UNIVERSITI PUTRA MALAYSIA

BIOCHEMICAL AND ELECTROPHYSIOLOGICAL RESPONSES IN GOATS SUBJECTED TO TRANSPORTATION, LAIRAGE, AND SLAUGHTER

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By

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Food animal production involves routine husbandry procedures including handling, transportation, introduction to novel environments, and eventually slaughter. These activities incur stress and pain to the animals. Pre-slaughter management of these animals could impact the experience of pain at slaughter. Therefore, this study was conducted to determine the effects of stressors in routine livestock management, by the changes in haematological parameters, serum biochemical, stress hormone, electroencephalogram (EEG), and neuro-histological findings, in goats subjected to 1-, 2-, and 6-h road transportation, pre-slaughter 3-, 6-, and 16-h lairage, and slaughter.

Goats exposed to 1-h road transportation showed higher neutrophil to lymphocyte ratio (N:L), neutrophil counts, plasma cortisol concentrations (CORT), and serum creatine kinase (CK) at post-transportation and post-slaughter than at pre-transportation (baseline) period. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), plasma protein (PP) and hematocrit (PCV) did not vary from baseline. The EEG median frequency (F_{50}) was significantly higher ($p<0.05$) at pre-slaughter than baseline suggesting increased arousal state, and remained high at post-slaughter period. It is suggested that the stress of handling, novel environment, and transportation increased slaughter pain threshold in goats through stress-induced analgesia (SIA) mechanism.

Goats transported for 2 (T2) and 6 h (T6) showed significantly higher ($p<0.05$) neutrophil counts, serum CK and ALT concentrations than those at pre-transportation period. Based on the high CORT, goats of both transportation groups were already stressed prior to transportation. However, CORT value decreased and EEG unchanged after transportation, which could be attributed to adaptation of goats to the transportation conditions. Increased PCV and PP were also observed in the T6 goats, indicating dehydration. The T2 and T6 goats were subjected to lairage for either 3 (L3), 6 (L6) or 16 (L16) h before slaughter. Among treatment groups, the pre-slaughter F_{50} was highest in the goats subjected to 2-h transportation and 3-h lairage. The F_{50} values increased further at post-slaughter, which is evidence for nociception. Other groups showed decreased post-slaughter F_{50}, indicating increased pain threshold.

Based on histological findings, the highest prefrontal cortex (PFC) pyramidal cells c-Fos activities were in the T2-L6 goats. Together with increased serum CK from muscle damage, this manifestation is suggested to be associated with aggressive behaviour among goats. Increased PFC c-Fos activities in T6-L6 goats suggest involvement of SIA that had increased pain threshold. Increased EEG δ and θ power at post-slaughter indicate state of unconsciousness, which is proposed to also occur via the SIA mechanism. Based on the findings from the study, it is recommended that the length of lairage period after 2- and 6-h transportation should be between 6 to 16 h when aggressive physical interactions had reduced, for animals to experience minimal slaughter pain.

The study showed that there is a relationship between activation of pyramidal cells c-Fos expression in the PFC of goats and the EEG responses to the stress and pain of slaughter. In conclusion, it is elucidated that emotional and cognitive elements of goats at pre-slaughter can affect stress regulation and influence the slaughter pain response in goats through the SIA mechanism.

Keywords: stress, pain, transportation, lairage, stress-induced analgesia.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

GERAK BALAS BIOKIMIA DAN ELEKTROFISIOLOGI PADA KAMBING YANG DIDEDAH KEPADA PENGANGKUTAN, LAIRAJ, DAN DISEMBELIH

Oleh

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Penghasilan haiwan makanan melibatkan prosedur penternakan rutin termasuk pengendalian, pengangkutan, pengenalan kepada persekitaran novel (baru), dan penyembelihan. Aktiviti-aktiviti ini menyebabkan stres dan kesakitan kepada haiwan. Pengurusan pra-penyembelihan haiwan ini boleh memberi kesan terhadap pengalaman kesakitan ketika disembelih. Justeru, kajian ini dijalankan untuk menentukan kesan penstres dalam pengurusan haiwan ternakan, melalui perubahan parameter hematologi, biokimia serum, hormon stres, elektroensefalogram (EEG), dan penemuan neuro-histologi pada kambing yang didedahkan kepada pengangkutan bertempoh 1, 2, dan 6 jam, dan lairaj pra-penyembelihan bertempoh 3, 6, dan 16 jam, dan penyembelihan.

Kambing yang didedah kepada pengangkutan jalan selama 1 jam menunjukkan nisbah neutrofil ke limfosit (N:L), bilangan neutrofil, kepekatan kortisol plasma (CORT), kreatin kinase (CK) serum pada masa pasca-pengangkutan dan pasca-penyembelihan yang lebih tinggi daripada pra-pengangkutan (garis asas). Kepekatan alanin aminotransferase (ALT), aspartat aminotransferase (AST), protein plasma (PP), dan hematokrit (PCV) tidak berbeza daripada garis asas. Frekuensi median (F_{50}) EEG lebih tinggi tererti ($p<0.05$) pada pra-penyembelihan daripada garis asas menyarankan keadaan kerangsangan, dan kekal tinggi pada tempoh pasca-penyembelihan. Adalah diusulkan bahawa stres pengendalian, persekitaran novel, dan pengangkutan meningkatkan ambang kesakitan pada kambing melalui mekanisme analgesia teraruh stres (SIA).

Kambing yang diangkut selama 2 (T2) dan 6 (T6) jam menunjukkan bilangan neutrofil, kepekatan CK and ALT serum lebih tinggi tererti ($p<0.05$) daripada masa pra-pengangkutan. Berasaskan CORT yang tinggi, kambing daripada kedua-dua kumpulan pengangkutan ini sudah berada dalam keadaan stres sebelum diangkut. Bagaimanapun, nilai CORT menjadi lebih rendah selepas pengangkutan dan EEG tidak berubah, dan ini disabitkan dengan penyesuaian kambing terhadap keadaan pengangkutan. Peningkatan PCV dan PP juga berlaku pada kambing T6, yang menunjukkan penyahhidratan. Kambing T2 dan T6 telah didedah kepada lairaj pra-penyembelihan selama 3 (L3), 6 (L6), atau 16 (16) jam sebelum penyembelihan. Di kalangan kumpulan terperlaku ini, F_{50} pra-penyembelihan paling tinggi dalam kambing yang didedahkan kepada 2 jam pengangkutan dan 3 jam lairaj. Nilai F_{50} ini terus meningkat pada masa pasca-penyembelihan dan ini merupakan bukti berlakunya nosisepsi. Kumpulan lain menunjukkan penurunan nilai F_{50}, menandakan peningkatan ambang kesakitan.

