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Physiological and electroencephalogram responses in goats subjected to pre-and during slaughter stress

Razlina Raghazli [a,b,*], Azalea-Hani Othman [c], Ubedullah Kaka [d], Ahmed A. Abubakar [e], Jurhamid C. Imlan [f], Hazilawati Hamzah [c], Awis Q. Sazili [e], Yong-Meng Goh [b,e]

[a] Department of Veterinary Services, Wisma Tani, Presint 4, 62630 Putrajaya, Malaysia
[b] Department of Preclinical Sciences, Faculty of Veterinary Services, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[c] Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[d] Department of Companion Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[e] Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[f] Department of Animal Science, College of Agriculture, University of Southern Mindanao, Kabacan 9407, North Cotabato, Philippines

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ABSTRACT

A comprehensive stress assessment is vital in understanding the impact of the pre-slaughter procedure on animal welfare. The transportation and handling process was commonly reported to cause stress in animals. This research utilises electroencephalography (EEG) as an alternative stress indicator to non-painful acute stress measurement. EEG has been proved to be instantaneous and sensitive with specific results. Therefore, this study was aimed to determine the stress level of goats subjected to two different transportation duration and the effect of lairage based on their EEG activities and blood parameters changes. Eighteen adult male goats were divided into two transportation stress groups based on the transport duration: the two-hour (TS2) and six-hour (TS6) groups. Then, each group was then again divided into three smaller groups according to the lairage duration, which was three-hour (L3), six-hour (L6), and overnight (L12) groups. Blood was sampled before transport, after transport, and during slaughter while EEG was recorded before transport, after transport, after lairage, and during slaughter. Results revealed that there was a significant decrease in beta wave activity compared to baseline in TS2 goats ($P < 0.05$) after transportation, whereas no significant difference was detected in the TS6 goats. At the same time, goats from the TS2 group showed increase in creatine kinase (CK) and lactate dehydrogenase (LDH) compared to that in TS6 goats. Together with the observed cortisol concentration, these findings showed that the TS6 goats were fully adapted to the transportation stress while the TS2 goats were still under stress. As for the lairage duration, it was observed that the TS2L3 goats showed lower EEG activities than the values obtained after two-hour transportation, while lower EEG activities were found from the TS6L6 goats after six-hour transportation. Therefore, it can be concluded that three-hour lairage was adequate to lower the impact of two hours transportation stress, whereas six-hour lairage was required to reduce the impact of six hours transportation stress. Finally, it was also found that the TS6L3, TS6L6, and TS6L12 groups took a long time to die after slaughter than the TS2L3, TS2L6, and TS2L12 goats based on the time their EEG activity reached isoelectric.

1. Introduction

A pre-slaughter process is as much as stressful to animals as the slaughter procedure itself. Pre-slaughter stress is the combination of various types of stressors such as transportation (Sporer et al., 2007), handling (B. Ekiz et al., 2012), heat stress (Kadim et al., 2006), and feed and water deprivation (Galipalli et al., 2004). Earlier research has reported the detrimental effect of pre-slaughter stress on the animal's immunity level (Maejima et al., 2005), meat quality (Abubakar et al., 2021) morbidity and mortality level

* Corresponding author at: Department of Veterinary Services, Wisma Tani, Presint 4, 62630 Putrajaya, Malaysia.
E-mail address: razlina@dvs.gov.my (R. Raghazli).
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(Samimi, 2017). Stress assessment often comprised the analysis of behaviour, autonomic nervous response, neuroendocrine and immunological changes (Moberg and Mench, 2000).

Few studies have reported that animals can experience excitement, pain, fear, and discomfort due to various stressors during the pre-slaughter procedure (Boissy et al., 2005; Forkman et al., 2007; Gregory and Grandin, 1998; Van Reenen et al., 2005). The fear and pain reaction will trigger animals' stress response (Moberg and Mench, 2000). The stress response begins when the brain activates the hypothalamus–pituitary–adrenal (HPA) axis, which regulates the release of corticotropin-releasing hormone (CRH) (Cechetto and Shoemaker, 2009). The CRH will then stimulate the secretion of adrenocorticotropic hormone (ACTH), which will then elevate the secretion of cortisol for the body to deal with stressor(s) (Cockrem, 2013). The cortisol is important in regulating stress and immune response and reinstates homeostasis (Herman et al., 2003; Hulbert and Moisá, 2016). On a typical unstressful day, the secretion of cortisol will follow the diurnal rhythm. Cortisol secretion plays a vital role in energy balance, body metabolism, and maintaining standard corticotropin-releasing factor (CRF) levels (Dallman et al., 2002). However, during stressful situations, HPA will be activated to initiate the secretion of ACTH. Nonetheless, cortisol secretion is also influenced by the diurnal cycle, environmental temperature, humidity and season (Budzyńska, 2014). Previous studies had also reported the delayed secretion of plasma cortisol of up to 20 h in faecal glucocorticoid metabolite (FGM) in African buffalo (Spaan et al., 2017). It has also been observed that the goat's cortisol increase 15 min after the beginning of the stressful events and remained high for only four hours before it decreases (Aoyama et al., 2008).

At the same time, the HPA axis also induces the release of catecholamine, which resulted in leukocyte changes (Pérez et al., 2002). Stress has been shown to cause neutrophilia, lymphopenia and eosinopenia (Latimer, 2011; Thrall et al., 2004). Animals that have been subjected to transportation and lairage usually experienced dehydration. Animals with severe dehydration normally shown to have high haemoglobin (Hb), haematocrit, total protein and red blood count concentration (B. Ekiz et al., 2012). Increased haematocrit, Hb and RBC level could also be due to the spleen contraction caused by the release of catecholamine secretion (Zhen et al., 2013). In addition, the process of transporting, handling, loading and unloading have caused injury to the animal due to fighting or falling (Ekiz et al., 2012), which increased the creatine kinase (CK) and lactate dehydrogenase (LDH) level in the blood (de la Fuente et al., 2012). Nonetheless, these types of responses have a time-lagged effect (Mormède et al., 2007) while some other conventional stress assessment methods that require samples from meat or blood will need specific equipment and analysis (Ozcan et al., 2014; Polycarp et al., 2016).

During the information process, the brain produced an electrical signal through the cortical pyramidal neurons (Babiloni et al., 2015; Bergamasco et al., 2006). These neural oscillations have a specific spatiotemporal pattern that varies in frequency, amplitude, and timing. The pattern can be related to the emotion of the animal (Cohen, 2017). Electroencephalogram (EEG), a brain imaging tool, was used to measure these neural oscillations during the stress period (Cohen, 2017; Sabow et al., 2018). Many studies show the relationship between stress and EEG activity in human (Abdul Hamid et al., 2010; Balconi et al., 2015; Hou et al., 2015). The analysis of the frequency from the EEG recording uses the Fast Fourier Transform (FFT) methods to obtain the signals' spectrum (Ang et al., 2017; Singh and Kanda, 2017). The signals' spectrum is categorised based on its frequencies which are delta frequency (<4.0 Hz), theta frequency (4.1–8.0 Hz), alpha frequency (8.1–12.0 Hz) and beta frequency (12.1–30.0 Hz). The slow-wave delta and theta frequency demonstrates the state of sleepiness. An increase in alpha waves indicates an animal is in a relaxed state and the high-frequency beta oscillation occurs when there is an increase in brain activity (Freeman and Quiroga, 2013). The integration of delta and theta activity indicates active brain activity during the unconscious state. The integration of alpha and beta activity indicates active brain activity during a sensible state (Seo and Lee, 2010).

