UNIVERSITI PUTRA MALAYSIA

EFFECTS OF ROAD TRANSPORTATION AND PRESLAUGHTER HANDLING ON WELFARE, PHYSIOLOGICAL STRESS RESPONSES, AND MEAT QUALITY OF HEIFERS IN TROPICAL CLIMATE

AHMED ABUBAKAR ABUBAKAR

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By

AHMED ABUBAKAR ABUBAKAR

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

August 2022

DEDICATION

This thesis is dedicated to my parents and my lovely sisters, Zainab, Fatimah (Ummi), Maimuma (Umaima), and Zainab (Ikram).

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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Chairman : Awis Qurni Sazili, PhD
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Stress has an adverse effect on animal welfare that cannot be ignored, making it imperative to control and minimise stress-inducing elements associated with transportation for ethical, quality, and economic reasons. However, there is little or no evidence in Malaysia regarding the influence of pre-slaughter transportation on stress thresholds and the welfare of heifers subjected to varying distances of road transportation and stocking density. Additionally, there has been a significant increase in demand for red meat, necessitating the movement of animals from their natural habitats to slaughter plants with little or no regard for how these animals are handled and transported before slaughter. Although transportation is stressful for animals, it is unknown if it affects stress thresholds when animals are transported for slaughter. In light of recent incidents, it is becoming increasingly difficult to overlook the risks to animal welfare posed by transportation and handling. No prior study has examined the effects of road transportation and pre-slaughter management on the welfare, physiological stress, and meat quality of heifers in Malaysia. As a result, a study was needed to ascertain the effect of transportation stress on EEG changes and establish a link between these findings and neurohumoral indicators of distress. Thus, this study investigated the effects of road transport and pre-slaughter handling on the welfare, physiological stress, apoptotic index, and meat quality of Brahman crossbred cattle maintained in hot, humid parts of the tropics. The current study examined the relationship between neuroendocrine acute phase proteins (APP), typical characteristics associated with probable stress as measured by electroencephalogram (EEG) activity, meat quality, and apoptotic markers following road transportation of animals. Sixty (60) Brahman crossbred heifers were transported from a cattle feedlot at Universiti Putra Malaysia (UPM) in Serdang to the Shah Alam abattoir complex, Selangor. All animals were transported for (9) nine hours (short-distance) or seventeen hours (long-distance) and unloaded at the slaughterhouse for slaughter. Both departures and arrivals at the slaughterhouse were formally documented. All animals were transported within the state of Selangor, using a 5-ton lorry fitted with a non-slip floor and rooftop coverings travelled along the highway. Animals were divided into two groups, long (850km) and short (450km) distances, and three stocking

densities of 600 kg/m^2 (high-SD), 400 kg/m^2 (medium-SD), and 200 kg/m^2 (low-SD) were used. Blood analysis revealed a significant increase in the intensity of the response to blood parameters cortisol and acute phase proteins (*Bovine alpha 1-acid glycoprotein and Serum amyloid–A*) ($p<.0001$). At the farm (baseline values), cortisol and acute phase proteins (*Bovine alpha 1-acid glycoprotein and Serum amyloid–A*) levels increased significantly ($p<.0001$) with increasing distance and stocking density and were statistically different from those obtained immediately after unloading and after neck cut. Similarly, the alpha, beta, delta, and theta wave frequencies and Ptot (total power) and MF (median frequency) of the EEG (electroencephalogram) were considerably higher ($p<.0001$). Long-distance transport also resulted in a significantly higher ($p<.0001$) response to nociception during slaughter than shorter-distance transport, as demonstrated by APPs (acute phase proteins), cortisol, and EEG. Additionally, results indicated that distances and stocking densities had significant ($p<.0001$) effects on the apoptotic index, colour, pH, shear force values, WHC (water holding capacity), glycogen levels, and MDA (malondialdehyde assay) content in meat. In conclusion, the current research found that distance and stocking density affected cortisol levels, EEG activity, APPs (*Bovine alpha 1-acid glycoprotein and serum amyloid–A*), apoptotic index, and meat quality parameters indicated by the significant changes in the parameters listed above. As a result, this data points to possible welfare issues related to animal handling (loading and unloading) and preslaughter following road transportation. Improved animal handling during transportation and decreased average stocking density may contribute to the welfare of animals hauled by road in Malaysia's hot and humid tropics.