Daripada penemuan histologi, aktiviti c-Fos sel-sel pyramidal korteks prefrontal (PFC) paling tinggi adalah dalam kambing T2-L6. Manifestasi ini, bersama dengan peningkatan CK serum daripada kecederaan otot, adalah terkait dengan kelakuan agresif di kalangan kambing. Peningkatan aktiviti c-Fos PFC pada kambing T6-L6 menunjukkan penglibatan SIA yang telah meningkatkan ambang kesakitan. Peningkatan kuasa δ dan θ EEG pada pasca-penyembelihan menunjukkan keadaan ketidaksedaran, yang diusulkan juga berlaku melalui mekanisme SIA. Berasaskan penemuan kajian ini, adalah dicadangkan yang tempoh lairaj selepas 2 dan 6 jam pengangkutan mestilah antara 6 sehinga 16 jam setelah interaksi sosial yang agresif telah berkurang dan haiwan mengalami kesakitan yang minimum ketika disembelih.

Kajian ini menunjukkan terdapat perkaitan antara pengaktifan ekspresi c-Fos sel-sel pyramidal dalam PFC kambing dan gerak balas EEG terhadap stres dan kesakitan penyembelihan. Kesimpulannya, di sini boleh dijelaskan yang elemen emosi dan kognitif kambing pada pra-penyembelihan boleh memberi kesan terhadap pengawaturan stres dan mempengaruhi gerak balas kesakitan penyembelihan kambing melalui mekanisme SIA.

Kata kunci: stres, kesakitan, pengangkutan, lairaj, analgesia teraruh stres.

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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

ACTH	Adrenocorticotropic hormone
ANS	Autonomic nervous system
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AVP	Arginine vasopressin
CK	Creatine kinase
CNS	Central nervous system
CRF	corticotrophin releasing factor
DAB	3,3’-diaminobenzidine
EDTA	Ethylene diamine tetra acetic acid
EEG	Electroencephalogram
FFT	Fast Fourier transformation
F_{50}	Median frequency
g/L	gram per liter
GC	Glucocorticoid
GR	Glucocorticoid receptor
Hb	Haemoglobin
HPA	Hypothalamic pituitary adrenal
IHC	Immunohistochemistry
LC	Locus ceruleus
LDH	Lactate dehydrogenase
N:L	Neutrophil to lymphocyte ratio
PCV	Haematocrit
PFC	Prefrontal cortex
POMC	Pro-opiomelanocortin
Ptot	Total power
PVN	Paraventricular nuclei
RBC	Erythrocytes
RMS	Root Mean Square
SAM	Sympatho-adrenal-medullary
SIA	Stress-induced analgesia
WBC	Total leucocyte count

CHAPTER 1

INTRODUCTION

Pain is a complex phenomenon that is manifested as unpleasant sensations and emotional experiences. Pain in animals have been an ongoing welfare concern and has become more so since the public now realise animals too feel pain (Weary et al., 2006; Coleman, 2007). There are numerous routine activities and procedures in animal husbandry that can incur pain to animals. Among the procedures in routine ruminant livestock farming that cause tissue injuries and pain in animals are castration (Mellor et al., 2002; Othman, 2015; Backus and McGlone, 2018), dehorning (Mellor et al., 2002; Gibson et al., 2007; Canozzi et al., 2019), tail docking (Mellor and Stafford, 2000; Backus and McGlone, 2018) and slaughter (Zulkifli et al., 2014; Sabow et al., 2016). Management practices such as handling (Ali et al., 2008; Kruger et al., 2016), transportation (D.M. Broom, 2003; Fazio and Ferlazzo, 2003; Honkavaara et al., 2003) and abrupt change of housing system (von Borell, 2001; Tarantola et al., 2016) are stressful to animals which also compromise animal welfare. Since animals have limited capabilities to communicate, there is a need for more research on their physiological and mental states when subjected to painful and stressful stimuli (Rushen, 2000; Anil et al., 2005).

Various methods have been used to mitigate pain and stress experiences in livestock management; among which are temperament genetic selection (Pighin et al., 2013; Haskell et al., 2014), provision of supplementations (Schaefer et al., 1997; Ferguson and Warner, 2008), improvement of animal's environment with enrichments (Grandin, 2003; Aschwanden et al., 2009; Backus and McGlone, 2018), improvement of handling techniques (Bulitta, 2015; Shahin, 2018), and improvement of interactions between animals and humans (Probst et al., 2013; Zulkifli, 2013; Mersmann et al., 2016; Nawroth, 2017; Tamioso et al., 2018). In pain management, different forms of analgesia had been used to provide relief from painful procedures (Valverde and Gunkel, 2005; Lizarraga and Chambers, 2012; Plummer and Schleining, 2013). Practical and cost-effective solutions are much needed to match the animal welfare with the financial state of the livestock industry in developing countries. Hence, improved methods to enhance animal welfare through the assessment of stress and pain in routine livestock management are required for implementation of efficient mitigation strategies. The overall aim is to refine agricultural policies that prioritise animal welfare concerns in livestock farming.

Physiological indices such as neuroendocrine, immunological, and pathological parameters, together with behavioural manifestations can be used as indicators of pain and stress in animals. However, abnormal changes in these parameters can be non-specific and require time to manifest, which may limit their utility as indicators of pain and/or stress (Weary et al., 2006). Furthermore, in livestock farming, the exposure of various painful and stressful stimuli to animals can be simultaneous and of various durations and intensities. Although difficult to quantify unequivocally, the experience of pain and stress is presumed to affect the physiological and mental states and welfare of animals. There is no measurement that on its own can assess animal welfare (von Borell, 2001; Nielsen et al., 2011), hence several methods are needed to assess pain and stress in farm animals to ensure that their welfare is preserved.

