal protein and serves an essential function in health of human (Troy *et al.*, 2016). Red meat is one the most sought after meat in the world due to its taste, flavor and juiciness which helps drive up its demand especially in the developing economies (Binnie *et al.*, 2014). Beef (cattle meat) is a vital source of quality protein, iron, and B-vitamins (Piatti-Farnell, 2013). Beef is the third most widely consumed meat in the world and it accounts for up to 25% of global meat production. In the world, the countries with the highest consumption of beef are United States, Brazil, and People's Republic of China. In 2015, the world's largest exporters of beef were India, Brazil, and Australia (Sharad, 2015). In Malaysia, beef production is considered an important agricultural industry. When the nation experienced steady economic prosperity in the post-independence era, beef consumption has been on an increasing trend (Ariff *et al.*, 2015). In general, the beef production in 2016 was up by 5 percent (Osothongs *et al.*, 2016). Beef is much more reachable for consumers now and higher quality of meat cuts is creating stronger market demand (Gellynck & Verbeke, 2001).

Slaughtering, which is the killing of domestic livestock for food, in the case of cattle for beef, and their management during this process is a focal point of discussion in the livestock industry. Stress which the livestock undergo before slaughter and their manhandling during the process can have detrimental impacts on the meat produced from these animals (Mpakama *et al.*, 2014).

Slaughtering of the animal for meat production is of utmost importance and has remained the subject of debate from welfare perspectives. Halal and Kosher slaughter methods are obligatory to practice by the Muslim and Jews, respectively. Halal and Kosher methods are also used commercially on a larger scale to meet the global demand of these meats (Sabow *et al.*, 2015). Slaughtering, if properly done according to the prescribed principles, religious slaughter takes care of animal welfare issues (Grandin, 2006). Current religious discussion on animal slaughtering emphasizes the stress which animals undergo during pre-slaughtering resulting from the use of restraints, the pain and distress they may feel during and after neck cutting, as well as the delayed period it takes before they lost their brain function and eventual death when stunning is not properly conducted (Anil, 2012a).

Halal slaughtered animals are slaughtered in left lateral recumbency, whereas, in Kosher animals are slaughtered in upright position. Research has been conducted in animals slaughtered in various positions such as, upright, 45°, 90° or lateral recumbency and 180° (Dunn, 1990; Gregory *et al.*, 2009; Grandin, 2013; Gerritzen *et al.*, 2014; Velarde *et al.*, 2014). It was reported that rotating causes acute stress in animals before slaughter, similarly, animals slaughtered by Kosher rite versus

traditional (with stunning) are subjected to higher stress conditions at exsanguination phase (Bozzo *et al.*, 2018).

In Halal slaughtering, the animals are killed by cutting the jugular vein, carotid artery, trachea, and esophagus with a sharpened knife in a single stroke which is usually less painful and stressful (Grandin & Regenstein, 1994; Gregory, 2005; Gibson *et al.*, 2009a; Ndou *et al.*, 2011). Knife sharpness is of utmost importance since the use of less sharp knives requires much force which may over time cause injury to the operator's hand. Over the years, studies have revealed that more than 80% of the workforce in abattoir use improperly sharpened knives (Claudon & Marsot, 2006). Duller knives necessitate the use of increased force, which results in slips, injuries, delayed production and lower profits (Karlton *et al.*, 2016). Besides this, less sharp knife results in smaller repetitive, un-even cuts to accomplish the slaughter; this can cause more trauma leading to pain and more stress to the animal, subsequently affecting the bleeding efficiency (Gibson *et al.*, 2009; Zulkifli *et al.*, 2014).

Following slaughter, blood parameters gives significant information in regards to some physiological changes related to stress and noxious stimuli (Nowak *et al.*, 2007). In farm animals, enhanced sympatho-adrenal activity caused by physical and psychological stress leads to hyperglycemia. This results from accelerated breakdown of glycogen in the liver (Mason, 2006; Adenkola & Ayo, 2010). Slaughtering cattle without stunning results in an increased blood lactate which is produced by an anaerobic glycolysis. Increased amounts of creatine kinase (CK) and lactate dehydrogenase (LDH) in serum indicate stress, muscle damage and muscle fatigue (Nakyinsige *et al.*, 2013; Nakyinsige *et al.*, 2014b). It widely believed that biochemical blood changes occur in response to physical stress condition such as those induced by slaughter procedures and this could compromise welfare (Sabow *et al.*, 2015). The possibility of pain, stress and onset of the unconsciousness during and after the neck cut are the major animal welfare concerns. Pain resulting from neck cutting is the focus of several discussions in recent time. It was recommended that slaughtering animals using exquisitely sharp knife brings about minimal behavioral reaction and this is perceived as un-painful to the animal (Regenstein, 2012; Awan & Sohaib, 2016). Sharp knife which results in quick bleeding causes physiological adjustments, loss of consciousness and eventually death. Less sharp knife can cause false aneurysm resulting in delay of animal death and consequently inflicts pain and stress to the animals. Blunt knife edge causes uneven and small repetitive cuts leading to more trauma and swelling at the cut ends of the vessels impeding the blood flow, leading to engorgement of vessels (ballooning) consequently, and false aneurysm (Rosen, 2004; Gregory, 2008; Gregory *et al.*, 2012b).

This can cause insufficient bleeding and other disturbances in physico-chemical properties imbalances, Poor bleeding efficiency result in more blood residue in the meat, favours multiplication of spoilage microorganisms (Lerner, 2009), causing retention of more hemoglobin resulting in higher oxidation of protein and lipid (Alvarado *et al.*, 2007) and affecting shelf life (Soyer *et al.*, 2010); thus, resulting in low standard meat quality (Pleiter, 2010).