EEG has been used throughout the years to determine the pain and nociception response in animals (Grint et al., 2015; Kaka et al., 2016; Sabow et al., 2016). Furthermore, EEG has been shown to have rapid, sensitive, and specific results (Bo et al., 2003; Coetzee, 2013). In humans, many studies have shown that EEG has a proven capacity to provide insightful information on the personal stress level. There was a significant correlation between EEG activities and the level of psychological stress in humans (Alshargie and Tang, 2017; Hou et al., 2015). Thus, it is acceptable to believe that EEG has great potential to investigate an animal's brain response to different kinds of stressor(s) (Freeman and Quiroga, 2013). Moreover, EEG is a non-invasive and practical method (Jun and Smitha, 2016; Sabow et al., 2018) and could give information on the animal's physiological state (Hosseini et al., 2013). Additional information on the animals' stress response is essential for animal welfare management strategies. Therefore, this study utilised EEG as an alternative indicator to non-painful acute stress measurement of goats subjected to different transportation and lairage duration during the pre-slaughter and slaughter procedures and the commonly used haematological and blood biochemical parameters. Information obtained can suggest a suitable lairage duration based on the transportation period and improve the animals' welfare and produce a better meat quality.

2. Materials and methods

2.1. Ethical statement

The research protocol in this study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Universiti Putra Malaysia (approve no: AUP: R042/2017).

2.2. Experimental conditions and treatments

This study was conducted in February and March 2018. The mean temperature during the study was 28.65 ± 1.07 °C (Mean ± SE), and the mean humidity was between 72.63 ± 3.79% (Mean ± SE). Goats were sourced from two local farms with the same management and were brought to Universiti Putra Malaysia in Selangor, Malaysia.

This study used eighteen adult male crossbred Boer goats divided into six treatment groups based on the transport (TS) and lairage (L) duration. Nine animals were transported by lorry for two hours (TS2) while another nine were transported for six hours (TS6) from the farm to the Research Abattoir of Animal Science Department, Faculty of Agriculture, Universiti Putra Malaysia (2°58′59.0″ N 101°44′06.4″ E). The space allowance for goats during transportation in this study complied with the space allowance set by the 'Council Regulation (EC) No 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No 1255/97′ (EUR-Lex – 32005R0001 - EN - EUR-Lex, 2005). Based on the guidelines, space allowance for goats <35 kg is between 0.20 and 0.30 m^2/animal. For both groups, transportation began at about 8.00 a.m. Goats were transported on a covered lorry with a slatted wooden floor on a straight flat surface highway road with an average speed of 50 km/h. The driver had been instructed to drive consciously and avoid speed and abrupt

braking to prevent additional stress to the animals. Water and feed were not given throughout the journey. Goats were handled carefully during the loading and unloading process to avoid stress from rough handling.

Upon arrival, animals from each transportation group were subdivided into three groups based on the lairage duration, which were three-hour (L3), six-hour (L6) and overnight lairage (L12). Goats were put in a holding space with a concrete floor and a solid fence beside the slaughter area. The area had a light cycle of 12:12. Water was available throughout the lairage period, but feed was not provided. The absence of feed was meant to reduce gut contamination during slaughter (Chulayo and Muchenje, 2016; Hogan et al., 2007). Goats were slaughtered at the end of every lairage period.

2.3. Blood sampling and analysis

The first samples of blood were taken at the farm before transportation and treated as a basal reading. The second samples were taken after transport, the third samples taken after lairage and the fourth samples after slaughter. Three ml of blood was taken from the jugular vein in each extraction and drawn into heparin and serum tubes (BD Vacutainer Systems, Plymouth, UK). Blood samples for haematology and biochemical analysis were analysed at the Haematology and Clinical Biochemistry Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia, within two hours after the collection. Blood samples for cortisol analysis were collected in the heparin tubes and put in an icebox. It was then centrifuged for 15 min at 1000g at 2–8 °C. Plasma was pipetted and transferred into centrifuge tubes and kept at −80 °C until assay to avoid the loss of bioactivity and contamination. The plasma that was earlier kept in the −80 °C were allowed to set at room temperature. 500 μl was then aliquot into a clean test tube. Few drops of 3.0 M of hydrochloric acid (HCl) was added into the aliquot to balance the pH plasma to 1.5–2.0 as much as 2 ml of methylene chloride was added into the sample. The sample was then mixed thoroughly using a vortexer and was later left to form a separated layer (Leme et al., 2012). The methylene chloride layer (lower layer) was pipetted into a clean tube. The procedure was performed three times. After the cortisol has been extracted, the methylene chloride is evaporated by heating it under a gentle stream of nitrogen. Subsequently, the extraction was dissolved in 0.5 ml of ELISA buffer. The extracted cortisol was then analysed using commercial Cayman's Cortisol ELISA kit (Cayman Chemical, Michigan, USA) according to the manufacturer's instructions with the reagents provided.

2.4. EEG sampling and recording

EEG activity was recorded at four sampling times when goats were at the farm before transportation (baseline), after transportation, after lairage and after slaughter until after the goats lost their sensibility. EEG recording was done using two conductive electrode patches affixed to the zygomatic process of the frontal bone and the mastoid area, as described by Sabow et al. (2017). Before EEG recording, each goat was shaved and degreased at the zygomatic process and the mastoid areas to better attach the electrodes. The recordings were done using Powerlab 4/20 data recording system (Powerlab data acquisition system, ADInstruments Ltd. Sydney, Australia). They were recorded into a laptop installed with Chart 5.0 (PowerlabTM data acquisition system, Sydney, Australia).

The EEG recording started after 30 s upon the placement of the electrodes and continued for about 2 min when taken at the farm and after transport. Observations were made during the EEG reading to record the artefacts resulted from the physiologic rhythmic movements such as ocular or cardiovascular movements, the electrical interference, and physical movements from the goats themselves, such as rumination or ear flapping. Goats were standing and restrained minimally during the whole process of the EEG reading. Goats in this study were slaughtered humanely based on the Malaysia Standard MS 1500:2009 (Halal Food: Production, preparation, handling, and storage - General Guidelines). The area of slaughter was cleaned after each slaughtering process to minimised stress to the animals. Electrodes were attached to the head of the goats throughout the slaughtering process. EEG activity was recorded after transport and during slaughter. The recording during slaughter ended when the frequency reached isoelectric. Isoelectric is a sign of silence or brain death in a living organism (Silva and Antunes, 2012). Isoelectricity was established when there was a stable trace of EEG activity with <1/8 of amplitude compared to the baseline of the EEG reading (Gibson et al., 2009).

EEG activities were analysed offline using the Chart 5.0 software (ADInstruments Ltd. Sydney, Australia). The EEG was recorded at a sampling rate of 1 kHz. The individual power spectrum of alpha, beta, delta and theta was calculated based on the amplitude and frequency of the EEG signals (Isley et al., 2009; Kongara, 2008). Artefacts were removed from the overall activity, and individual waves were subjected to FFT analysis. The Total power (Ptot), Root mean square (RMS) and Median Frequency (F50) were calculated repeatedly for non-overlapping of one-second epochs, yielding 60 epochs per minute.

2.5. Statistical analysis

Statistical analysis was performed using SPSS Statistics 23 software (SPSS Inc., Chicago, IL, USA). The one-way ANOVA was used to examine the treatments and sampling time on the haematological, blood biochemical, cortisol and EEG reading. Duncan's multiple range test was used to test the differences between means. All values were expressed as mean ± SE, and the confidence interval was set at 95%. Data were presented as the percentage changes after transportation, after lairage, and after slaughter, against the baseline value.