Food animals are slaughtered for meat. The pre-slaughter process begins at the farm, where the animals are herded, and then transported and restrained at the abattoir (Ferguson and Warner, 2008). The pre-slaughter logistics is a complex of activities that are physiologically and psychologically stressful to animals (Ferguson and Warner, 2008). The factors that contribute to stress in these animals include handling, loading and unloading, transportation, road conditions, transport motion and vibration, noise, unfamiliar animals, food and water deprivation, and novel environments (Broom, 2000; Ferguson and Warner, 2008). These stressors do not only impact animal welfare but also quality of meat (De la Fuente et al., 2010; Ekiz et al., 2012; Miranda-de la Lama et al., 2012; Chulayo and Muchenje, 2016; Jama et al., 2016). At the abattoir, animals are usually placed in lairage before slaughter. The time spent in lairage is presumed to allow animals to recover from the pre-slaughter stresses. However, this may not be true, because poor lairage conditions, insufficient period, and the company of other unfamiliar animals that could induce aggression, are factors compounding instead of relieving stress in these animals (Ferguson and Warner, 2008; Faucitano, 2011).

Most slaughter practices perform pre-slaughter stunning to render the animal unconscious and oblivious to pain at slaughter. The unconscious animal would become insensitive to stimulants from its environment that would cause the brain incapable to deal with painful sensory information (Terlouw et al., 2016). However, in certain religions, pre-slaughter stunning is still contentious among conservative and partisan followers. Thus, slaughter without stunning is still commonly practiced (Aghwan and Regenstein, 2019). The loss of blood from exsanguination would induce the loss of consciousness in these animals that eventually leads to death.

Neck-cut is a painful experience in slaughtered animals. The neck incision represent a noxious stimulus perceived by the animal prior to the onset of insensibility (Gibson et al., 2009a). Measurable data for pain in animals at slaughter are currently limited due to complexities of measuring pain in animals and limitations in interpreting the animal's behavioural and physiological responses towards the act of slaughter. It is also not known how pre-slaughter stresses affect blood parameters, electroencephalography (EEG), and sensation of pain in animals at slaughter. Although it has been asserted that good treatment of animals during the pre-slaughter processes will improve animal welfare at slaughter (Nakyinsige et al., 2013), it has not been unequivocally substantiated. Currently, there is a dearth of evidence on effect of pre-slaughter stresses on pain experience of animals at slaughter.

1.1 Problem Statement

Pain and inability to cope with stress are signs of compromised animal welfare. However, detecting and quantification of physiological, physical, and psychological pain and stress parameters in ruminants are complicated by poor correlation with behaviour and the time-lag between exposure to stimuli and physiological changes. In addition, painful stimuli also generate non-specific alterations in homeostasis. Hence, there is a need for more specific means to discriminate the types of stressors in the assessment of animal welfare. It is proposed that the EEG is a more accurate and immediate assessment of pain and stress in animals at slaughter.

1.2 Justification of the study

Small ruminants like goats are gregarious animals and are evidently fearful towards handling and novel environment and conditions. Animals that are not familiar with handling and new environment would perceive these elements as stressful. Most studies on livestock animals primarily focused on the effects of stress on the meat quality, and not on animal welfare (Terlouw et al., 2008). The effect of handling, transportation, loading and unloading, introduction to and confinement in novel environment, and separation from familiar herds on measurable parameters of pain and stress in livestock animals at pre- and post-slaughter period is currently limited and non-conclusive.

Fear, anxiety, and stress can modulate pain experience through stress-induced analgesia (SIA) (Rhudy and Meagher, 2000; Kambur et al., 2008). Although, pre-slaughter stress may affect pain experience in livestock animals at slaughter, there are still uncertainties on the involvement of SIA in the experience.

The EEG is a real-time measurement of brain activity and cognitive state. The EEG frequency bandwidths oscillations represent different brain operations including perception, memory, and cognition involved in the pain modulatory system. It was shown that the EEG median frequency (F_{50}) is a reliable indicator of arousal and associated pain (Gibson et al., 2009b; Kaka et al., 2015). However, the role of EEG bandwidths in stress and pain modulatory system in animals, particular at slaughter, is still not clear.

1.3 Objectives

Based on the problem statement mentioned, the objective of this study is to determine the effects of routine production and management activities on pain and stress in goats. The outcome of this study is to establish recommendations on the animal management activities that are known to cause stress, hence to minimise the animal's experience of pain at slaughter.

1.4 Specific Objectives

1. To determine the effects of road transportation under the hot and humid tropical conditions on blood haematological and biochemical parameters of goats.
2. To assess the quantitative EEG changes and association with haematological and biochemical parameters in goats subjected to transportation, lairage, and slaughter.
3. To determine the role of c-Fos expression in the prefrontal cortex in association with stress and pain of slaughter in goats.

1.5 Hypotheses

1. The psychological and physical stress of transportation and lairage can decrease the SIA system-modulated pain experience in goats.
2. Prolonged period of lairage can reduce the experience of pain at slaughter in goats subjected to transportation.
3. The prefrontal cortex c-Fos expression increases at slaughter in goats subjected to pre-slaughter stress.

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APPENDICES

APPENDIX A

UPM
UNIVERSITI PUTRA MALAYSIA

PEJABAT TIMBALAN NAIB CANSELOR (PENYELIDIKAN DAN INOVASI)

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Date:	15 December 2015
Ref.:	UPM/IACUC/AUP- R040/2015
Project Title:	Effects of polyphenol rich oil palm frond extract on goat meat quality.
Principal Investigator:	Assoc. Prof Dr Goh Yong Meng
Associates:	Dr Mahdi Ebrahim
Student:	Mr Wisam Salim Al-Jumali
Committee Decision:	The committee has reviewed and approved the proposed animal utilization protocol
AUP No.:	R040/2015
Project Classification:	Chronic
Category of Invasiveness:	B
Source of Animals:	A Sapphire Enterprise, 45 Jalan Indah 1/22, Taman University Indah, 43300 Seri Kembangan, Selangor Darul Ehsan
Number of Animals Approved:	24 goats
Accommodation:	Ladang Dua, UPM
Duration:	01 January, 2016 – 31 December, 2017