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

KESAN PENGANGKUTAN JALAN RAYA DAN PENGENDALIAN PRA-SEMBELIHAN TERHADAP KEBAJIKAN, TINDAK BALAS TEKANAN FISIOLOGI DAN KUALITI DAGING DI LEMBU DALAM IKLIM TROPIKA

Oleh

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Tekanan mempunyai kesan buruk terhadap kebajikan haiwan dan tidak boleh diabaikan, justeru adalah penting untuk mengawal dan meminimumkan unsur-unsur yang menjurus kepada tekanan semasa pengangkutan atas sebab etika, kualiti dan ekonomi. Walau bagaimanapun, hanya terdapat sedikit maklumat atau tiada bukti di Malaysia berhubung dengan pengaruh pengangkutan sebelum sembelihan terhadap tahap tekanan dan kebajikan lembu tertakluk kepada jarak pengangkutan jalan raya dan kepadatan stok yang berbeza-beza. Selain itu, terdapat peningkatan yang ketara dalam permintaan bagi daging lembu, lantas memerlukan haiwan untuk dibawa dari habitat semula jadi mereka ke pusat penyembelihan yang mana cara haiwan ini dikendalikan dan diangkut sebelum disembelih tidak diberikan perhatian sewajarnya. Walaupun diketahui bahawa pengangkutan akan memberikan tekanan terhadap haiwan, namun masih tidak diketahui sama ada ianya akan menjejaskan tahap tekanan apabila haiwan diangkut untuk disembelih. Berdasarkan insiden baru-baru ini, semakin sukar untuk kita mengabaikan risiko yang ditimbulkan oleh proses pengangkutan dan pengendalian kepada kebajikan haiwan. Tiada kajian terdahulu yang mengkaji kesan pengangkutan jalan raya dan pengurusan pra-sembelihan terhadap kebajikan, tekanan fisiologi, dan kualiti daging lembu kacukan Brahman yang dilaksanakan di Malaysia. Lantas, sebuah kajian diperlukan bagi memastikan kesan tekanan semasa pengangkutan ke atas perubahan EEG dan mewujudkan perkaitan diantara beberapa penemuan semasa kajian ini dan indikator kesakitan neurohumoral. Oleh itu, kajian ini telah menyiasat kesan pengangkutan jalan raya dan pengendalian pra-sembelihan ke atas kebajikan, tekanan fisiologi, indeks apoptosis, dan kualiti daging lembu kacukan Brahman di kawasan tropika yang panas dan lembap. Kajian semasa mengkaji hubungan diantara protein fasa akut neuroendokrin (APP), ciri tipikal yang dikaitkan dengan bakal tekanan seperti yang diukur oleh aktiviti electroencephalogram (EEG), kualiti daging dan penanda apoptotic yang berkaitan dengan pengangkutan haiwan melalui jalan raya.

Enam puluh (60) ekor lembu kacukan Brahman telah diangkut dari fidlot lembu di Universiti Putra Malaysia (UPM) di Serdang ke rumah penyembelihan komersial di Shah Alam, Selangor. Semua haiwan telah diangkut selama (9) sembilan jam (jarak dekat) atau (17) tujuh belas jam (jarak jauh) dan diturunkan di rumah sembelihan sebelum disembelih. Kedua-dua masa berangkat dan sampai di rumah sembelihan telah didokumentasikan. Kesemua haiwan telah diangkut di dalam kawasan negeri Selangor, menggunakan lori 5 tan yang dilengkapi dengan lantai anti-gelincir, beserta penutup bumbung sepanjang perjalanan. Haiwan dibahagikan kepada dua kumpulan, jarak jauh (850km) dan jarak pendek (450km), dan tiga kepadatan stok 600 kg/m2 (tinggi-SD), 400 kg/m2 (sederhana-SD), dan 200 kg/m2 (rendah). -SD) telah digunakan. Analisis darah mendedahkan peningkatan ketara dalam keamatan tindak balas kepada parameter darah cortisol dan protein fasa akut (APP) (Bovine alpha 1-acid glycoprotein and Serum amyloid–A) ($p<.0001$). Di ladang (nilai asas), paras kortisol dan APP (bovine alpha 1-acid glycoprotein dan Serum amyloid–A) meningkat secara signifikan ($p<.0001$) dengan peningkatan jarak dan kepadatan stok, dan ianya berbeza secara statistik daripada yang diperolehi sejurus selepas pemungahan dan selepas sembelihan. Begitu juga dengan frekuensi gelombang alfa, beta, delta dan theta serta Ptot (jumlah tenaga) dan MF (frekuensi median) EEG adalah tinggi ($p<.0001$). Pengangkutan jarak jauh juga menghasilkan tindak balas yang lebih tinggi ($p<.0001$) kepada nociception semasa penyembelihan berbanding pengangkutan jarak pendek, seperti yang ditunjukkan oleh APP, kortisol dan EEG. Tambahan pula, hasil kaian menunjukkan bahawa jarak dan kepadatan stok mempunyai kesan yang ketara ($p<.0001$) ke atas indeks apoptosis, warna, pH, nilai daya memotong, kapasiti pegangan air (WHC), paras glikogen, dan kepekatan malondialdehyde assay (MDA) dalam daging.

