UNIVERSITI PUTRA MALAYSIA

PHYSIOLOGICAL STRESS RESPONSES AND MEAT QUALITY OF GOATS SUBJECTED TO DIFFERENT TRANSPORTATION AND LAIRAGE DURATION

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By

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August 2020

Chairman : Professor Goh Yong Meng, PhD
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The main objective of this study was to determine the effect of acute pre-slaughter stress of different transport (TS) and lairage (L) duration on the physiological stress indicators and meat quality of goats. Eighteen male goats were grouped into six groups which are the TS2L3, TS2L6, TS2L12, TS6L3, TS6L6 and TS6L12. Goats were transported for two or six hours, kept in the lairage according to the designated time and slaughtered. Samples were obtained at the farm level (T1), after transportation (T2), after lairage (T3) and during slaughter (T4). EEG were recorded and blood were samples for the analysis of the haematological, blood biochemistry changes, cortisol, Triiodothyronine (T3) and thyroxine (T4) concentration. *Longissimus lumborum* (LL) muscles were sampled on day 0, 1, 3 and 7 post-mortem days for the analysis of meat quality, lipid peroxidation, HSP27 expression and apoptosis rate. Two-hour transportation (TS2) resulted in higher EEG activity than the six-hour transportation (TS6) duration ($P < 0.05$). At the same time, transportation stress significantly increased the neutrophil and monocyte counts and neutrophil-lymphocyte ratio (N/L ratio). Lairage time has a significant impact on the EEG analysis during slaughter. Long transport and lairage duration increased the time for the EEG to reach isoelectric, increase the RBC, PCV and plasma protein level,cortisol concentration, cellular apoptosis index and affect the meat quality ($P < 0.05$). The TS6 group produced meat which were higher in pH, darker in colour than the TS2 group. However, the TS2 group produced juicier meat than the TS6 group. More than three-hour lairage in the TS2 group and more than six-hour lairage for the TS6 group was needed for the meat quality to improve. Ageing was crucial in improving meat quality. Most of the stress effects were diminished after three to seven days of ageing. In conclusion, transport and lairage duration produce a significant impact on the physiological stress response and the meat quality of goats.

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

GERAKBALAS TERHADAP TEGASAN FISIOLOGI DAN KUALITI DAGING PADA KAMBING YANG MELALUI TEMPOH PENGANGKUTAN DAN LAIRAJ YANG BERBEZA

Oleh

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Ogos 2020

Pengerusi : Profesor Goh Yong Meng, PhD
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Objektif utama kajian ini adalah untuk menentukan gerakbalas fisiologi dan kualiti daging kambing terhadap tegasan pre-penyembelihan akut akibat tempoh pengangkutan (TS) dan lairaj (L) yang. Lapan belas ekor kambing jantan diletakkan diagihkan dalam enam kumpulan iaituTS2L3, TS2L6, TS2L12, TS6L3, TS6L6 and TS6L12. Kambing diangkut selama dua atau enam jam, diikuti dengan tempoh lairaj yang telah ditetapkan sebelum disembelih. Sampel diperolehi di peringkat ladang (T1), selepas pengangkutan (T2), selepas lairaj (T3) dan semasa penyembelihan (T4). Bacaan EEG direkodkan dan sampel darah digunakan bagi tujuan analisis hematologi, perubahan biokimia darah, kortisol, triiodotironina (T3) dantiroksina (T4). Otot *Longissimus lumborum* (LL) disampel pada hari 0, 1, 3 dan 7 selepas penyembelihan bagi tujuan analisa kualiti daging, pemperoksidaan lipid, ekspresi HSP27 dan kadar apoptosis. Kambing yang diangkut untuk selama dua jam (TS2) menunjukkan peningkatan aktiviti EEG berbanding dengan kambing yang diangkut untuk selama enam jam (TS6) ($P< 0.05$). Dalam masa yang sama, tegasan pengangkutan meningkatkan bilangan neutrophil dan monosit. Malahan, nisbah neutrophil-limfosit (nisbahN/L) turut meningkat. Jangka masa lairaj menghasilkan impak yang signifikan ke atas parameter EEG semasa tempoh penyembelihan. Jangka waktu pengangkutan dan lairaj yang panjang meningkatkan tempoh EEG untuk mencapai isoelektrik, meningkatkan bilangan RBC, PCV dan plasma protein, kepekatan kortisol, indeks apoptosis dan kualiti daging kambing ($P < 0.05$). Kambing dari kumpulan TS6 menghasilkan daging dengan pH yang tinggi dan warna yang lebih gelap berbanding daging yang dihasilkan daripada kumpulan haiwan yang diangkut untui 2 jam sahaja (TS2). Walaubagaimanapun, kambing dari kumpulan TS2 menghasilkan daging yang lebih berjus berbanding dengan daging dari kumpulan TS6. Tempoh lairaj yang melebihi tiga jam bagi kumpulan TS2, dan lebih daripada enam jam bagi kumpulan TS6 menunjukkan kualiti daging yang lebih baik. Kebanyakan kesan tegasan pra-penyembelihan