(Prof. Dr. Mohd Hair Bejo)
Chairman,
Institutional Animal Care and Use Committee
Universiti Putra Malaysia

Pejabat Timbalan Naib Canselor (Penyelidikan dan Inovasi), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
Pejabat Timbalan Naib Canselor (P&I) 603-8947 1293 603-8945 1646, Pejabat Pentadbiran TNCPI 603-8947 1608 603-8945 1673, Pejabat Pengarah, Pusat Pengurusan Penyelidikan (RMC) 603-8947 1601 603-8945 1596, Pejabat Pengarah, Putra Science Park (PSP)

PEJABAT TIMBALAN NAIB CANSELOR (PENYELIDIKAN DAN INOVASI)
OFFICE OF THE DEPUTY VICE CHANCELLOR (RESEARCH AND INNOVATION)

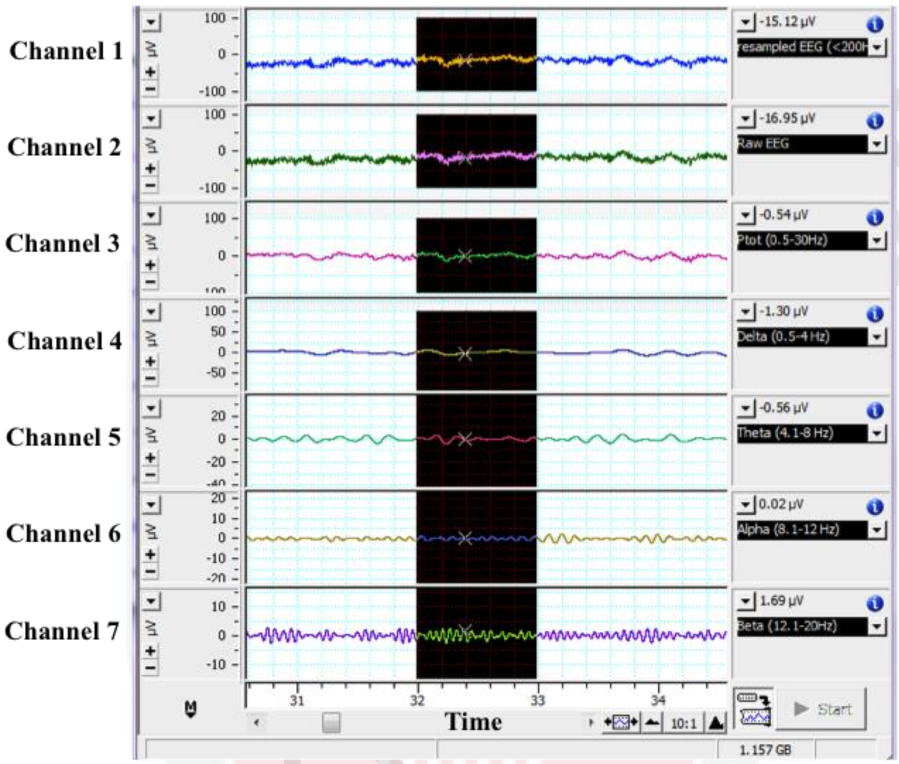
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Date:	14 August 2017
Ref.:	UPM/IACUC/AUP- R042/2017
Project Title:	The Behavioural and Physiological Responses of Goats Subjected to Stresseors In Hot and Humid Tropical Environment.
Principal Investigator:	Prof. Madya Dr. Goh Yong Meng
Members:	Dr. Azalea Hani Bt Othman; Razlina Bt Raghazli
Committee Decision:	The committee has reviewed and approved the proposed animal utilisation protocol, subject to relevant permit and/ or owner's consent.
AUP No.:	R042/2017
Project Classification:	Acute
Category of Invasiveness:	B
Source of Animals:	Commercial Farm, Pn. Mahani, Dengkil Farm.
Number of Animals Approved:	36 F1 crossbred Boer
Accommodation:	Lairage area at Research Abbatoir, Faculty of Agriculture, Universiti Putra Malaysia
Duration:	14 August 2017 – 14 August 2018

(PROF. DR. MOHD HAIR BEJO)
Chairman
Institutional Animal Care and Use Committee
Universiti Putra Malaysia

Pejabat Timbalan Naib Canselor (Penyelidikan dan Inovasi), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
Pejabat Timbalan Naib Canselor (P&I) 603-8947 1002 603-8945 1646, Pejabat Pentadbiran TNCPI 603-8947 1608 603-8945 1673, Pejabat Pengarah, Pusat Pengurusan Penyelidikan (RMC) 603-8947 1601 603-8945 1596, Pejabat Pengarah, Putra Science Park (PSP) 603-8947 1291 603-8946 4121 http://www.tncpi.upm.edu.my

APPENDIX B



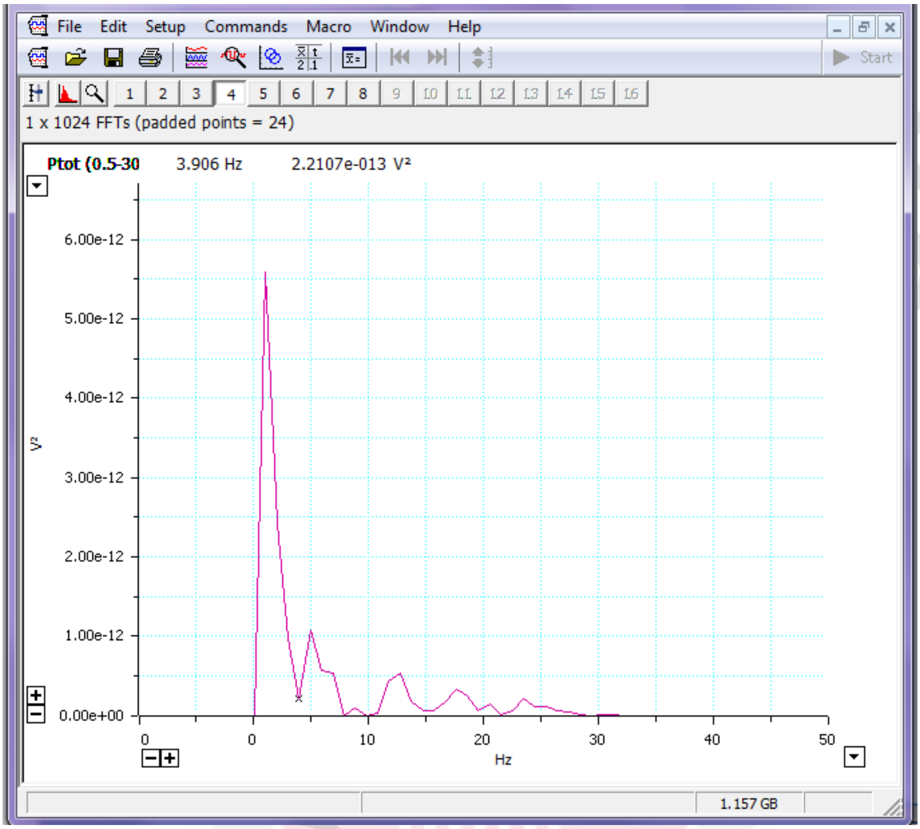
Appendix B1: The 'chart view' sample of Chart 5.0 Software (ADInstruments) representing electroencephalography (EEG) activity signals after subjecting to fast fourier transformation (FFT). Channel 1 = resampled EEG with low pass filter of 200 Hz; Channel 2 = raw EEG; Channel 3 = Total power (Ptot); Channel 4 = δ wave; Channel 5 = θ wave; Channel 6 = α wave; Channel 7 = β wave. The highlighted area (black background) represents a selected 1-sec epoch (0:00:32-0:00:33) within 1-min block.