3. Results

3.1. Haematological changes

Table 1 shows the haematological values (mean ± S.E.) for goats in this study. The table was organised to show the difference of haematological parameters between the values taken in the farm (baseline values) with values obtained after animals subjected to transportation and lairage. Analysis between treatments showed that the RBC, Hb, and PCV values of the TS2L3, TS2L6 and TS2L12 with the TS6L3, TS6L6 and TS6L12 groups were significantly different when recorded on the farm. The differences in the baseline values caused similar significant differences in the RBC between the TS2 and TS6 groups after goats being subjected to transportation stress. However, the RBC values did not differ significantly throughout the lairage period in all groups. The Hb and PCV values were found to be insignificant after transportation. Overnight lairage caused a significant increase in the Hb and haematocrit concentration in the TS6L12 groups. It can be observed that the concentrations of lymphocytes and eosinophils were higher in the TS2L3 group. On the other hand, the values of plasma protein, basophil, and N/L ratio were higher in the TS6L3 group. No significant changes in the total WBC counts were observed in TS2L3 and TS6L3. The WBC counts were high until in the TS2L3 and TS2L6 and returned to the baseline value in the TS2L12 group. In the TS6 group, the WBC remained high in the TS6L3 and TS6L6 groups

Table 1
Effects of transport and lairage duration on the haematological parameters (Mean ± S.E.) in goats.

Blood parameters	Sampling Time	Treatments					
		TS2			TS6		
		L3	L6	L12	L3	L6	L12
RBC ($\times 10^{12}$/L)	Farm	12.41 ± 0.14^{a}			11.58 ± 0.32^{b}		
	After transport	12.59 ± 0.23^{a}			11.45 ± 0.32^{b}		
	Slaughter	12.4 ± 0.1^{a,b}	12.57 ± 0.44^{a,b}	12.8 ± 0.1^{b}	11.43 ± 0.38^{a,b}	11.07 ± 0.33^{a}	11.73 ± 0.88^{a,b}
Hb (g/L)	Farm	113.47 ± 2.25^{a}			105.22 ± 1.86^{b,x}		
	After transport	112.6 ± 2.76			110.06 ± 2.19^{x,y}		
	Slaughter	117.67 ± 3.33	110.77 ± 2.62	105.6 ± 7.2	103.63 ± 5.52^{x}	106.0 3.46^{x}	117.87 4.07^{y}
Haematocrit (L/L)	Farm	0.36 ± 0.02^{a}			0.31 ± 0.02^{b,x}		
	After transport	0.35 ± 0.01			0.35 ± 0.01^{x,y}		
	Slaughter	0.37 ± 0.00^{a,b}	0.35 ± 0.02^{a,b}	0.33 ± 0.02^{a}	0.33 ± 0.02^{a,x}	0.33 ± 0.01^{a,x}	0.38 ± 0.01^{b,y}
Plasma protein (g/L)	Farm	76.89 ± 0.21^{y}			79.11 ± 2.56		
	After transport	74.22 ± 1.68^{a,x,y}			79.33 ± 1.11^{b}		
	Slaughter	67.33 ± 0.67^{a,x}	75.33 ± 0.67^{a,y}	71.33 ± 0.67^{a,x,y}	85.0 ± 2.08^{b}	84.0 ± 1.15^{b}	86.0 ± 1.15^{b}
WBC ($\times 10^{9}$/L)	Farm	12.87 ± 0.64^{x,y}			13.64 ± 0.62^{y}		
	After transport	13.27 ± 0.21^{y}			12.86 ± 0.27^{x,y}		
	Slaughter	14.37 ± 0.22^{b,y}	14.4 ± 0.15^{b,y}	11.17 ± 0.03^{a,x}	12.1 ± 1.08^{a,b,x,y}	14.37 ± 1.14^{b,y}	10.80 ± 0.76^{a,x}
Neutrophil ($\times 10^{9}$/L)	Farm	3.55 ± 0.10^{w}			3.72 ± 0.14^{x}		
	After transport	7.51 ± 0.22^{y}			7.71 ± 0.23^{y}		
	Slaughter	8.26 ± 0.16^{b,z}	7.41 ± 0.16^{a,x,y}	6.77 ± 0.05^{a,x}	7.05 ± 0.57^{a,y}	7.40 ± 0.06^{a,y}	6.95 ± 0.13^{a,y}
Lymphocyte ($\times 10^{9}$/L)	Farm	5.52 ± 0.32^{y,z}			6.1 ± 0.32^{y}		
	After transport	6.58 ± 0.32^{a,z}			5.29 ± 0.16^{b,x,y}		
	Slaughter	6.75 ± 0.01^{c,z}	4.32 ± 0.35^{a,x,y}	4.20 ± 0.06^{a,x}	4.54 ± 0.22^{a,x}	5.18 ± 0.62^{a,b,x,y}	6.11 ± 0.06^{b,c,y}
Monocyte ($\times 10^{9}$/L)	Farm	0.25 ± 0.01^{x}			0.25 ± 0.01^{x}		
	After transport	0.48 ± 0.01^{y}			0.48 ± 0.04^{y}		
	Slaughter	0.46 ± 0.01^{a,b,y}	0.47 ± 0.01^{a,b,y}	0.49 ± 0.01^{a,b,y}	0.55 ± 0.02^{b,y}	0.42 ± 0.07^{a,y}	0.54 ± 0.02^{b,y}
Eosinophils ($\times 10^{9}$/L)	Farm	0.18 ± 0.03			0.2 ± 0.03^{x,y}		
	After transport	0.26 ± 0.08^{a}			0.04 ± 0.02^{b,x}		
	Slaughter	0.00	0.1 ± 0.1	0.15 ± 0.07	0.00^{x}	1.33 ± 0.33^{y}	1.33 ± 0.33^{y}
Basophils ($\times$109/L)	Farm	0.14 ± 0.03^{x,y}			0.18 ± 0.03^{y}		
	After transport	0.18 ± 0.04^{a,y}			0.32 ± 0.02^{b,z}		
	Slaughter	0.19 ± 0.1^{b,y}	0.00^{a,x}	0.00^{a,x}	0.08 ± 0.04^{a,b,x,y}	0.12 ± 0.06^{a,b,x,y}	0.07 ± 0.03^{a,b,x}
N/L	Farm	0.66 ± 0.03^{x}			0.62 ± 0.04^{x}		
	After transport	1.17 ± 0.08^{a,y}			1.47 ± 0.07^{b,z}		
	Slaughter	1.22 ± 0.03^{a,b,y}	1.73 ± 0.13^{c,z}	1.61 ± 0.01^{c,z}	1.55 ± 0.11^{b,c,z}	1.47 ± 0.19^{a,b,c,z}	1.14 ± 0.03^{a,y}

N/L neutrophil/ lymphocyte ratio.
^{a,b,c} Values with different superscripts in the same row are significantly different ($p < 0.05$).
^{w,x,y,z} Values with different superscripts in the same column are significantly different ($p < 0.05$).

and decreased significantly from the baseline value in the TS6L12 group. The significant increase can be seen in the neutrophil, monocyte, basophil counts, and N/L ratio in TS2L3 and TS6L3 groups. The N/L ratio increased significantly in the TS2L6 and TS2L12 but decreased in the TS6L6 and TS6L12 groups.

