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WELFARE AND PHYSIOLOGICAL STRESS RESPONSES IN CATTLE DUE TO EFFECTS OF SEA AND ROAD TRANSPORTATION IN TROPICAL CLIMATE

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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 Master of Science

March 2018

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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 Master of Science

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March 2018

Chairman : Awis Qurni Sazili, PhD
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Currently, there is an increasing global demand for meat. However, animal welfare legislation in many developing countries is not fully implemented and therefore remains debatable on how animals should be handled, loaded, transported, unloaded, and time to spend in lairage and slaughter without causing unnecessary pain or distress. Therefore, stress induced by transport is very important in meat production; whose time and or mismanagement pose some risks to both animal welfare and meat quality. In fact, transportation does not only affect animal welfare, but it can also negatively influence meat quality or worse still, cause economic losses. The impact of stress on animal welfare is too important to ignore and this makes it necessary to control and minimise transport related stress-inducing factors for ethical, quality and economic reasons. There is dearth of scientific information on the effects of sea and road transport on welfare, physiological stress response and electroencephalogram.

Therefore, the current study was aimed at evaluating the effects of sea and road transport on physiological stress responses, electroencephalogram in compliance with animal welfare ethics.

The current study, describes the link between blood biochemistry, hematology, neuroendocrine, and acute phase proteins (APP), typical characteristics associated with possible stress with electroencephalogram (EEG) activities after subjecting animals to sea and road transport and effects on welfare. Sixty (60) heifers of Brahman crossbred were subjected to a 14 d transport by sea and 14 h of road transport.

Blood analysis revealed that the intensity of response for most biochemical blood parameters - *alanine transaminase, aspartate transaminase and creatinine kinase* significantly increased ($p<0.05$) and were different from the baseline values taken while animals were in Darwin Port, Australia. Hematological results obtained also revealed that the (*White blood cells, Red blood cells, neutrophils and lymphocytes*) increased significantly ($p<0.05$) and were different from the baseline values taken while animals were in Darwin Port, Australia. Additionally, cortisol and APP (*Bovine alpha 1-acid glycoprotein and Serum amyloid–A*) results increased significantly ($p<0.05$) and were different from the baseline values taken while animals were in Darwin Port, Australia. Similarly, RMS of alpha, beta, delta, theta, Ptot and MF of the EEG were significantly ($p<0.05$) high during both sea and road transport.

In conclusion, results of the current study revealed that the concentration of liver enzymes, total blood count, cortisol, electroencephalogram (EEG) activities and acute phase proteins (*Bovine alpha 1-acid glycoprotein and Serum amyloid–A*) were more detrimental by sea transport as evidenced by the significant change of the parameters above. Hence, this finding is suggestive of potential welfare problems due to animal handling and transport in relation to heat stress. Suggestively, improved animal handling during transport, stocking density and regulated vessel temperature will improve the welfare of animals subjected to long distance transport by sea.

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

TINDAKBALAS LEMBU KEATAS KEBAJIKAN DAN TEKANAN FISIOLOGI TERHADAP KESAN PENGANGKUTAN LAUT DAN DARAT PADA IKLIM TROPIKA

Oleh

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Terdapat peningkatan permintaan global untuk daging. Walaubagaimana pun, perundangan kebajikan haiwan di kebanyakan negara membangun tidak dilaksanakan sepenuhnya dan oleh itu perihal bagaimana haiwan perlu dikendalikan, dimuatkan, diangkut, dibebaskan, dan masa untuk diluangkan dalam lairaj dan penyembelihan tanpa menyebabkan kesakitan atau ketegasan yang tidak sepatutnya masih lagi menjadi isu perdebatan. Oleh yang demikian, cetusan ketegasan yang disebabkan oleh pengangkutan adalah sangat penting dalam pengeluaran daging. Ada kemungkinan masa dan/atau masalah pengurusan menimbulkan beberapa risiko kepada kebajikan haiwan dan kualiti daging. Malah, pengangkutan bukan hanya memberi kesan kepada kebajikan haiwan, tetapi ia juga boleh mempengaruhi kualiti daging atau lebih buruk lagi, menyebabkan kerugian ekonomi. Kesan ketegasan terhadap kebajikan haiwan adalah terlalu penting untuk diabaikan dan faktor yang berkaitan dengan pencetusan ketegasan pengangkutan perlu dikawal dan diminimumkan bagi kepentingan etika, kualiti dan ekonomi. Terdapat sedikit kekurangan maklumat saintifik mengenai kesan pengangkutan laut dan jalan raya ke atas aspek kebajikan, tindakbalas fisiologi ketegasan dan elektroensefalogram.

Oleh yang demikian, kajian semasa ini adalah bertujuan untuk menilai kesan pengangkutan laut dan jalan raya di kawasan tropika yang lembap dan panas terhadap tindakbalas fisiologi ketegasan, elektroensephalogram dan kebajikan haiwan.