1000 points from t=31.99 to t=32.989

A Comment Time	B Time	C Comment Text	D resampled EEG (<200Hz) RMS	E Ptot (0.5-30Hz) RMS µV	F Alpha (8.1-12 Hz) RMS µV	G Beta (12.1-20Hz) RMS µV	H Delta (0.5-4 Hz) RMS µV	I Theta (4.1-8 Hz) RMS µV
0:00:07.567	0:00:31.99	start	14.0218	4.8546	0.614	1.0548	3.5007	1.4873
0:00:07.567	0:00:10.99	start	13.7211	11.1316	0.9377	1.053	10.0179	1.6622
0:00:07.567	0:00:11.99	start	11.1187	7.2706	1.2357	1.0882	5.4626	2.6495
0:00:07.567	0:00:12.99	start	8.7715	7.4668	0.8743	1.3203	5.5346	2.0284
0:00:07.567	0:00:13.99	start	11.1313	7.3611	0.964	1.3093	5.2676	2.3142
0:00:07.567	0:00:14.99	start	8.1913	5.54	0.8782	1.2765	4.3753	1.4439
0:00:07.567	0:00:15.99	start	67.2949	60.2386	3.1072	2.4069	56.8938	6.2055
0:00:07.567	0:00:16.99	start	30.7012	14.7589	1.3831	1.1545	13.8597	2.9272
0:00:07.567	0:00:17.99	start	18.41	10.6422	0.8898	1.539	9.5746	1.9139
0:00:07.567	0:00:18.99	start	14.274	5.3013	0.5706	1.0494	4.3692	1.3062
0:00:07.567	0:00:19.99	start	15.2734	4.4958	0.7873	1.2597	2.5725	1.9481
0:00:07.567	0:00:20.99	start	26.626	7.902	0.8336	1.0198	6.796	1.928
0:00:07.567	0:00:21.99	start	23.1719	5.9076	0.7147	0.9984	4.7599	1.8901
0:00:07.567	0:00:22.99	start	17.41	4.4402	0.5795	1.4202	2.4405	1.8996
0:00:07.567	0:00:23.99	start	13.4829	3.6953	0.7205	0.9582	2.0748	1.3414
0:00:07.567	0:00:24.99	start	18.5197	3.5136	0.499	1.0133	2.4162	1.0668
0:00:07.567	0:00:25.99	start	28.3553	8.8933	1.0651	0.9719	7.568	2.4245
0:00:07.567	0:00:26.99	start	25.7119	6.8336	0.8967	1.1165	5.2686	2.032
0:00:07.567	0:00:27.99	start	30.8481	5.7316	0.945	1.4163	3.6859	2.6473
0:00:07.567	0:00:28.99	start	51.4997	50.3529	5.6645	3.3857	42.817	11.0543
0:00:07.567	0:00:29.99	start	39.6311	17.9041	0.5589	1.1575	17.4851	1.4132
0:00:07.567	0:00:30.99	start	22.5621	4.8532	0.554	0.9964	3.0886	1.8549
0:00:07.567	0:00:31.99	start	14.0218	4.8546	0.614	1.0548	3.5007	1.4873
0:00:07.567	0:00:32.99	start	16.1348	5.0938	1.0109	1.0156	3.4917	1.7644
0:00:07.567	0:00:33.99	start	17.0921	7.5666	1.0295	1.0239	6.049	2.1449
0:00:07.567	0:00:34.99	start	64.2487	57.5172	6.3597	3.671	49.1217	12.1781
0:00:07.567	0:00:35.99	start	25.1268	17.992	0.9052	1.2594	14.6367	4.8865
0:00:07.567	0:00:36.99	start	12.9202	6.3614	1.0958	1.7044	4.331	2.0559
0:00:07.567	0:00:37.99	start	10.3298	6.2853	1.0105	1.0887	5.4689	1.0997