berkurangan selepas tiga hingga tujuh hari dalam tempoh penuaan. Kesimpulannya, tempoh pengangkutan dan lairaj member kesan yang signifikan terhadap gerakbalas tegasan fisiologi dan kualiti daging kambing.

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This thesis was submitted to the Senate of the 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

°C	degree celcius
5-HT	serotonin
a*	redness (meat colour)
AChE	cortisol acethylcholinesterase
ACTH	adrenocorticotropic hormone
ADP	adenosine diphosphate
am	ante meridiem: Before noon.
ANOVA	analysis of variance
ANS	autonomic nervous system
APAF1	Apoptotic protease activating factor 1
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
b*	yellowness (meat colour)
Bcl-2	B-cell lymphoma 2
BW	body weight
CK	Creatine kinase
Cl	chloride
CL	cooking loss
cm	centimeter
CNP	circulating pool
CNS	central nervous system
COMb	carboxymyglobin
CRF	corticotropin-releasing factor

CRH	corticotropin-releasing hormone
DFD	dark, firm and dry
DISC	death-inducing signalling complex
DMb	Deoxymyoglobin
DNA	deoxyribonucleic acid
EEG	electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
EPSP	excitatory postsynaptic potential
ER	endoplasmic reticulum
EU	European union
F50	median frequency
FADD	Fas-associated protein with death domain
FAO	Food and Agriculture Organization of the United Nations
FE^{2+}	ferrous
FFT	Fast Fourier Transformation
FGM	faecal glucocorticoid metabolite
FWD	feed-water deprivation
GABAA	gamma-aminobutyric acid
GH	Growth hormone
GHRH	Growth-hormone-releasing hormone
GHSR	Growth hormone secretagogue receptor/ ghrelin
g	gram
GRP	glucose-related protein
GSH	glutathione
h	hour(s)

H_2O_2	hydrogen peroxide
Hb	haemoglobin
HCW	hot carcass weight
HO-1	heme oxygenase-1
$HO^{\cdot}$	hydroxyl radical
HOCl	hypochlorous acid
HPA	hyphothalamic-pituitary-adrenal
HCl	hydrochloric acid
HSE	heat shock element
HSF	heat shock factor
HSP	Heat shock protein
IACUC	Institutional Animal Care and Use Committee
IGF	insulin-like growth factors
IL	Interleukin
IPSP	inhibitory postsynaptic potential
KCl	Potassium chloride
kDa	kiloDalton
kg	kilogram
km	kilometer
L*	lightness (meat colour)
L3	three-hour lairage
L6	Six-hour lairage
L12	Overnight lairage
LDH	Lactate dehydrogenase
LH	luteinizing hormone

LL	*Longissimus lumborum*
M	molar
µl	microliter
µm	micrometer
m^2	Meter square
mm	milimeter
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDA	malondialdehyde
Mg	miligram
ml	mililiter
MNP	marginating pool
MOMP	mitochondrial outer membrane permeabilization
MMb	metmyoglobin
mRNA	Messenger RNA
N/L ratio	neutrophil/ lymphocyte ratio
Nm	Nanometer
$NO^{\cdot}$	Nitric oxide
O_2^-	Superoxide
OIE	Office International des Epizooties/ World Organisation for Animal Health
OMb	oxymyoglobin
ONOO-	peroxynitrite
PBS	phosphate-buffered saline
PCV	packed cell volume