Kajian semasa juga menerangkan hubung kait di antara biokimia darah, hematologi, neuroendokrin, dan protein fasa akut (APP), ciri khas yang berkaitan dengan kemungkinan tegasan dengan aktiviti elektroensefalogram (EEG) selepas haiwan

diangkut melalui pengangkutan laut dan jalan raya, serta kesan ke atas kebajikan. Enam puluh (60) lembu dara dari baka kacukan Brahman diangkut melalui pengangkutan laut selama 14 hari dan pengangkutan jalan raya selama 14 jam. Analisis darah mendedahkan bahawa tindakbalas keamatan untuk kebanyakan parameter biokimia darah, kortisol, dan APP adalah sangat ($p < 0.05$) berbeza daripada paras awal yang diambil semasa haiwan masih berada di Darwin Port Australia. Begitu juga dengan RMS alpha, beta, delta, theta, Ptot dan MF EEG yang turut terjejas dengan ketara oleh pengangkutan ($p < 0.05$). Bertentangan dengan parameter darah lain, laktat dehidrogenase (LDH) tidak terjejas oleh pengangkutan.

Sebagai kesimpulan, hasil kajian semasa mendedahkan bahawa paras enzim hati, jumlah kiraan darah, hormone kortisol, aktiviti elektroensefalogram (EEG), dan protein fasa akut (*Bovine alpha 1-acid glycoprotein and Serum amyloid–A*) lebih terjejas melalui pengangkutan laut dan ini jelas terbukti melalui perubahan signifikan ke atas parameter tersebut. Justeru, dapatan ini menunjukkan potensi masalah kebajikan yang disebabkan pengendalian dan pengangkutan haiwan berkaitan ketegasan haba. Oleh yang demikian, penambahbaikan ke atas pengendalian haiwan semasa pengangkutan, kepadatan stok dan suhu dalam kapal yang dikawal atur akan menambah baik kebajikan haiwan yang diangkut jauh melalui laut.

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I certify that a Thesis Examination Committee has met on 5 March 2018 to conduct the final examination of Ahmed Abubakar Abubakar on his thesis entitled "Welfare and Physiological Stress Responses in Cattle Due to Effects of Sea and Road Transportation in Tropical Climate" 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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Name of Member of Supervisory Committee: Associate Professor Dr. Goh Yong Meng

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AGP	α-1 glycoprotein
ALP	Alanine phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
APPs	Acute phase proteins
APR	Acute phase response
ATP	adenosine triphosphate
CK	creatine kinase
cm	Centimeter
CNS	central nervous systems
d	Day
ddH2O	deionized distilled water
EC	European Community Council Regulations
ECG	Electrocardiogram
EDTA	ethylene diamine tetra acetic acid
EEG	Electroencephalogram
EFSA	European Food Safety Association
g	Gram
HCT	Hematocrit
h	Hour
HGB	Hemoglobin
HPA	hypothalamic pituitary adrenal
KCl	potassium chloride
kg	Kilogram
l	Liter
LDH	lactate dehydrogenase
LYM	Lymphocytes
MF	Median Frequency
min	Minute
µg	micro gram
µl	micro liter

ml	Milliliter
NaCl	sodium chloride
NAD	β-nicotinamide adenine dinucleotide
ng	Nanogram
nm	Nanometer
NEU	Neutrphils
ºC	degree Celsius
OD	optical density
OIE	World Organization for Animal Health
%	Percent
SAA	serum amyloid-A
PLT	Platelets
Ptot	Total Power
RMS	Root mean square
s	Second

CHAPTER 1

INTRODUCTION

1.1 Background

Transportation is an inevitable animal husbandry practice and may expose animals to challenging conditions which may compromise their welfare. Livestock are subjected to transportation as a result of marketing and the need to slaughter them for meat in abattoirs which are often located outside places where the animals are raised (Fisher et al., 2004; Adenkola & Ayo, 2010). The welfare of livestock transported by ship is of great concern due to significant risks caused by transporting them in ships, especially over long distances.

Long distance transport of livestock by ship poses a different set of challenges to welfare. Ship transportation is used to export animals because it is the most appropriate way to move them, compared with road or rail transport. According to Norris et al. (2003), the main challenges faced by cattle transported by sea from Australia to Asia are heat stress, respiratory disease, trauma and conjunctivitis with an average mortality rate of all shipments between 1995-2000, documented as 0.2-0.5%. Similarly, feed intake in some species of cattle was affected during shipment (Beatty et al., 2014).

Thus, transporting livestock by road remains the most common form of moving animals in many countries around the world (Adenkola et al., 2009). Recently, the plight of animals during handling, loading, transportation and disembarkation is increasingly gaining attention (Sporer et al., 2008; Werner & Gallo, 2008).