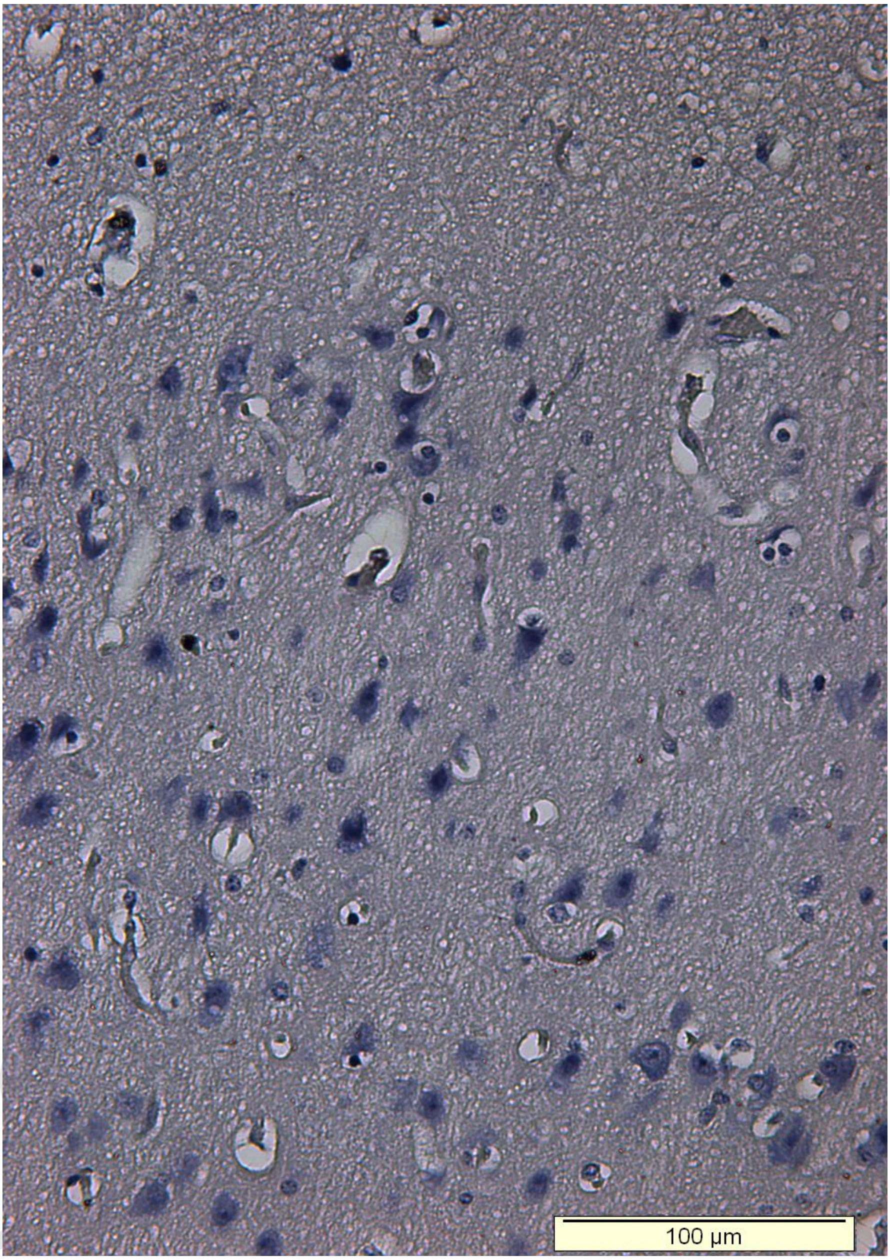
1.157 GB

Appendix B2: The 'Data Pad' view of Chart 5.0 Software (ADInstruments) representing the analysed data in route mean square (RMS) of total power (Ptot), δ, θ, α, and β band waves. Highlighted row (black background) represents the analysed data for the 1-sec Epoch (0:00:32-0:00:33).

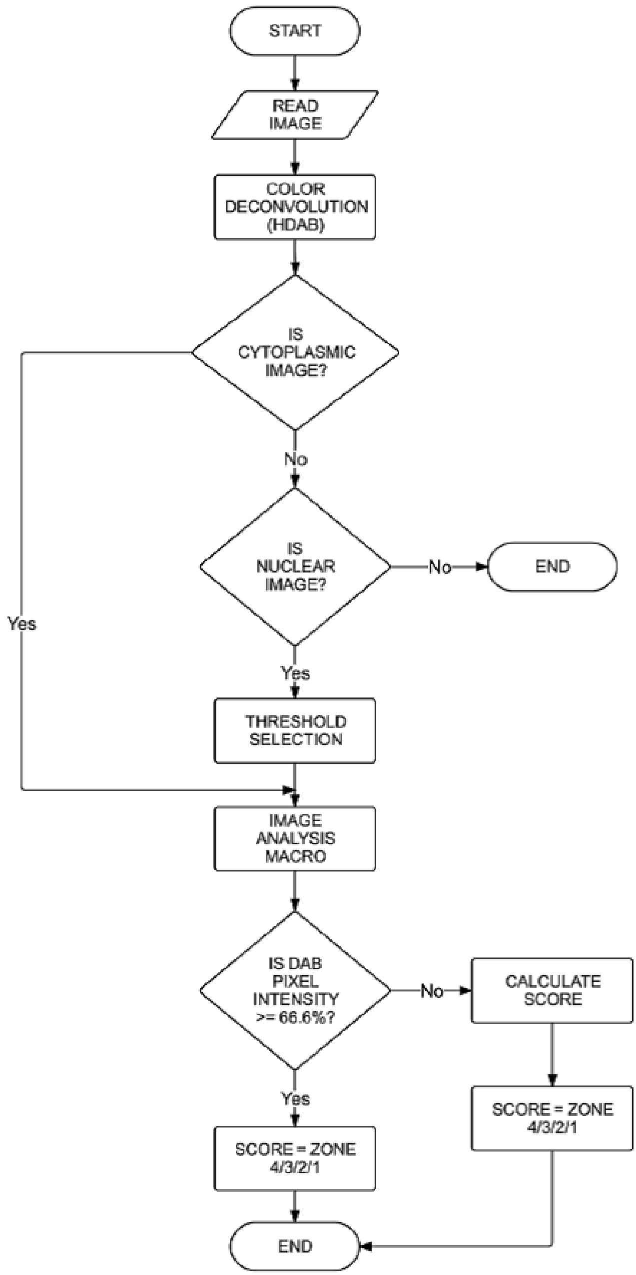


Appendix B3: The 'Power Spectrum' view of the selected epoch to derive the median frequency (F_{50}). The F_{50} for the selected 1-sec epoch is 3.906 Hz.