PE	polyethene
pH_u	ultimate pH
pm	post meridiem: After noon
POMC	pro-opiomelanocortin
PRL	prolactin
PSE	pale, soft and exudative
Ptot	total power
PVN	Paraventricular nucleus
RBC	red blood cell
RMS	root mean square
RNA	ribonucleic acid
ROS	reactive oxygen species
s	second(s)
S.E.	standard error
SF	shear force
sHSP	small heat shock protein
SRIF	somatostatin
T3 hormone	Triiodothyronine hormone
T4 hormone	Thyroxine hormone
TS2	two-hours transported goats
TS6	six-hours transported goats
TS2L3	two-hours transport, three-hours lairage goats
TS2L6	two-hours transport, six-hours lairage goats
TS2L12	two-hours transport, twelve-hours lairage goats
T6L3	six-hours transport, three-hours lairage goats

T6L6	six-hours transport, six-hours lairage goats
T6L12	six-hours transport, twelve-hours lairage goats
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substance
TdT	terminal deoxynucleotidyl transferase
TH1	T helper-1 mediated cellular immunity
TH2	T-helper-2 mediated humoral immunity
TMB	tetramethylbenzidine
TNF	tumour necrosis factor
TNFR	tumor necrosis factor receptor
TRH	thyrotrophin releasing hormone
TSH	thyroid stimulating hormone
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
U/L	Units/ litre
WBC	White blood cell
WHC	Water holding capacity

CHAPTER 1

INTRODUCTION

1.1 Background

Over the years, goats have become one of the most important livestock as part of Malaysia's food security strategy. In 2018 alone, Malaysians consumed 4,572 metric tonnes of mutton (Department of Veterinary Services Malaysia, 2018). From 2008 until 2017, the recorded slaughtered goats increased tremendously from 16,743 to 63,875 animals, which corresponds to a 382% increase in the total volume (Department of Veterinary Services Malaysia, 2017). As a developing country, there is an urgent need to establish a proper goat welfare assessment, particularly during the pre-slaughter procedure under Malaysia's hot and humid tropical conditions. Apart from that, an increasing number of public demand that animal products are derived from livestock with good welfare conditions (Boissy, Fisher, Bouix, Hinch, & Le Neindre, 2005; Stilwell, 2018). Decreased performance, high mortality, and morbidity together with lower quality of animal products will occur if the welfare of the animals being jeopardised (Llonch, King, Clarke, Downes, & Green, 2015; Marino et al., 2016; Mcmanus, Louvandini, Gugel, Paiva, & Paim, 2011; Ozcan et al., 2014). During 2012-2018, the goat/ sheep sector's ex-farm value was RM 178.42 million (Department of Veterinary Services Malaysia, 2018). In view that meat quality is very much related to the animal's pre-slaughter welfare, low welfare standards will lead to significant economic losses to the producers, retailers, and consumers (Fathurahman & Jamilah, 2019).

A thorough pre-slaughter stress assessment in a local environment will provide valuable information in preparing useful welfare guidelines for the animals' benefits and improving the meat industry. Functional stress assessment is critical to ensure sustainable welfare in the animals' production systems (Fraser, 2008; Sørensen, Sandøe, & Halberg, 2001; Stilwell, 2018). Pre-slaughter procedures that include transportation, lairage, and handling process were commonly reported as a source of stress to farm animals (Alcalde et al., 2017; Polkinghorne, Philpott, & Thompson, 2018; Zimerman, Domingo, Grigioni, Taddeo, & Willems, 2013). Previous research has reported the adverse effect of the pre-slaughter stress on the animals' immunity level (Maejima, Aoyama, Abe, & Sugita, 2005), meat quality (Kadim, Mahgoub, Al-Marzooqi, & Khalaf, 2017), and on the morbidity and mortality level (Saeb, Baghshani, Nazifi, & Saeb, 2010; Samimi, 2017). Stress recognition is vital in providing more efficient stress and welfare management, particularly to farm animals. Measurement of stress is generally very complicated and involves various parameters (Moberg & Mench, 2000).

