EFFECTS OF PRESLAUGHTER STRESS INTENSITIES ON ANIMAL WELFARE, PHYSIOLOGICAL RESPONSES, MUSCLE BIOCHEMISTRY, CARCASS AND MEAT QUALITY IN GOATS

By

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

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DEDICATION

This thesis is dedicated to my cherished wife, Smt. Meenakshi Vashishtha, whose unwavering support and encouragement fueled my journey. To my beloved children, Ishant Sharma and Gauranga, your joyous presence and boundless love have been my constant motivation.

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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Preslaughter stress intensities, such as inappropriate handling of animals prior to slaughter and slaughter ambient, have a significant impact on animal welfare and meat quality. The present study evaluated the effects of training of livestock handlers and exposure to the act of slaughter on various behavioral, physiological, muscle biochemistry, and meat quality parameters in goats. A total of 6 handlers were divided into trained (trained in animal handling, behavior, and welfare), contact trained (not trained directly but interacted and visualized the handling by trained handlers), and untrained groups (not undergone training), with 2 handlers in each group. A total of 18 goats (Boer cross, bucks, 8-12 months of age, 25-30 kg live weight) were used in the study. Various behavioral, physiological, electroencephalogram variable, and blood-biochemical parameters were recorded before handling at the lairage and after handling at the slaughter point goats. The goats were halal slaughtered into three groups viz., Control (slaughtered alone), E (exposed to act of slaughter), and S (slaughtered in front of E). The *Longissimus thoracis et lumborum* (LTL) muscle was

used to evaluate various meat quality parameters on days 0, 1, and 5 during aging under refrigeration. The goats handled by untrained and contact-trained handlers were recorded with significant ($p<0.05$) increase in the electroencephalogram (EEG) spectrum (beta waves, theta waves, and median frequency), intense vocalization, significant ($p<0.05$) increase in heart rate and blood glucose, and nor-adrenaline. The exposure of the goats to the slaughter resulted in a significant ($p<0.05$) increase in the EEG spectrum (beta waves, theta waves, and median frequency), lactate dehydrogenase, nor-adrenaline, and β-endorphin. The exposure to slaughter ambient in goats was observed to have a significant ($p < 0.05$) effect on muscle pH, glycogen content, and cooking loss. Thus, the present study highlighted the importance of training livestock handlers to improve human-animal interactions and slaughtering goats without exposure to the slaughter of conspecific to improve animal welfare and meat quality. These findings could help in harmonizing all practices of slaughter and improving animal welfare while fulfilling religious and customary demands.

Keywords: Emotional stress, Meat quality, Preslaughter handling, Stress biomarkers, Training

SDG: GOAL 12: Responsible consumption and production

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

INTENSITI STRESS SEBELUM SEMBELIHAN DAN KESAN TERHADAP KEBAJIKAN HAIWAN, RESPON FISIOLOGIKAL, BIOKIMIA OTOT, KARKAS DAN PARAMETER KUALITI DAGING KAMBING

Oleh

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Tahap intensiti stress sebelum sembelihan, seperti pengedalian haiwan dan keadaan persekitaran yang tidak sesuai sebelum sembelihan akan mempengaruhi kebajikan haiwan dan kualiti daging. Kajian ini telah dijalankan untuk menilai kesan latihan pengendali haiwan ternakan dan pendedahan kepada proses sembelihan terhadap pelbagai tingkahlaku fizikal, fisiologikal, biokimia otot, dan parameter kualiti daging kambing. Sejumlah 6 orang pengadali telah dibahagikan kepada kumpulan terlatih (mempunyai latihan asas tentang pengendalian haiwan, tingkah laku haiwan, dan kebajikan haiwan), separa terlatih (tidak dilatih secara terus tetapi telah berinteraksi dan melihat pengendalian yang dilakukan oleh pengendali terlatih), dan tidak terlatih (tiada latihan secara formal), di mana setiap kumpulan diwakili oleh dua orang pengendali. Sejumlah 18 ekor kambing (baka Boer cross, tidak dikebiri, berumur 8-12 bulan, berat hidup 25-30 kg) telah digunakan di dalam kajian ini. Pelbagai tingkahlaku, fisiologikal, pembolehubah elektroensefalogram, dan parameter biokimia-darah telah direkod sebelum pengedalian di kendang haiwan dan selepaas kambing di kendalikan