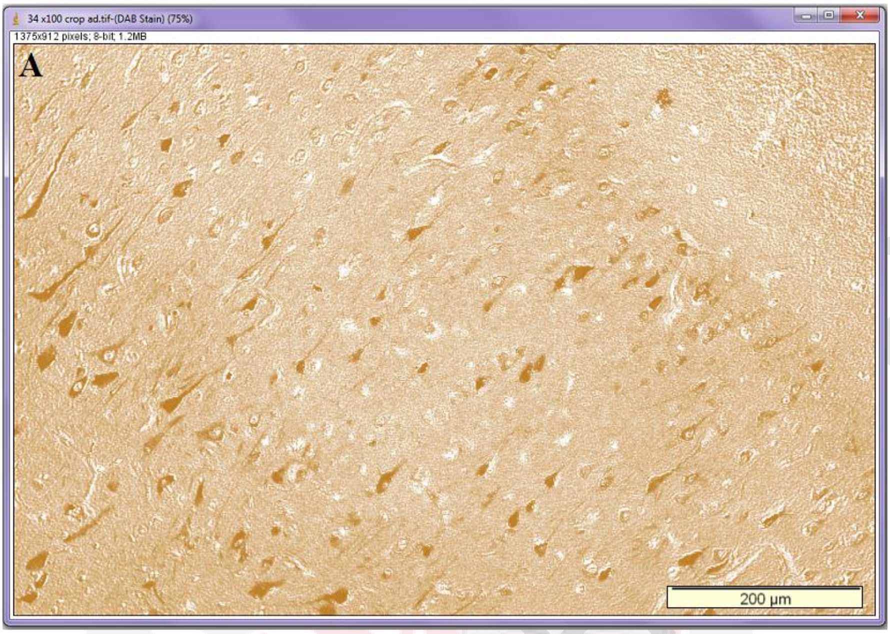
APPENDIX C

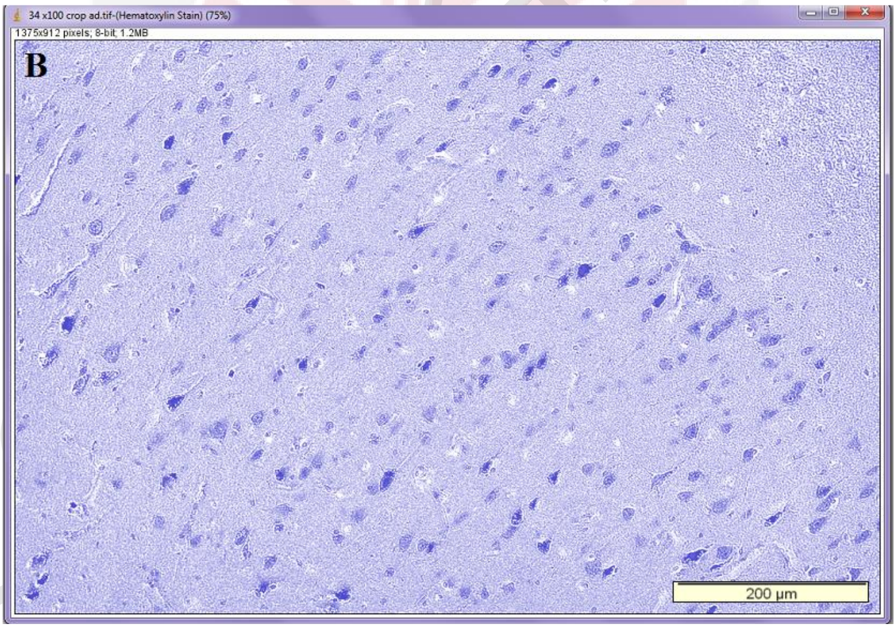


Appendix C1: Negative control of goat prefrontal cortex incubated with normal goat serum. (Magnification ×200, scale bar: 100 µm)