Evaluation of stress frequently involves the measurement of behaviour, autonomic nervous response, neuroendocrine, and immunological changes (Moberg & Mench, 2000). These measurements are performed during the pre, at the time, and post transport to determine the stress level. Nonetheless, animals may not use all parameters mentioned above to maintain their homeostasis during the stress period (Moberg & Mench, 2000). Various studies have shown that each stressor would evoke a specific response in humans and animals, and no parameter is suitable to detect all types of stressors (Cockrem, 2013; Pacak & Palkovits, 2001; Rubio-González et al., 2015; Zimerman et al., 2011). The search for a different type of parameter is crucial to give more additional information on the animal stress response against transportation stress. The current research utilised electroencephalography (EEG) and apoptosis as an alternative indicator to non-painful acute stress measurement in addition to the commonly used haematological, biochemical, and meat quality parameters. The heightened interest in the animals' neuroscience through EEG analysis generates a new branch of research in the animal stress study (Jia et al., 2006; Tidswell, Blackmore, & Newhook, 1987). EEG is widely used to quantify pain and nociception response in animal (Jongman, Morris, Barnett, & Hemsworth, 2000; Kaka, Goh, Chean, & Chen, 2016; Sabow et al., 2016) and to measure emotions and stress level in human (Choi, Kim, & Chun, 2015; Ke et al., 2015). It has been proved to give real-time stress response to other stress parameters (Zulkifli et al., 2019). Thus in this study, EEG was used to determine the stress level of goats during the transportation and lairage period by quantifying the root mean square (RMS) of each band wave in the EEG activity. In the last decade, scientists start to address cellular stress response as part of animals' stress indicators (Hu et al., 2016; Rubio-González et al., 2015). Nonetheless, most of the studies used apoptosis studies to measure the ageing rate and meat quality in animals (Lana & Zolla, 2016; Ouali et al., 2006; Sierra & Oliván, 2013). However, this study utilised the apoptosis rate as stress parameters to detect the effect of transportation in animals, especially goats, in the local environment. Therefore, with many results and parameters reported, a specific stress assessment approach is required for transported goats under the hot and humid tropical climate.

1.2 Hypothesis and General Objectives

Overall, the work presented in this thesis focused on the effects of two different transport and lairage durations on some of the physiological and meat quality parameters of adult male crossbred Boer goats in the hot and humid tropical environment. It is hypothesized that the optimum lairage span based on the transportation duration would reduce the impact of transportation stress and improve the welfare and quality of goats in this study.

Therefore, the specific objectives of this study were:

1. To assess the EEG responses of goats subjected to pre-slaughter transportation stress and the effect of lairage on the EEG responses during slaughter.
2. To evaluate the impact of transportation stress and lairage durations on the hormones, haematological, and blood biochemicals of goats.
3. To investigate the oxidative stress level based on the malondialdehyde (MDA) concentration, HSP27 expression, and the apoptotic rate in the *Longissimus lumborum* (LL) muscle of goats subjected to different transportation and lairage duration.
4. To evaluate the effect of different transportation and lairage durations to Boer goats' carcass, and meat quality

1.3 Significance of the study

Environmental factors contribute to a different effect on the stress response (Alamer, 2006; Ouraouia, Ahmarb, Ajdoubc, Jemalic, & Elyead, 2002; Rojas-Downing, Nejadhashemi, Harrigan, & Woznicki, 2017a; Singh, Vaidya, Upadhyay, & Aggarwal, 2016). Most of the research published was based in a country with different climate and temperature compared to Malaysia. Furthermore, farm animals' transportation is unavoidable, and animals are often moved to the slaughterhouse (Ekiz, Ekiz, Kocak, Yalcintan, & Yilmaz, 2012) or relocated to another farm (Hulbert & Moisá, 2016). It is impossible to eliminate the effect of transportation stress on animals. Adequate information on the stress response and its impact is important for animal welfare management strategies. Thus, the transportation stress and lairage duration research need to be done in the local background to give realistically attainable guidelines to the livestock sector and its stakeholders. This study also aimed to provide additional information on the stress response parameters and suggest a suitable lairage duration depending on the transportation period, improving the animals' welfare and producing a better meat quality.

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