semasa di titik sembelihan. Kambing-kambing tersebut telah disembelih secara halal dah dibahagikan kepada tiga kumpulan iaitu, kawalan (disembelih berseorangan), E (didahkan kepada proses sembelihan), dan S (disembelih di hadapan E). Otot longissimus thoracis et lumborum (LTL) telah digunakan untuk menilai pelbagai parameter kualiti daging pada hari 0, 1 dan 5 semasa proses penuaan kering di dalam kondisi sejuk. Kambing-kambing yang telah dikendalikan oleh kumpulan yang tidak terlatih dan separa terlatih telah merekodkan perubahan yang signifikan ($p<0.05$) terhadap spektrum EEG (gelombang beta, gelombang theta, dan frekuensi median), penyuaraan yang sangat tegang, kenaikan signifikan ($p<0.05$) untuk kadar degupan jantung dan glukosa darah, dan nor-adrenaline. Pendedahan kambing-kambing kepada penyembelihan telah mengakibatkan peningkatan signifikan ($p<0.05$) kepada spektrum EEG (gelombang beta, gelombang theta, dan frekuensi median), lactate dehydrogenase, nor-adrenaline, dan β-endorphin. Pendedahan kepada suasana penyembelihan kambing telah dilihat memberikan kesan signifikan ($p<0.05$) terhadap pH otot, kandungan glycogen, dan kehilangan air selepas memasak. Justeru, kajian semasa telah menunjukkan kepentingan latihan kepada pengendali haiwan agar dapat menambahbaik interaksi antara haiwan dan manusia, dan kambing yang disembelih tanpa didedahkan kepada proses sembelihan sebelumnya dapat menambahbaik kebajikan haiwan dan kualiti daging. Penemuan-penemuan ini dapat membantu dalam mengharmonikan kesemua amalan penyembelihan dan menambahbaik kebajikan haiwan dan dalam masa yang sama memenuhi keperluan agama dan adat.

Kata kunci: Tekanan emosi, Kualiti daging, Pengendalian sebelum penyembelihan, Penanda bio tekanan, Latihan

SDG: Matlamat 12: Tanggungjawab Penggunaan dan pengeluaran

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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

%	Percent
µg	Micro gram
µl	Microliter
ATP	Adenosine triphosphate
b*	Yellowness
C*	Chrom
CK	Creatine kinase
cm	Centimeter
CNS	Central nervous systems
d	Day
ddH2O	Deionized distilled water
DFD	Dark, firm, and dry
dUTP	Deoxyuridine Triphosphate
EC	European Community Council Regulations
ECG	Electrocardiogram
EDTA	Ethylene diamine tetra acetic acid
EEG	Electroencephalogram
EFSA	European Food Safety Association
g	Gram
h	Hour
HGB	Hemoglobin
HPA	Hypothalamic pituitary adrenal
KCl	Potassium chloride
kg	Kilogram

Km	Kilometers
L	Liter
L*	Lightness
LDH	Lactate dehydrogenase
m	Muscle
m^2	Square Meter
MDA	Malondialdehyde
MF	Median Frequency
min	Minute
ml	Milliliter
NaCl	Sodium chloride
NAD	β-nicotinamide adenine dinucleotide
ng	Nanogram
nm	Nanometer
ºC	Degree Celsius
OD	Optical density
OIE	World Organization for Animal Health
PGD	Programmed cell death
pHu	Ultimate pH
PSE	Pale, soft and exudative
Ptot	Total Power
RMS	Root mean square
s	Second
SAA	Serum amyloid-A
TUNNEL	Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling
WHC	Water Holding Capacity

CHAPTER 1

INTRODUCTION

1.1 Background

Slaughter is the most important operation in transforming an animal into pieces to make it fit for human consumption. This crucial moment of killing is governed by strict regulations related to food hygiene and safety, religious laws, working conditions, and animal welfare. Various pre-slaughter handling practices and procedures of animals at the farm, during transport and marketing, and at the slaughterhouses may subject the animals to various stressors. These preslaughter stress intensities/ stressors can adversely affect the welfare of animals by eliciting various behavioral and physiological responses (Terlouw & Bourguet, 2022), such as increasing excitability score, respiration rate, rectal temperature, and increased plasma cortisol and catecholamines concentrations (Sejian et al., 2021; Kadim et al., 2006; Kannan et al., 2002), which in turn may negatively influence the carcass and meat quality attributes (Ferguson & Warner, 2008).