3.2. Blood biochemical changes

Effects of transportation and lairage duration on goats' blood biochemical are shown in Table 2. The blood biochemical values obtained on the farm was treated as the baseline values. In the TS2L3 group, almost all blood biochemical were significantly increased ($P < 0.05$) after transportation except for LDH, creatinine and total protein values. On the contrary, in the TS6L3 group, the only significant changes detected is for the CK and LDH values. Overall, the CK values showed the highest increased to almost two-fold after transportation from the baseline values in both TS2L3 and TS6L3 groups. Glucose increased significantly from TS2L3 to TS2L6 and TS2L12 but was insignificant in the TS6L3, TS6L6 and TS6L12 groups. The effect of lairage duration can be seen in the changes in all of the blood biochemical parameters. The CK concentration did not change during the TS2L3 compared to CK collected after transportation. TS2L6 has the highest CK concentration and decreased significantly in the TS2L12 group. No significant changes were detected in the TS6L3 and TS26L6 CK concentration compared to the CK after transport value. A significant increase in CK concentration was detected in the TS6L12 ($P < 0.05$). The LDH concentration increased significantly in the TS6L3 compared to LDH concentration after transport and decreased significantly in the TS6L6 and TS6L12 groups ($P < 0.05$). No significant changes in LDH concentration detected between TS6L3, TS6L6 and TS6L12 groups. Lactate concentration in TS2 and TS6 groups was found to be not significant when sampled after transport. Lairage has no effect on the lactate concentration in the TS2 groups. However, the lactate concentration decreased below the baseline value in the TS2L6 group. The concentration of lactate was highest in the TS6L3 group and started to decrease after the TS6L6 group.

TS2L3 shows a significantly reduced creatinine level compared to the level after transport and remains significantly unchanged in the TS2L6 and TS2L12 groups. However, no significant changes detected in the TS6L3, TS6L6 and TS6L12 groups. The urea concentration was significantly decreased in the TS2L3 group and remain unchanged in the TS2L6 and TS2L12 groups. Contrarily, the urea concentration slightly decreased in the TS6L6 group but increased again in the TS6L12 group. Total protein level was unaffected by the lairage duration in all groups. Glucose concentration decreased significantly in the TS2L3 group compared to the concentration obtained after transportation and increased significantly in the TS2L6 group but decreased significantly in the TS2L12 group. On the other hand, the glucose concentration returned to the baseline value In the TS6L12 group.

3.3. Cortisol changes

The cortisol concentration levels data were expressed as the percentage of the mean changes after transportation and slaughter

Table 2
Effects of both transportation and lairage duration (mean ± S.E) on blood biochemical parameters and cortisol concentra in goats.

Blood parameters	Sampling time (ST)	Treatments (T)					
		TS2			TS6		
		TS2L3	TS2L6	TS2L12	TS6L3	TS6L6	TS6L12
CK (U/L)	Farm	280.89 ± 2.09^{x}			283.56 ± 5.92^{x}		
	After transport	425.22 ± 36.28^{y}			410.11 ± 36.11^{y}		
	Slaughter	455.33 ± 1.33^{a,y}	586.33 ± 7.31^{b,z}	473.33 ± 12.77^{a,y}	457.00 ± 4.36^{a,y}	437.00 ± 37.01^{a,y}	586.33 ± 7.31^{b,z}
LDH (U/L)	Farm	322.44 ± 3.26^{x}			320.00 ± 3.01^{x}		
	After transport	363.00 ± 18.16^{x,y}			342.11 ± 4.54^{y}		
	Slaughter	448.00 ± 0.58^{c,z}	405.00 ± 1.53^{b,y,z}	372.33 ± 8.95^{a,x,y}	362.33 ± 2.19^{a,y,z}	344.67 ± 10.91^{a,y}	368.00 ± 16.01^{a,z}
Lactate (mmol/L)	Farm	10.66 ± 0.8^{x,y}			9.09 ± 0.42		
	After transport	11.96 ± 0.86^{y}			11.56 ± 0.94		
	Slaughter	9.10 ± 0.61^{a,b,x,y}	7.60 ± 0.17^{a,x}	7.57 ± 0.22^{a,x}	11.67 ± 0.88^{c}	9.27 ± 0.37^{a,b}	10.00 ± 0.58^{b}
Creatinine (μmol/L)	Farm	84.44 ± 0.71^{y}			86.78 ± 2.16		
	After transport	88.47 ± 1.58^{y}			85.67 ± 3.32		
	Slaughter	77.80 ± 3.09^{a,x}	83.40 ± 3.32^{a,x,y}	78.4 ± 0.85^{a,x}	86.67 ± 2.40^{a,b}	85.33 ± 2.73^{a,b}	94.0 ± 4.51^{b}
Urea (μmol/L)	Farm	4.28 ± 0.18^{x}			4.6 ± 0.14^{x}		
	After transport	5.58 ± 0.14^{a,z}			5.04 ± 0.11^{b,x,y}		
	Slaughter	4.73 ± 0.15^{a,x,y}	4.90 ± 0.06^{a,x,y}	5.0 ± 0.06^{a,y,z}	5.33 ± 0.33^{a,b,y,z}	4.8 ± 0.15^{a,x,y}	5.73 ± 0.27^{b,z}
Total protein (g/L)	Farm	78.58 ± 2.24			76.04 ± 1.96		
	After transport	79.33 ± 1.29			83.04 ± 2.34		
	Slaughter	84.9 ± 4.81	81.5 ± 4.45	80.37 ± 1.33	80.1 ± 3.23	84.67 ± 1.76	82.73 ± 4.41
Glucose (mmol/L)	Farm	4.09 ± 0.09^{y}			3.78 ± 0.13^{x,y}		
	After transport	4.71 ± 0.15^{z}			4.78 ± 0.16^{y}		
	Slaughter	3.17 ± 0.32^{x}	4.53 ± 0.09^{y,z}	3.07 ± 0.35^{x}	4.4 ± 0.46^{x,y}	4.37 ± 0.54^{x,y}	3.67 ± 0.72^{x}

^{a,b,c,d,e} Values with different superscripts in the same row are significantly different ($p < 0.05$).
^{v,w,x,y,z} Values with different superscripts in the same column are significantly different ($p < 0.05$).

compared to the values obtained in the farm (Table 3). From the table, it can be seen that no significant differences in the cortisol concentration level were obtained in all groups after transportation. However, the cortisol concentration significantly increases in the TS2L3 group and decrease significantly to lower than the baseline value in the TS2L12 group. In the TS6 group, it can be seen that the cortisol concentration decreased significantly from TS6L3, TO TS6L6 and TS6L12 group.

3.4. EEG activity

Fig. 1 demonstrates the percentage difference of the values obtained after transportation over the baseline value on the transportation effect on the goats' EEG activity. For the TS2 goats, there was a significant decrease in the Ptot, alpha, delta, and theta values ($P < 0.05$) during the after-transportation period compared to the EEG values before transportation. Conversely, no significant difference was detected in all the EEG activities of the TS6 goats after transportation than the EEG activity recorded before transportation.