The effects of handling, loading, transportation and disembarkation of animals can be significant on their welfare. The welfare of an animal is said to be its ability to cope with environments which include both difficulty and it ease in coping (Broom, 2003a). During transportation, animals are exposed to a wide range of effects which includes physical and psychological responses (motion, noise, vibration, novelty, mixing with unfamiliar animals, food and water deprivation, and extreme weather conditions). The process of moving animals in and out of transport vehicles may cause unnecessary suffering. A major factor which brings about physiological stress during transportation in poultry and livestock is high ambient temperature (Broom, 2000; Zulkifli et al., 2010).

A study on cattle and sheep indicates that stocking rate is vital for the welfare of animals during transportation and becomes critical at high stocking rates. Physiological stress response and poor quality of meat have been linked strongly with high stocking rates as compared with medium and low stocking rates during transportation (Broom, 2000; Zulkifli et al., 2010). Protecting animals is the

responsibility of all humans as it is aimed at improving poor welfare. Improving welfare during transportation will not only reduce mortality rates, carcass downgrading but will result in improved finance which is an advantage (Broom, 2005).

Stress responses due to fear or novelty in animals are universal feelings amongst animals to avoid predators. Restraining an animal in a squeeze chute may not significantly cause pain but fear which is a major psychological stressor in animals reared under extensive management system (Grandin, 1997).

The procedures of loading and unloading animals into and out of transport vehicles may also result in severe distress. The transports' main adverse effects on animal welfare are considered dehydration, muscular damage, thermal and physical discomfort, and behavioural restrictions. Although, there is a considerable amount of documented work on road transport in farm animals, many studies are conducted in the temperate regions. High ambient temperature is considered a major factor in the elicitation of physiological stress reactions during road transportation.

Stress is a part of life and is not inherently bad. All life forms have evolved mechanisms to cope with the stresses of their lives. However, the impact of stress on animal welfare is too important to ignore (Moberg, 2000). According to Moberg (1985) the animal stress model evolved from an earlier model that divided the stress response into three general stages: the recognition of a stressor, the biological defence against the stressor and the consequences of the stress response.

A stress response begins with the central nervous system perceiving a potential threat to homeostasis. Electroencephalogram (EEG) is the real time graphical representation of tiny (of the microvolt range) spontaneously generated electrical currents of neurons from cerebral cortex through electrodes located at different locations on the scalp in human or the head in other species (Murrell & Johnson, 2006). Recent advances in the quantitative interpretation of the EEG have identified changes in cerebrocortical function in response to noxious stimulation (Kongara et al., 2010; Zulkifli et al., 2014; Kaka et al., 2015; Kells et al., 2017; Lehmann et al., 2017). There have been few studies that have investigated the effects of road transportation and duration on haul, on neurophysiological response in animals (Azaleaet al. (2016) and Raghazli et al. (2016).

There have been few studies that have investigated the effects of transport in a vessel by sea, followed by 14 h road transport duration on haul, on acute phase proteins, and physiological stress response before and after transport. In addition, no studies have investigated the effects on the animals' physiological responses and electroencephalogram allowed to rest for 12 h on a vessel without unloading following a sea journey (14 days) and land transport (14 h).

Thus, this study was aimed at investigating the effects of sea and road transportation on physiological stress response in Brahman crossbred cattle.

It was hypothesized that the physiological stress response will be affected by sea and road transportation in Brahman crossbred cattle.

1.2 Problem Statement

Animal welfare remains an issue of debate in some countries as there is little or no consideration on how these animals are handled and transported. In Malaysia the Animal welfare Act 2015 and regulations came into existence after a thorough legislation which requires that animal's right be upheld and these can only be achieved with scientific evidence. There is, however, little or no information on the welfare of cattle during road and sea transport in Malaysia.

1.3 Justification of the Study

Animal welfare legislation in many developing countries is not fully implemented and therefore remains an issue of debate. Although, numerous studies have been conducted on transportation and welfare of road transported farm animals in Malaysia, in goats (Rajion et al., 2001; Zulkifli et al., 2010b), poultry (Zulkifli et al., 2001; Zulkifli, 2003; Al-Aqil and Zulkifli, 2009; 2010; 2012) and rabbits (Nakyinsige et al., 2013). There is a need for scientific evidence to assess the effects of sea and road transportation in cattle using stress biomarkers to provide useful information with facts. These will add to the implementation of Acts and regulations for animal transportation and Animal Welfare Act 2015.

1.4 General Objective

To evaluate the effects of sea and road transportation on physiological responses and electroencephalogram activities in Brahman crossbred cattle under hot, humid tropical climate.

1. To assess the effects of sea and transport on hematological and biochemical parameters.
2. To assess the effects of sea and road transport on acute phase proteins (α-1 glycoprotein and serum amyloid-A).
3. To assess the effects of sea and road transport on electroencephalogram activity.

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