Pre-slaughter stress has a marked impact on meat quality, such as higher drip losses, lower WHC, DFD, downgrading of carcass due to bruises, hemorrhage, poor sensory score, and reduced shelf life (Ponnampalam et al., 2017; Kadim et al., 2006). The mishandling of animals during various pre-slaughter operations also leads to muscle injuries, consequently leading to an increase in blood CK and LDH levels (Ekiz et al., 2012). Proteomic studies have associated the regulation of small heat shock proteins

(sHSPs) with various meat quality attributes, including tenderness, color, juiciness, and flavor. Due to the anti-apoptotic and chaperone functions of sHSPs, they are proposed to be involved with the eating quality of meat (Lomiwes et al., 2014).

According to the Universal Declaration of Animal Rights that was proclaimed in Paris, France in 1978, all animals' life has the right to be respected and if it is necessary to kill an animal, it must be instantaneous, painless, and without apprehension (Chapouthier & Nouët, 1998). Humane slaughtering concerns being sympathetic to the animals being killed for meat production through minimizing animal suffering and respect for animals' intrinsic worth. Islam respects the intrinsic worth of animals and emphasizes the animal welfare aspect. Islam has set explicit rules for using animals for human purposes. Islam has set out clear rules for the humane slaughter of animals for human consumption with particular emphasis on handling prior to slaughter and after slaughter. Prophet Muhammad (peace be upon Him) said: "Allah calls for mercy in everything, so be merciful when you kill and when you slaughter; sharpen your blade to relieve its pain." (Al-Qaradawi, 1960).

Goat rearing is very common in developing countries of Asia and Africa reared for meat, milk, and leather. Goats can be reared with minimum feed and housing inputs and provide extra income and sustenance to poor people (Umaraw et al., 2017). The lack of proper infrastructure, marketing facilities, and awareness about good animal handling practices makes these smart and intelligent animals prone to mishandling, physical abuse, distress, pain, and suffering. Recent developments in cognitive science have established goats' ability to differentiate between positive and negative emotion-linked vocalization and more alertness toward negative stimuli (Baciadonna et al.,

2018; Baciadonna, 2017). The expression of negative emotions is more important and intense than positive emotions due to their role in animal survival and defense. Goats exhibited different behavioral reactions to images of faces photographed in a different situation ($p<0.05$) and gave more attention to images or videos of conspecific with negative situations (Bellegarde et al., 2017).

Gregarious animals like goats also have emotional reactions and share feelings with conspecifics. Empathy in ruminants, such as ewe to kids and cow to calves, has been well documented (Khan et al., 2018). This emotional transmission plays a vital role in coordination, strengthening social bonds, prey defense, productivity, and mitigating stress by social buffering and animal welfare (Baciadonna et al., 2018). The vision and smell of the blood of distressed animals (vocalized or struggling during slaughter) cause panic in the animal to be slaughtered due to semiochemicals released from the blood of a distressed animal (Grandin, 2019; Grandin & Vogel, 2011).

1.2 Problem statement

Animals are often beaten and dragged while restrained just prior to the slaughter, which is an extreme violation of animal welfare guidelines (Rahman, 2017; Farouk et al., 2016; Rahman & Aidaros, 2012). Grandin (2013) also observed the mishandling of young calves dragging by ear pulling. This cruelty to animals during slaughter has been reported to occur in most countries where Islam is a major religion (Alam et al., 2020; Ahsan et al., 2014). Most of the people involved in this, such as those involved in the transport of animals, animal handlers, and butchers, are Muslims (Rahman, 2017). Peres et al. (2014) noted low-intensity stressors as mild handling that does not

cause physical injury and agitation, while high-intensity handling evokes agitation, and stress and may cause injury to the animals.