Fig. 2 shows the percentage difference of EEG activity of the lairage subgroups recorded after lairage and after the slaughter period over the baseline value. In the lairage subgroups of two-hour transported animals, only the Ptot value from the TS2L6 goats showed a significant increase by two-fold after lairage compared to the baseline value. However, only Ptot value from the TS2L3 and TS2L12 but not TS2L6 were significant after slaughter. In the

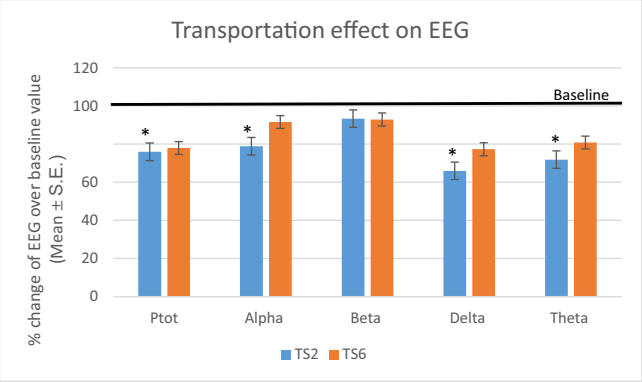

Fig. 1. Effect of 2 h transportation and 6 h transportation on EEG activity of goats. Data were expressed as percentage changes of EEG activity (Mean ± SE) after the transportation period over baseline values. Baseline values were recorded at the farm (*) indicate a significant difference ($P < 0.05$) between the effect of transportation (TS2 or TS6) when compared to the baseline value.

TS6 transportation group, it was found that there was a significant increase of the Ptot values after lairage than before transportation of the TS6L6 and TS6L12 group. All TS6 groups showed a significant increase in the Ptot values during slaughter than the value obtained at the farm (P < 0.05).

For the RMS of the alpha waves, no significant changes were detected in the TS2 group. At the same time, there was a significant

Table 3
Percentage differences in cortisol concentration levels (mean ± S.E) compared of farm values of goats after subjected to different transport and lairage duration.

Blood parameters	Sampling time	TS2			TS6		
		L3	L6	L12	L3	L6	L12
Cortisol (ng/ml)	Farm	100^{x,y}			100^{x}		
	After transport	91.79 ± 9.53^{x,y}			84.7 ± 10.59^{x}		
	Slaughter	112.03 ± 7.02^{b,y}	153.05 ± 10.54^{c,z}	79.21 ± 9.81^{a,x}	152.51 ± 5.07^{c,y}	99.05 ± 7.19^{a,b,x}	84.06 ± 2.73^{a,x}

^{a,b,c,d} Values with different superscripts in the same row are significantly different ($P < 0.05$).
^{x,y,z} Values with different superscripts in the same column are significantly different ($P < 0.05$).

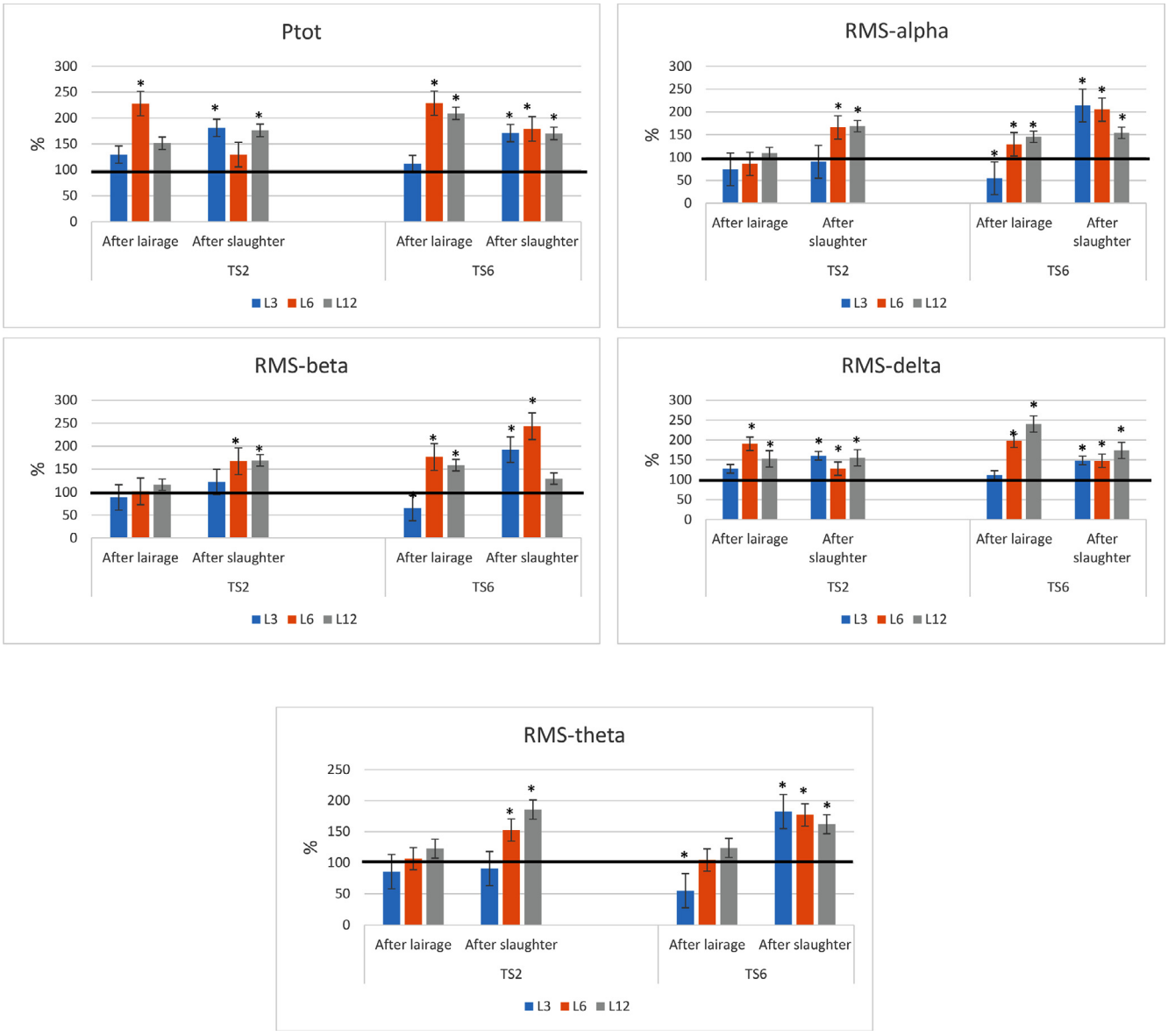

Fig. 2. EEG Total power (Ptot) and Root mean square (RMS) (Mean ± SE) expressed as a percentage difference from the baseline values recorded at the farm for individual band waves taken after lairage and after slaughter. The baseline value is set at 100%. (*) indicate a significant difference ($P < 0.05$) between the time of sampling to the baseline value.

difference after lairage compared to the baseline value for the TS6 group. After slaughter, only TS2L6 and TS2L12 showed a significant increase in the alpha values (P < 0.05). The TS6L3 and TS6L6 have doubled the value of alpha waves compared to the value attained at the farm.

As for beta waves, no difference was recorded after lairage for the TS2 groups compared to the baseline value. A significant increase (P < 0.05) was seen during slaughter in goats from the TS2L6 and TS2L12, while a slight decrease was detected from the TS2L3 goats. In the TS6 group, TS6L6 and TS6L12 beta waves increased significantly after slaughter than the values at farm level, while a significant decrease was observed for the TS6L3 group. The beta waves of TS6L3 increased significantly by two-fold while TS6L6 by two-and-a-half fold after slaughter. However, the beta waves of TS6L12 decreased significantly compared to values obtained after lairage after slaughter.

Delta waves increased significantly after lairage compared to the baseline value in both transportation groups except for goats in TS2L3. During the slaughter period, the delta waves of the TS2L6 goat increased by two-fold and continue to increase by almost 2.5-fold in the TS2L12. All delta waves in the TS6 group, except for goats in TS6L3, increase significantly after slaughter compared to the value obtained at the farm level.