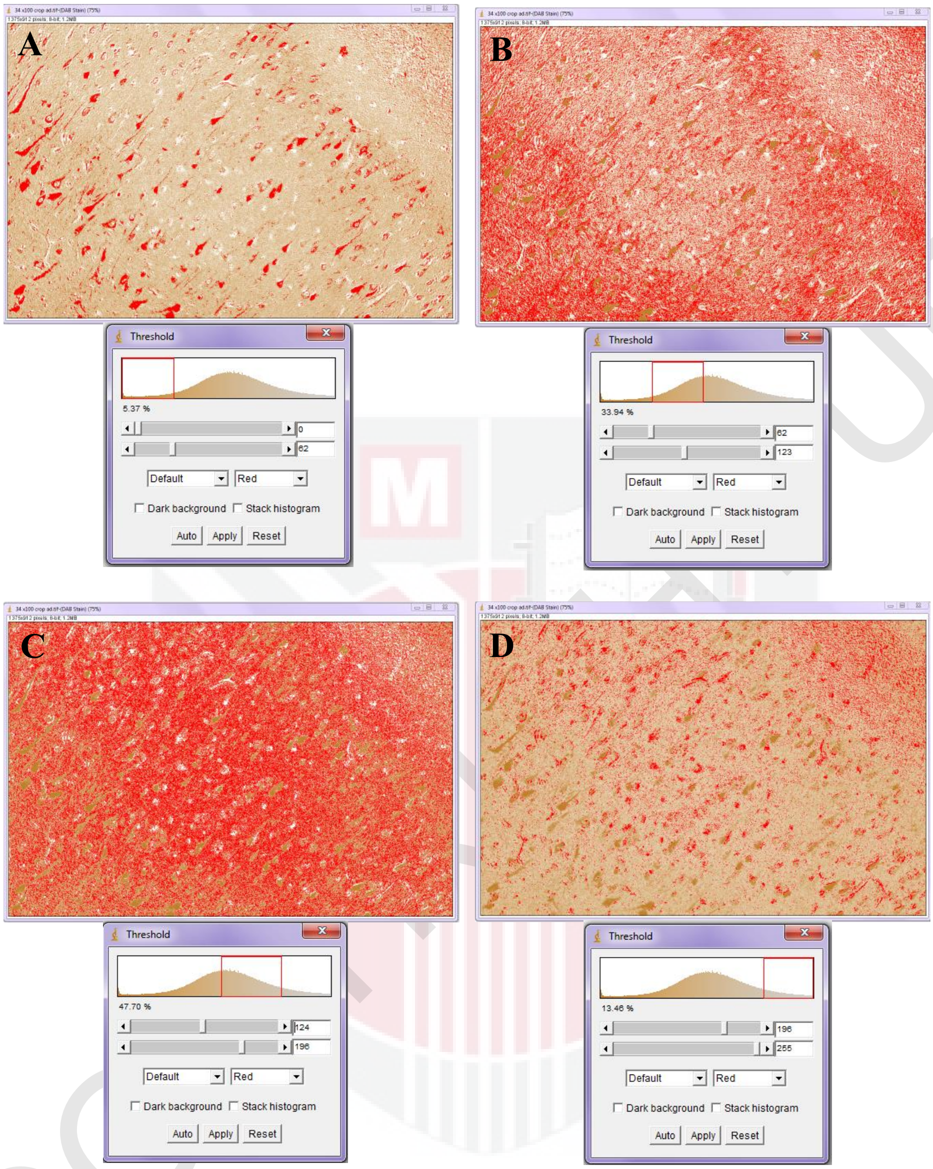


Appendix C2: Flow chart showing the computer steps involved in ImageJ IHC Profiler. [Adapted from Varghese et al. (2014)]

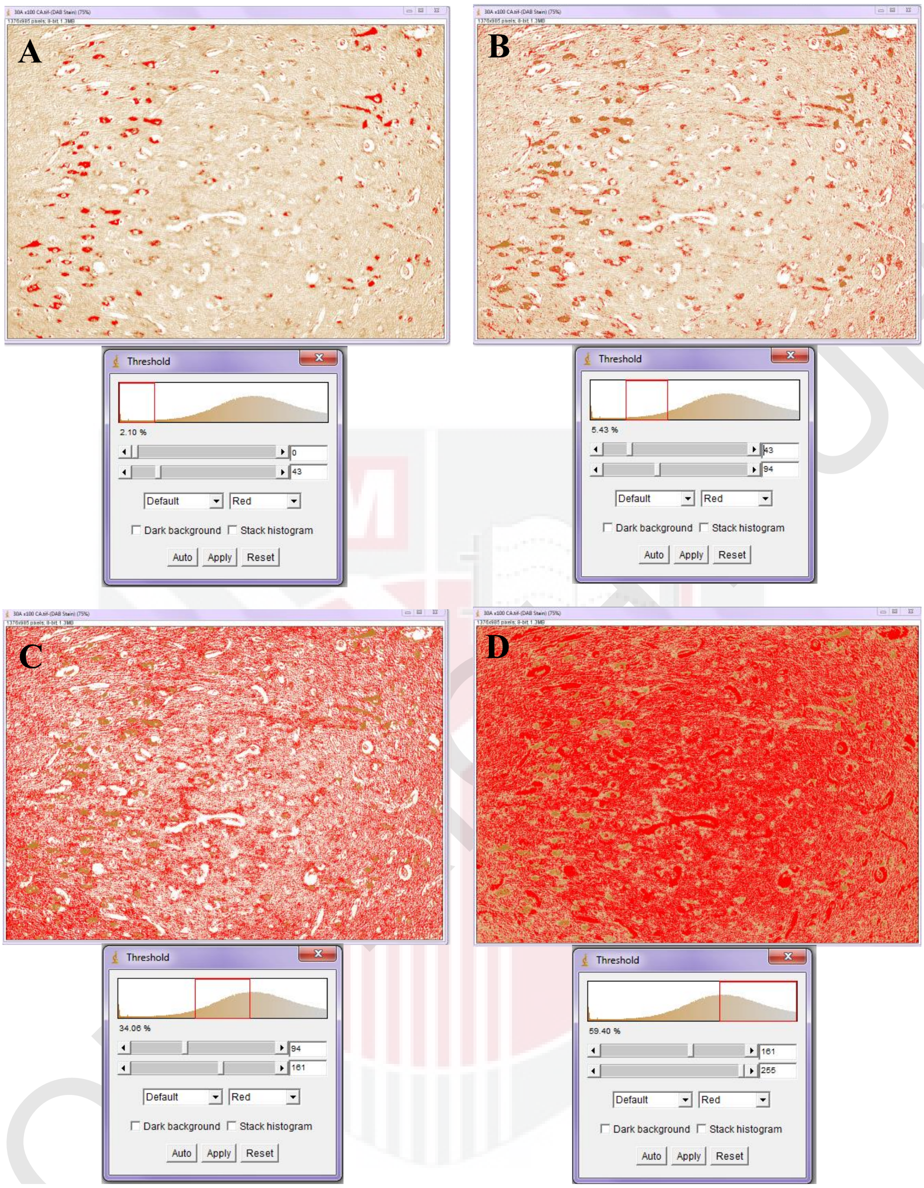




Appendix C3: Representative images of IHC Profiler splitting the sample image into DAB (A) and Haematoxylin (B) staining. (magnification ×100, scale bar: 200 µm)



Appendix C4: DAB-stained image sample with computed score of positive. Percentage of positive DAB-stained cells image based on ImageJ threshold zones: A=High Positive, B=Positive, C=Low Positive, D=Negative.



Appendix C5: DAB-stained image sample with computed score of low positive. Percentage of DAB intensity based on ImageJ threshold zones: A=High Positive, B=Positive, C=Low Positive, D=Negative

BIODATA OF STUDENT

Azalea Hani Othman was born in Manchester, United Kingdom on 27th June, 1984. She did her primary education at Sekolah Rendah Zainab (I), Kota Bharu. Her secondary education were from Sekolah Menengah Dato' Ahmad Maher, Kota Bharu (1997-1998) and Sekolah Menengah Kebangsaan Batu Lintang, Kuching (1999-2001). She continued with her Form 6 education at Sekolah Menengah Abdul Rahman Talib, Kuantan.

In 2004, she pursued her dream in veterinary medicine at the Faculty of Veterinary Medicine, UPM and graduated with a DVM degree in 2009. Her final year project was on transportation stress in chickens. Following year, she joined the Faculty of Veterinary Medicine, UPM academia as a tutor in Veterinary Clinical Pathology. She was awarded with Australia Prime Minister Endeavour Award scholarship which enabled her to continue her studies at the University of Queensland, Australia where she graduated with Master of Philosophy in Veterinary Medicine. Her master research was on stress and pain assessment in sheep castration. Upon returned from her Masters, she continued her postgraduate studies in PhD which her main research interest is the physiological assessment of animal welfare in ruminants.

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