Kesimpulannya, kajian semasa mendapati bahawa jarak dan kepadatan stok mempengaruhi tahap kortisol, aktiviti elektroensefalogram (EEG), protein fasa akut (glikoprotein asid alfa 1 lembu dan amiloid-A serum), indeks apoptosis, dan parameter kualiti daging, seperti yang ditunjukkan oleh perubahan ketara dalam parameter yang disenaraikan di atas. Justeru, data ini menunjukkan perkaitan antara isu-isu kebajikan dan cara pengendalian haiwan dan penyembelihan selepas pengangkutan jalan raya. Pengendalian haiwan yang lebih baik semasa proses pengangkutan dan pengurangan purata kepadatan stok boleh menambahbaik tahap kebajikan haiwan yang diangkut melalui jalan raya di kawasan tropika panas dan lembap seperti Malaysia.

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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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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent
µg	micro gram
µl	microlitre
AGP	α-1 glycoprotein
APPs	Acute phase proteins
APR	Acute phase response
ATP	adenosine triphosphate
b*	Yellowness
C*	Chrom
CK	Creatine kinase
cm	Centimetre
CNS	central nervous systems
CORT	Cortisol
d	Day
ddH_2O	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
HCT	Hematocrit
HGB	Haemoglobin
HPA	hypothalamic pituitary adrenal
KCl	potassium chloride
kg	Kilogram
Km	Kilometres
l	Litre
L*	Lightness
LDH	lactate dehydrogenase
m	muscle
m^2	square Metre
MDA	Malondialdehyde
MF	Median Frequency
min	Minute
ml	Milllilitre
NaCl	sodium chloride
NAD	β-nicotinamide adenine dinucleotide
NEU	Neutrophils
ng	Nanogram
nm	Nanometer
ºC	degree Celsius
OD	optical density
OIE	World Organization for Animal Health

PGD	Programmed cell death
pHu	Ultimate pH
PLT	Platelets
PSE	Pale, soft and exudative
Ptot	Total Power
RMS	Root mean square
s	Second
SAA	serum Amyloid-A
SD	Stocking Density
TUNNEL	Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling
WHC	Water Holding Capacity

CHAPTER 1

INTRODUCTION

1.1 Background

Transportation is an unavoidable aspect of animal husbandry and may subject animals to stressful situations that jeopardise their welfare. Increasing market demand and the slaughter of animals in abattoirs frequently located in locations other than their natural habitat result in freight (Adenkola & Ayo, 2010; Fisher et al., 2004). Thus, livestock transportation by road continues to be the most popular mode of animal transport in many nations worldwide (Adenkola et al., 2009). Recent years have seen an increased awareness of animals' predicament during handling, loading, transportation, and disembarkation (Sporer et al., 2008; M. Werner & Gallo, 2008).

Animal welfare may be impacted significantly by handling, loading, transportation, and disembarkation. An animal's welfare is defined as its ability to cope with environments that involve problems and ease of coping (Broom, 2003). Animals are subjected to various consequences during transportation, including physical and psychological responses (motion, noise, vibration, novelty, mixing with unfamiliar animals, food and water deprivation, and extreme weather conditions). Animals may suffer unnecessarily during the process of entering and exiting transport vehicles. A prominent component contributing to physiological stress in poultry and cattle during the transportation is a high ambient temperature (Broom, 2000).