No difference in theta waves was observed in both transportation groups after the lairage period except for the significant decrease in the TS6L3 goats compared to the theta waves at the farm. After the slaughter period, all groups showed a significant increase from the T1 value except for goats in the TS2L3 group.

Fig. 3 shows the effect of lairage on the EEG activities of goats obtained after lairage and slaughter. For the TS2 group, the Ptot value significantly high (P < 0.05) in the TS2L3 group, reached the highest value in the TS2L6 group, and significantly low in the TS2L12 group. The delta waves were found significantly high (P < 0.05) in the TS2L3 group but showed a slight decrease in the TS2L12 group. No significant difference was observed for the alpha, beta, and theta values for goats in the TS2 group after the lairage

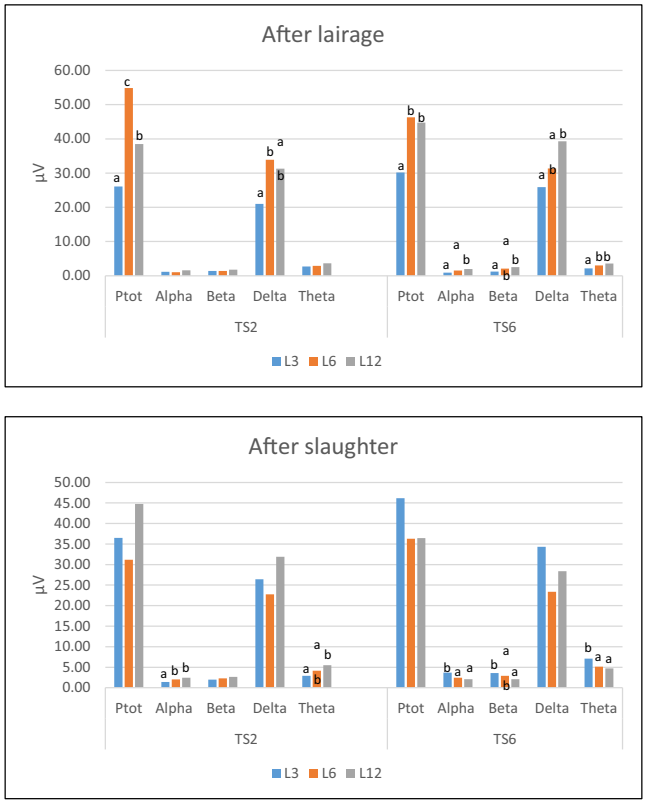

Fig. 3. Difference in the RMS power (Mean ± SE) of each band waves and the total power in every sampling point [a,b] Different letters indicate the significant difference ($P < 0.05$) between each band of waves.

period. For the TS6 group, lairage plays a significant role in determining the EEG activity of the goats. A significantly high value ($P < 0.05$) of the EEG activities in the TS6L3 and TS6L12 group in most individual waves.

Values obtained after slaughter shows that noxious stimuli caused significant EEG response in all individual waves (Fig. 3). The values obtained after lairage show that lairage had a big impact on the goats' overall EEG activity during slaughter. In the TS2 group, significant differences between treatment groups were only detected from the alpha and theta waves. Alpha waves showed a significant increase when goats were put in L6 compared to the goats in L3. For beta waves, significant differences were from the L3 and L12 goats, while the highest theta values were from the TS2L12 goats. As for the TS6 group, a significant difference between treatments were found from the alpha, beta and theta waves. The highest values were from the TS6L3 group for alpha and theta waves and decreased significantly throughout the lairage period. Beta waves were highest in the TS6L3 and significantly lowest in the TS6L12 group.

Table 4 shows the median frequency (F50) of goats taken in each sampling time. No difference was detected between both transport groups when sampled at farm level and after transport. No significant difference was detected between the transport groups during the slaughter period. A significant increase ($P < 0.05$) of F50 was observed after slaughter compared to the value obtained at the farm level in both transportation groups. Lairage has shown to have a significant impact on the F50 values of animals. The highest F50 values in all treatment groups were observed after the slaughter period ($P < 0.05$).

3.5. Isoelectric

The presence of isoelectric in the EEG recording marked the disappearance of brain activity (Shaw, 2002). The length of time between slaughters until EEG became isoelectric was counted and shown in Fig. 4. Different letters indicate that the time shown is significantly different between treatments ($P < 0.05$). It was observed that the time for EEG to become isoelectric was significantly different between goats from the TS2 and TS6 group ($P < 0.05$). Within the TS2 animals, TS2L3 goats had a significantly shorter time than the TS2L6 goats. Goats in the TS6 group required a longer time to reach isoelectric than goats in the TS2 group ($P < 0.05$).

4. Discussion

No significant changes in cortisol concentration were observed after transport in both transport groups in this study. Various findings were obtained from earlier studies. There were reported cases of increased cortisol concentration after transport (Kadim et al., 2006; Tajik et al., 2016), while another study on cattle reported a reduced cortisol concentration after transport (Tadich et al., 2005). The significant difference between the studies was the distance travelled by the animals. The fluctuation of cortisol concentration could cause these conflicting results due to the adrenal function during transportation (Nwe et al., 1996). At the same time, the adaptation during the transportation period could lead to a fear reduction, thus lowering the cortisol secretion in the animals (Kannan et al., 2007). Increased cortisol concentration sampled three hours after lairage in both transport duration in this study shows that the novelty of the environment could act as an additional stressor to the animals (Díaz et al., 2014). It was later observed that the cortisol concentration decreased after six-hour lairage until the end of the lairage period. The decrease in the cortisol concentration throughout lairage in both transport duration signifies that the activity of the HPA axis was reduced when goats were allowed to rest for a longer period resulting in the deterioration of cortisol concentration (Galipalli et al., 2004; Liste et al., 2009).

Increased activity in the brain would elevate the beta waves from the EEG recording (Freeman and Quiroga, 2013; Reisman, 1997). It was found that there were insignificant changes of beta waves compared to the baseline value at the end of the journey, with a significant decrease in the alpha waves for the TS2 transport group. Based on previous findings, the transportation process has triggered a stress response in goats (Rajion et al., 2001). For goats in the TS2 transport group, all band waves except for the beta waves showed a significant decrease after transportation, similar to the findings made in humans, which found that the decrease in alpha waves and increased beta waves indicates stress characteristics (Jun and Smitha, 2016). It is suggested that goats in the TS2 groups were under stress and unable to adapt to the transportation procedure due to the short time between transportation and sampling period. It could be due that the most stressful part of transportation is at the beginning of the transportation process (Nwe et al., 1996).

On the other hand, goats in the TS6 transportation group showed no stress response. This situation could be due to the adaptation strategy by the animals during the six hours of transport, which match with previous studies by Hall, Kirkpatrick, Lloyd, and Broom (1998) and Tadich et al.(2005) which shows the animals ability to adapt during the journey. Increased activity in the brain would elevate the beta waves from the EEG recording (Freeman and Quiroga, 2013; Reisman, 1997). It was found that

Table 4
The median frequency (F50) of goats in each treatment based taken at every sampling time (Mean ± SE.)