Proper preslaughter handling of animals is regarded as the first step in improving the productivity and welfare of farm animals (Grandin, 1998). The training to livestock handlers in basic animal behavioral and handling principles improve the skills, attitude, and behavior, thereby improving the human-animal interactions. Basic knowledge about the behavioral principles of animal handling among livestock handlers and slaughterhouse staff is very important in ensuring animal welfare by alleviating stress, pain, and fear and improving good quality meat (Farouk et al., 2014). Trained handlers are the livestock handlers that have successfully undergone training on basic animal behavior and handling principles, stress responses and animal welfare principles. Contact-trained handlers are handlers that have not undergone training on basic animal behavior and handling principles, stress responses and animal welfare principles but have visualized the handling of trained handlers and interacted with them. Untrained handlers are the handlers that did not undergone training program on animal behavior and handling principles and did not have direct contact with trained or contact-trained handlers (Cellabos et al., 2018).

It has been observed that at the actual point of slaughter, animals are being slaughtered in front of other animals; consequently, they resist moving towards the end they know what awaits them (they are certainly "dying twice") (Ahsan et al., 2014; Rahman & Aidaros, 2012). This practice is more prevalent in situations where commercial slaughter co-exists in parallel with both backyard slaughter and religious slaughter. This exposure of goat to negative stimuli such as smell of blood, vocalization, noise,

movement of equipment and unfamiliar workers could initiate stress responses, thereby affecting animal welfare and meat quality. There have been several scientific reports on the role of olfactory senses through stress pheromones in modifying physiological status and animal welfare conditions (Bombail, 2019). Scientific studies of animals getting panicked and stressed by the vision and auditory senses are still lacking. Thus, there is a need to safeguard and improve practices in all institutions or situations (smallholder, religious/ceremonial set-up) where animals are slaughtered in view of each other.

1.3 Justification of the study

Mitigation of preslaughter stress is crucial for animal welfare and production of good quality meat. Various preslaughter stress intensities such as improper handling of animals during moving from lairage to slaughter point, novelty of environment, and exposure to slaughter ambient initiate stress, pain and fear responses in animals, thereby compromising animal welfare and deteriorating meat quality. Out of 40 animal welfare hazards occurring during preslaughter handling of animal, livestock handlers and slaughter house staffs (Nielson et al., 2021).

Thus, the present study is designed to generate baseline data and scientific evidences of the importance of proper training on preslaughter handling and animal behavior principles to livestock handlers in ensuring animal welfare, alleviating stress, and the production of good quality meat. With the recent developments in cognitive science and research on animal emotions, it is imperative to study various emotional distress and their effect on animal behavior, physiological parameters, and meat quality. The

outcome of the present study will be helpful to convince the people involved in this business to follow proper animal handling principles as well as to the law enforcement organizations. In addition, due to the rather limited number of studies in this area and the relevance of the current situations in most countries where commercial slaughter co-exists in parallel with both backyard slaughter and religious slaughter, the data from our study would be the most appropriate source of reference.

1.4 Hypothesis

The hypothesis of the present study is that behavioral, physiological, and neurohormonal indicators of stress, electroencephalography, meat quality, and apoptotic markers can be linked with responses to training to livestock handlers and emotional stress during the exposure to act of slaughtering.

1.5 Objectives of the study

1.5.1 General objective

The main objective of the present study is to assess the effects of training of livestock handlers and effects of exposure to slaughter ambient on the welfare, behavioral, physiological stress, and electroencephalogram response, muscle biochemistry, meat proteomics, and meat quality traits in goats.

1.5.2 Specific objective

1. To evaluate the effect of training to livestock handlers on behavioral, physiological, and hormonal responses during preslaughter handling in goats.

2. To assess the effect of training to livestock handlers on electroencephalogram variables and muscle biochemistry during preslaughter handling in goats.

3. To evaluate the electroencephalogram and physiological responses as affected by slaughter empathy in goats.

4. To assess the effect of exposure to slaughter ambient on behavioral, carcass parameters, catecholamines, beta-endorphin, and plasma enzymes in goats.

5. To evaluate the effect of exposure to slaughter ambient on physico-chemical, shear force, apoptotic index and sensory attributes of *Longissimus thoracis et lumborum* muscle in goats.

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