According to research on goats and sheep, stocking rate is essential for animal comfort during transportation and becomes critical at high stocking rates. High stocking rates have been strongly linked with physiological stress responses and poor meat quality instead of goats' medium and low stocking rates (Broom, 2000). Protecting animals is everyone's duty since it aims to avoid poor well-being. Not only would enhancing well-being during transit result in lower death rates and carcass degradation, but it will also result in increased financial viability, which is a benefit (Broom, 2005).

Stress reactions in animals caused by fear or novelty are universal sensations shared by all animals to escape predators. While restraining an animal in a squeeze chute may not result in severe discomfort, it may result in dread, a significant psychological stressor in animals raised under a strict management system (Temple Grandin, 1997). Secure confinement prevents anguish, suffering, bruising, and harm to the animal (Lambooij et al., 2012b). Hoisting by hindleg, casting with a rope or chain, restraint in a V-shaped or straddled conveyor, complete or partial inversion in a rotating pen, and upright restraint system are all conventional ways of restricting animals before stunning and or slaughter (Gregory, 2005).

Stress is a natural occurrence in social animals, and the cerebrum, hypothalamus-pituitary axis, glucocorticoids, and a cascade reaction are the key components. Glucocorticoids are delivered into cells via the bloodstream and enter the nucleus (Siegel & Honaker, 2014). Stress-related reactions might be physiological, behavioural, anatomical, or immunological. Stress is initiated when the central nervous system detects a danger to equilibrium. Whether or whether the stimulus constitutes a threat, what matters is the sense of risk (Moberg, 2000).

According to (Broom, 2009), well-being refers to an animal's attempt to adapt to its surroundings based on its current status. Earlier research utilized various techniques to measure animal well-being, including physiological, behavioural, endocrine, and immunological suppression. However, the endocrine and immune systems have certain disadvantages. For example, a single sample taken at a given moment may not accurately reflect the amount of discomfort experienced by an animal.

Similarly, changes in the plasma levels of stress-related hormones may result from other stress reactions. Additionally, biochemical and hormonal-based techniques frequently exhibited a lag period in response to stress-induced alteration. By contrast, the autonomic nervous system, which comprises action potential transmission across brain neurons, has emerged as a powerful and vital platform providing the quickest and most immediate reaction.

The electroencephalogram (EEG) is a real-time visual depiction of the small spontaneously produced electrical currents of neurons (in the microvolt range) emanating from the cerebral cortex using electrodes placed at various sites on the scalp or skull of other animals (Murrell & Johnson, 2006). Recent advancements in quantitative EEG interpretation have shown alterations in cerebrocortical activity in response to noxious stimuli (Kaka et al., 2015; Kongara et al., 2010; Zulkifli et al., 2014). Electroencephalography is a non-invasive, stress-free technology for recording conscious and anaesthetised animals' immediate physiological responses to stress, pain, and nociception. EEG is fast becoming a key instrument in assessing an animal's well-being due to its utility.

Transportation-related stress has been linked to decreased meat quality. According to research on cattle and sheep, stocking rate is essential for animal comfort during transportation and becomes critical at high stocking rates. The physiological stress response and poor meat quality have been substantially associated with high stocking rates during transportation instead of medium and low stocking rates (Broom, 2000). According to (Tarrant, 1990), behavioural, physiological, and pathological markers have been quantified during transportation. Pre-slaughter stress depletes muscle glycogen, raising pH and increasing the likelihood of meat rotting and pathogen growth with a dark hue, which reduces the meat's overall acceptability (Gregory, 1996). Glycogen loss is the most critical metabolic activity lost due to preslaughter stress on meat quality.

Additionally, various circumstances, particularly those associated with transportation and the slaughter process, substantially impact the development of rigour mortis and, consequently, meat (Gregory et al., 2008; Van de Perre, Permentier, De Bie, Verbeke, & Geers, 2010). Ante-mortem stressors, such as heat stress and struggle before slaughter, have been demonstrated to accelerate the rate and degree of post-mortem proteolysis, resulting in more soft meat (Gregory, 1994). Quality refers to the composite of attributes that distinguishes units of a product and is critical in determining the degree of acceptance by a consumer.