Sampling time	F50 (Hz)					
	TS2			TS6		
	L3	L6	L12	L3	L6	L12
Farm (Baseline)	6.41 ± 0.24^{x}			6.5 ± 0.23^{c,x,y}		
After transport	7.74 ± 0.18^{y}			7.74 ± 0.31^{y}		
After lairage	3.97 ± 0.47^{a,b,w}	3.3 ± 0.37^{a,w}	4.53 ± 0.33^{a,b,w}	5.0 ± 0.51^{b,x}	4.13 ± 0.69^{a,b,w}	3.9 ± 0.01^{w}
During slaughter	7.98 ± 0.49^{z}	7.78 ± 0.54^{z}	9.00 ± 0.14^{z}	10.01 ± 1.49^{z}	8.5 ± 0.69^{z}	8.49 ± 0.85^{z}

TS2: two-hour transportation group; TS6: six-hour transportation group.
L3: 3 h lairage; L6: 6 h lairage; L12: overnight lairage.
$^{a,b,\ c}$ Different letters indicate the significant difference ($P < 0.05$) between each columns.
^{w,x,y,z} Different letters indicate the significant difference ($P < 0.05$) between each row.

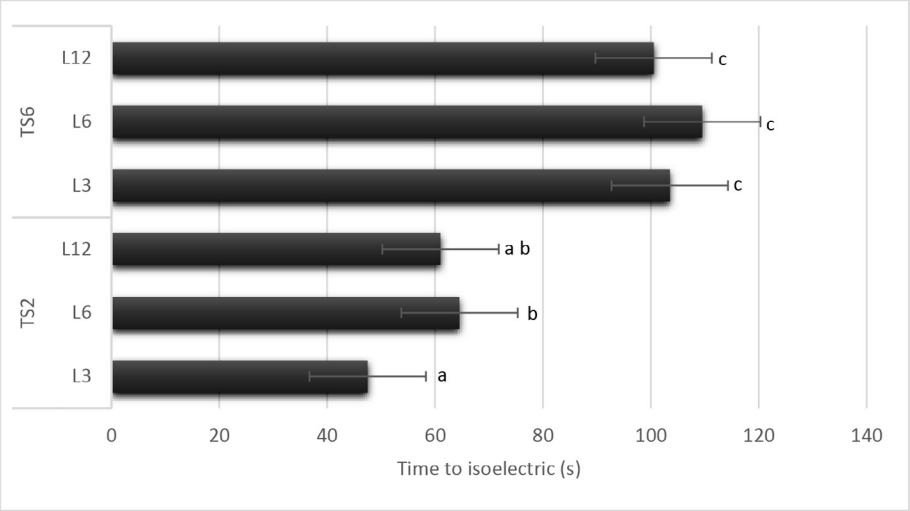

Fig. 4. The time to the isoelectric activity of goats after slaughter (Mean ± SE). Different letters indicate that the time shown is significantly different between treatments ($P < 0.05$).

there were insignificant changes of beta waves from the baseline value at the end of the journey, with a significant decrease in the alpha waves for the TS2 transport group. In human studies, the decrease in alpha waves and increased beta waves indicate stress characteristics (Jun and Smitha, 2016). Generally, the transportation process is undoubted may trigger a stress response in goats (Rajion et al., 2001). For the goats in the TS2 transport group, all band waves except for the beta waves showed a significant decrease after transportation. It is suggested that goats in the TS2 groups were under stress and still unable to adapt to the transportation procedure due to the short time between transportation and sampling period.

Multiple studies in human have shown a positive correlation between the cortisol level and EEG during stress (Brouwer et al., 2011; Seo and Lee, 2010). In the current study, it has been found that cortisol concentration was not significantly affected after both transport duration. The insignificant cortisol changes could be due to the animal's adaptation after the initial phase of the transport (Nwe et al., 1996). However, according to the previous study, cortisol secretion to the bloodstream is delayed for 10 min after the peak of the beta activities, which explained the insignificant changes of cortisol detected from this study (Chapotot et al., 1998). Nonetheless, cortisol secretion is not necessarily after the increase in beta activities, and the secretion can also occur from the stimulation of other origins (Chapotot et al., 1998).

The differences of RBC, Hb, and haematocrit observed at the farm level in this study show that the handling process and farm conditions between the two transportation groups would cause some differences (Bórnez et al., 2009b). At the same time, the three haematological parameters can also be used as a sign of dehydration in animals (Tadich et al., 2009). Nonetheless, the RBC, Hb, and haematocrit were not affected by this study's transport and lairage duration. No sign of dehydration was detected in goats after the transportation and lairage period. The indifference could be because the transport durations were not long enough to change the RBC, Hb, haematocrit and plasma protein concentration levels. Another possible explanation might be that goats in this study were provided with water throughout the lairage period. At the same time, goats' physiological mechanism enables them to stand long period without water by forming dry faeces and concentrated urine (Knowles et al., 1993).

The increased number of neutrophils and monocytes observed after both transport duration in this study matched with findings from a previous study in Cashmere goats (Samimi et al., 2018). However, similarly to prior reports, the neutrophil counts decreased with increased lairage time (Bórnez et al., 2009a). The secretion of catecholamine and cortisol due to stress resulted in the elevation of neutrophil counts caused by shifting the neutrophils to the peripheral circulation from the body pools (Latimer, 2011). As a result, the N/L ratio of goats in this study increased tremendously after both transportation time. These results were similar to the earlier findings, which found a sudden increase in the N/L ratio of goats after 1.5 h and 2.5 h transportation (Rajion et al., 2001). In addition, there was an increase in the neutrophil counts and N/L ratios compared to the baseline value until after overnight lairage (L12) in the TS2L12 and TS6L12

groups, which shows that more than twelve hours lairage is needed for the N/L ratio to return to its baseline values. The present findings were consistent with earlier research that found that it may take at least three days up to two weeks for blood to stabilise or return to the standard value in goat (Polycarp et al., 2016).

In this study, it has been found that lairage significantly impacts the transported goats in this study. It was observed that there was a significant increase of Ptot and delta waves activity in the TS2L6 group after lairage. As for TS2L12, a significant increase was only detected in the delta waves activity. The oscillation of the alpha and theta activities were related to the neuronal activities linking to the sensory and emotional processing. Animals in the relaxed states showed lower Ptot activities, higher theta activities, and decreased alpha waves (Bergamasco et al., 2005; Choi et al., 2015; Madan et al., 2017). All goats except for those in the TS6L6 and TS6L12 showed a decrease in alpha activities. The decrease indicates that there were in a relaxed state after the lairage period. Earlier studies in humans have found that the reduction in alpha activity is associated with physical relaxation (Freeman and Quiroga, 2013; Reisman, 1997). However, excessive lairage duration has been proved to cause another set of stressors which may have been resulted from dehydration, fighting, and emotional stress caused by the unfamiliarity of the environment (Dokmanovi et al., 2014; B. Ekiz et al., 2012). Goats in both TS6L6 and TS6L12 groups spend a longer time during transport and lairage duration, and the increase in stress level might be from the restlessness of the animals. The decrease percentage in beta waves after the lairage procedure except for the same two groups of TS6L6 and TS6L12 also support the assumptions made earlier.