Stress before or during slaughter to animal can disturb the bleeding efficiency. Efficient bleeding is one of the prime goals of slaughter procedures. Complete removal of the blood during exsanguinations has major impact on the quality of meat, its physico-chemical characteristics and shelf life. Stress results in stimulation of sympathetic nervous system (a built-in mechanism of fight-or-flight response) leading to changes in the blood pressure leads to vasoconstriction (narrowing of blood vessels) and changes in blood constituents of the animals such as concentrations of creatine kinase (CK), lactate dehydrogenase (LDH), glucose, cortisol, and packed cell volume (Knowles *et al.*, 2014). Stress has been reported to greatly influence the toughness of beef (Gruber *et al.*, 2010), high pH in cattle, sheep and pigs resulting in darker piece of meat (Ponnampalam *et al.*, 2017). The pH is affected by the conversion of glycogen to lactic acid, the immediate and long-term stress being experienced by the animals affect the rate of glycogen metabolism (Stajković *et al.*, 2017).

### **Problem statement**

There is a dearth of knowledge on the effect innovative restraining methods on the welfare of ruminants. Thus, there is a need for further study on the impact of positioning and animal rotation. In this context various methods of restraining chutes such as Facomia, Weinberg pen, ASPCA pen, MARK IV, (Dunn, 1990; Grandin, 1992; Velarde *et al.*, 2014; Bozzo *et al.*, 2018) and slaughter positions such as, upright, 45°, 90° or lateral recumbency and 180° (Dunn, 1990; Gregory *et al.*, 2009; Grandin, 2013; Gerritzen *et al.*, 2014; Velarde *et al.*, 2014) have been studied. The results of some studies reported that animals slaughtered by Kosher rite versus traditional (with stunning) are usually exposed to intense stress conditions during the exsanguination phase (Bozzo *et al.*, 2018). Likewise, it has been revealed that there is an acute stress effect caused by animal rotation, and it was reported that repeated restraining and rotation is more stressful on the animal.

Although, the sharpness of the knife is not possible to be measured quantitatively everywhere or at every slaughterhouse, however, ANAGO® sharpness tester has been influential in accomplishing notable improvements in knife sharpness. It ensures accurate, independent and efficient monitoring of sharpening tools, people and practices allowing sharpening to be constantly and effectively controlled, optimized and upgraded. ANAGO® knife sharpness tester can be used as an objective tool to measure the sharpness of the knife at commercial slaughterhouses at a very large scale. There have been no studies on the effect of knife sharpness on the physiology, stress and pain of slaughtered animals. Furthermore, dull or knife of less sharpness have been reported to lead to more than one cut to severe the required structure (Gregory *et al.*, 2012b) for a slaughter to be considered as “Halal”. More cuts at the vessels due to less sharp knife have been reported to cause false aneurysm leading to a delayed period before the animal is unconscious (Gregory, 2008; Gregory *et al.*, 2012a) in the animal with subsequent more stress and pain.

Studies regarding the effects of slaughter positions in Halal and Kosher as well as less sharp compared to sharp knife in similar experimental condition, which could provide

understandings into the effects of positions and sharpness of knife on animals various physiological parameters such as blood biochemistry, meat quality, stress as well as pain, have not been reported. Therefore, this study was conducted to achieve the following objectives

1. To determine blood biochemical and electroencephalographic changes associated with slaughter in cattle subjected to different halal slaughter positions and knife sharpness.
2. To assess the effect of different halal slaughter positions and knife sharpness on the bleeding efficiency and storage stability of cattle meat.
3. To evaluate the meat quality characteristics in cattle subjected to different Halal slaughter positions and knife sharpness.
4. To identify the bleeding efficiency, lipid and protein oxidation and microbiological quality of cattle meat subjected to different halal slaughter positions and knife sharpness.

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## BIODATA OF STUDENT

Jurhamid Columbres Imlan was born on the 30<sup>th</sup> day of August 1977 at USM, Kabacan, North Cotabato. He finished his primary education at USM Annex Elementary School, Kabacan, Cotabato, Philippines in 1990 with honors. He pursued his secondary education at University Laboratory School – University of Southern Mindanao and graduated in 1994. He took his Bachelor of Science degree in Agriculture major in Plant Breeding and Genetics at University of Southern Mindanao and successfully graduated in 1998.

He joined the University of Southern Mindanao as Research Assistant in 1998 and later became lecturer in the Department of Animal Science, College of Agriculture. With his desire to advance his professional education, he enrolled his Master of Science in Animal Science (MSAS) at University of Southern Mindanao and successfully finished in 2002.

He believed that career advancement through a doctorate degree in a prestigious University would benefit himself professionally and as well as his students in his home country so he applied for SEARCA Scholarship grant. He was accepted in the year 2014 and was given the scholarship grant to pursue his Doctor of Philosophy degree in Animal Production (Meat Science) at Universiti Putra Malaysia. He had successfully conducted this research study on the meat quality attributes and physiological, metabolic and electroencephalographic responses to slaughter position and knife sharpness in cattle.

## LIST OF PUBLICATIONS

- Jurhamid Columbres Imlan, Ubedullah Kaka, Yong-Meng Goh, Zulkifli Idrus, Elmutaz Atta Awad, Ahmed Abubakar Abubakar, Tanbir Ahmad, Hassan N. Quaza Nizamuddin and Awis Qurni Sazili (2020). Effects of Slaughter Knife Sharpness on Blood Biochemical and Electroencephalogram Changes in Cattle *Animals* 2020, 10, 579.  
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