Tenderizing meat is an enzymatic process that incorporates a variety of intracellular proteolytic systems. The first stage in converting muscle to meat is the initiation of apoptosis, a complex and precisely regulated energy-dependent cell death process (Ouali et al., 2013). Apoptosis is a physiological process that occurs naturally in living organisms due to cell death. It eliminates cells that are either severely damaged or potentially hazardous to the organism's health without causing damage to the surrounding cells. Caspases are a category of cysteine peptidases that initiate the apoptotic process (Kemp & Parr, 2012).

Apoptosis can also be a defensive mechanism, such as an immunological response or reaction to illness or toxic substances (Elmore, 2007). Apoptosis is a suitable method for determining the degree of tension in muscles following slaughter.

Although transportation is stressful for animals (Zulkifli et al., 2019), it is unknown if it affects stress thresholds when animals are transported for slaughter in tropical climates. In light of recent incidents, it is becoming increasingly difficult to overlook the risks to animal welfare posed by transportation and handling. No prior study has examined the effects of road transportation and pre-slaughter management on the welfare, physiological stress, and meat quality of cattle carried out in Malaysia. As a result, a study was needed to ascertain the effect of transportation stress on meat quality traits and establish a link between these findings and neurohumoral indicators of distress and EEG alterations.

Thus, this study examined the effects of road transportation and pre-slaughter handling on the welfare, physiological stress, and meat quality of heifers in tropical climates.

The neurohumoral indicators of stress, electroencephalography, and meat quality are associated with apoptotic markers in heifers subjected to road transportation stress.

1.2 Problem Statement

Animal welfare continues to be a concern in many nations (particularly developing countries) since little or no attention is given to how these creatures are treated and transported before slaughter. Malaysia's Animal Welfare Act 2015 and rules were enacted following extensive laws requiring humane treatment of animals, which can only

be accomplished via scientific proof. There is, however, little or no evidence of the effect of pre-slaughter transportation on stress thresholds (alteration in the homeostasis due to a stimulus) and the welfare of heifers subjected to varying lengths of road transit and stocking density. Additionally, there is a sharp increase in the demand for red meat, which necessitates the movement of animals from their habitats to slaughter plants, with little or no consideration of how these animals are handled and transported before slaughter. Transportation has been significantly associated with welfare concerns and degradation in meat quality, necessitating gathering scientific data to elucidate the relationship between transportation stress and distance and stocking in Malaysia's hot, humid tropical climate.

1.3 Justification of the Study

Animal welfare legislation is not fully enforced in many developing nations and remains a source of worry. However, numerous studies on the transportation and welfare of road-transported farm animals in Malaysia have concluded that the experience is stressful for goats (Rajion, Mohamed Saat, Zulkifli, & Goh, 2001), poultry (Zulkifli et al., 2001; Zulkifli, 2003; Al-Aqil and Zulkifli, 2009; 2013), and rabbits (Nakyinsige et al., 2013). To fully comprehend this phenomenon, empirical data is needed to quantify the impact of stress on-road transportation and pre-slaughter handling on cattle welfare, physiological stress responses measured using various stress biomarkers, and meat quality. Assessing the effects of transportation stress on meat at chemical, cellular, and molecular levels is critical. These additions will aid in implementing animal transportation statutes and regulations and the Animal Welfare Act 2015.

1.4 General Objective

To determine the effects of road transportation and preslaughter handling on welfare, physiological stress responses, and meat quality of heifers in a tropical climate.

1.4.1 Specific Objective

1. To determine the effects of distance and stocking density on acute-phase proteins (α-1 glycoprotein and serum amyloid-a), neuroendocrine, and electroencephalogram changes in heifers as welfare indicators in a tropical climate

2. To determine the effects of distances and stocking density on the physicochemical properties and oxidative stability of *Longissimus lumborum, Semitendinosus, and Infraspinatus* muscles in heifers in a tropical climate.

3. To determine the influence of distances and stocking densities on the apoptosis and oxidative stress levels in heifers in a tropical climate.

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