Findings in the current study confirm the association of transportation stress to increased CK concentration (Galipalli et al., 2004). The CK concentration has been found to reach its peak value in the TS2L6 and TS6L12 goats. During some of the stages, a higher concentration of CK suggested the physical exertion experienced by the animals during the lairage (Fabrega et al., 2002). Longer transportation duration caused a significant increase in LDH, observed from the TS6 transportation group. This result was similar to the findings made by Bórnez et al. (2009) and Minka and Ayo (2013). The increase in these plasma enzymes could be due to the expanded muscle destruction caused by transportation stress (Adenkola et al., 2009). It can be observed that the LDH from TS2L3 and TS2L6 during slaughter was significantly higher than LDH from the TS6L3, TS6L6 and TS6L12 groups. Similar findings were also reported in lambs, indicating the longer time needed by animals to fully adapt to the transportation stress and lower the LDH concentration (De La Fuente et al., 2010). However, the LDH concentration in the TS6L12 increased significantly higher than the value recorded after transportation which shows that excessive lairage could impose another stressor to the goats. The increased LDH concentration continued until the TS2L6 from the two hours transported goats to the TS6L12 in the six hours transported goats. These findings proved that lairage was beneficial in the recovery process of the plasma enzyme activity. However, the combination of long transport and lairage further increased the LDH concentration in the goats. In contrast, a study by Yalcintan et al. (2018) found no effect of lairage on the LDH concentration in lambs. It seems possible that the discrepancy of the results with current findings may be due to the length of the lairage duration. In those study, the lairage duration was only 30 min and 2.5 h long, which showed the insufficient time for the animals to recover compared to the longer duration of lairage from the current study.

Lactate is the product from the glycogenolysis process, activated during high energy demand by the animal (Tadich et al., 2009). No significant changes in the lactate concentration from TS2 and TS6 show that low energy was required during the transport period. However, high lactate concentration, especially in the TS6L3 group, indicate that goats were highly stressed by the new lairage environment. Yet, sufficient time to rest and adapt to the new surrounding caused a decrease in lactate concentration, as shown in the TS6L6 and TS6L12 groups. The increase of glucose after transportation in the TS2 group is consistent with other earlier studies (Pascual-Alonso et al., 2016; Sabow et al., 2016). However, the concentration of plasma glucose found to fluctuate throughout the different lairage period. The glucose level reached its peak in TS2L6 goats but decreased in TS2L12 goats. However, the glucose level in the TS6L3, TS6L6 and TS6L12 groups seemed unaffected by transportation or lairage. The reduction of glucose concentration in the TS2L12, TS6L3, TS6L6 and TS6L12 showed animals were already adapted to the lairage or transportation stress (Ekiz and Yalcintan, 2013).

Slaughter would cause pain, especially for un-stunned animals (Gibson et al., 2007; Grandin and Smith, 2004). It was observed that the noxious stimulation during the slaughter process has triggered the neural response and caused an increase in the EEG activities. There was a significant increase in all group activities after slaughter except for goats in the TS2L3 group. The two-hour lairage duration has been found to cause an increase, while six-hour lairage resulted in a decrease of Ptot value of goats during the after slaughter. However, after overnight lairage, the decreased Ptot value shows that goats experienced more stress and possibly pain compared to goats with higher Ptot value (Gibson et al., 2007; Jongman et al., 2000). Goats in this study were all fully awake during the slaughter procedure. The neck cut process has been observed to cause changes in EEG activity (Devine et al., 1986). During the transformation process from the state of consciousness to the state of unconsciousness, it was found that all treatment groups showed an increase in the Ptot, alpha, beta, theta and F50 values. These results show that noxious stimuli increased the EEG activities and were shown in other species as well, such as calves (Gibson et al., 2009), lambs (Johnson et al., 2009; Jongman et al., 2000), dogs (Kaka et al., 2016) and horses (Murrell et al., 2003). High alpha activity signifies the increase in the auditory and visual stimulations with the memory-related event, while the increase in theta activity signifies increased arousal and alertness (Basar et al., 2001). The increase in the EEG activities might be because of the movement of animal during the slaughter process (Gibson et al., 2009). It was observed that the delta waves and Ptot were lower in the TS2L6, TS6L6 and TS6L12 group after slaughter compared to after lairage, was due to stress. The delta waves in this study are related to the observations made by Ong et al. (1997) during the experiment on inducing pain in sheep by electrical stimulation.

Studies in human have found out that the occurrence of isoelectric EEG happens when the brain is dead (Bales and Kim, 2019). In slaughtered animals, the decreased cortical electrical activity during exsanguination leads to isoelectric (Gibson et al., 2009). It has been shown that goats from the TS6L6 group have the highest beta waves value at slaughter, which is caused by a painful stimulus experienced during slaughter. The pain response could be associated with the longer time taken for the animals to reach the unconscious state, which increases the chance of pain and distress to the animals (Sabow et al., 2016). It was previously observed that insensibility happened before the EEG activity becomes isoelectric. An earlier study has shown that EEG activity in pigs took almost 115 s to reach isoelectric even though pigs in the study have lost their insensibility within 25 s after slaughter (Blackmore and Petersen, 1981). Based on the findings, it can be presumed that the longer time of EEG activity to reach isoelectric of goats in this study means that the animals took a longer time to reach insensibility after slaughter, while the shorter time of EEG to reach isoelectric means animals becomes insensible and possibly death in a shorter time. The longest time of the EEG

activity to reach isoelectric in this study comes from animals in the TS6L6 group. It was observed in the earlier study that a calmer animal would lose its sensibility earlier than a stressed animal (Grandin, 2010). In another study, it was noticed that distressed cattle have a prolonged insensibility time after the slaughter (Gregory et al., 2010). Goats in the TS2 groups have low beta waves, which was parallel with the shorter time of the EEG to reach isoelectric. An increase in beta waves is generally associated with stress response in humans (Seo and Lee, 2010). In this study, no significant increases in beta waves were observed in all goats after transport, suggesting that either goat in this study did not experience transportation stress or were already fully adapted during the EEG recording.

Goats in most treatment groups except for the TS6L3 are in a relaxed state based on increased alpha activities during after lairage period. The increase in alpha oscillations is related to physical relaxation in human and animals (Freeman and Quiroga, 2013). A positive relation was observed between the cortisol concentration and alpha waves activities when animals were slaughtered in this study. This result matches previous findings that found the association between alpha activities and cortisol response in humans (Brouwer et al., 2011). The TS2L6 and TS6L3 groups, which had the highest cortisol concentration during slaughter, had the lowest delta activities. Cortisol, ACTH, and CRH can suppress and restrain the delta waves activities, thus increasing alertness during the stress period (Gronfier et al., 1998). The results obtained in this study confirm the association between EEG activity and cortisol concentrations as a pain indicator in animals (Bergamasco et al., 2011). These findings provide further evidence of EEG characteristics as a non-pain stress indicator and can be used in future stress studies involving animals.

5. Conclusion

The current findings have shown that transportation, regardless of its duration, imposed stress on goats in this study. Based on EEG reading and cortisol concentration analysis, goats were well adapted to the transportation stress at the end of the journey period. A six-hour transportation (TS6) duration provides ample time for goats to adapt to transportation stress, while two-hour transported goats (TS2) were still experiencing stress at the end of the journey. These findings proved that the most critical part of transport is during the initial stage of the journey. Muscle fatigue and muscle damage were seen in goats after transportation. Severe muscle damage was observed in TS2 group due to insufficient time for goats to adapt to the transportation stress. Optimum lairage duration plays a significant role in reducing the transportation stress impact on the animals. This study found that six hours lairage was needed to reduce the detrimental effect from transportation stress while more than six hours and up to overnight lairage was required for goats transported for six hours. In conclusion, the duration of lairage depended on the duration of transport to minimise the adverse effect to the animals in order to produce better quality meat. Lastly, the slaughter procedure without any intervention (such as stunning) will undoubtedly cause pain to animals. Therefore, the imposition of another potentially painful stimulus before slaughter must be justified and done by prevailing welfare standards harmonised with cultural and religious practices. However, minimising the stress level during the pre-slaughter period will facilitate the rapid attainment of insensibility, thus reducing the pain and distress level.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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