J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
49(3):215-223, September 2024
DOI: 10.14710/jitaa.49.3. 215-223

Electroencephalogram, creatine kinase, and lactate dehydrogenase responses during preslaughter handling in goats handled by trained and untrained handlers

P. Kumar[1,2], A. A. Abubakar[1], M. A. Adewale[1], M. N. Hayat[3], G. Adamu[1], M. Ajat[4], Y-M. Goh[4], U. Kaka[5,6]*and A. Q. Sazili[3,6]*

[1]Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2]Department of Livestock Products Technology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, Punjab, India
[3]Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[4]Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[5]Department of Companion Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[6]Halal Products Research Institute, Universiti Putra Malaysia, Putra Infoport, 43400 UPM Serdang, Malaysia
*****Corresponding E-mail: dr_ubedkaka@upm.edu.my; awis@upm.edu.my*

Received: January 10, 2024; Accepted: August 12, 2024

ABSTRACT

The present study evaluated the effect of training to livestock handlers on electroencephalogram (EEG) and plasma enzymes levels during preslaughter handling in goats. Six handlers were divided into three groups viz., trained (T-trained in basic animal handling practices, animal behavior, and animal welfare principles), contact trained (CT-not trained directly but interacted and saw the working of trained handlers), and untrained (UT-no formal training). Eighteen Boer cross bucks were used in the study by following a cross-over design and electroencephalogram spectrum, creatine kinase (CK), and lactate dehydrogenase (LDH) concentrations were recorded before handling (in lairage) and after handling (at slaughter point). The training of livestock handlers had a significant ($p<0.05$) effect on the total power (Ptot) and median frequency (MF) of the EEG spectrum. Whereas no significant ($p>0.05$) change was recorded in the alpha, beta, delta, theta, and gamma bands activity. The goats handled by trained handlers exhibited significantly ($p<0.05$) lower CK and LDH concentrations than goats handled by CT and UT handlers, thereby indicating higher preslaughter stress in goats handled by CT and UT groups. Therefore, the current study emphasizes the significance of providing training to livestock handlers in order to mitigate preslaughter stress levels in goats.

Keywords: Animal welfare, Electroencephalogram, Plasma enzymes, Preslaughter handling

INTRODUCTION

Human-animal interaction (HAI) plays an important role in animal production by affecting handling stress and animal productivity. Proper preslaughter handling of animals, especially between the lairage and slaughter point, was crucial for mitigating stress, animal welfare, and meat quality (Kumar *et al.*, 2023a). To train livestock handlers is very important for improving their handling skills, attitude, and behavior, thereby it can minimize the stress levels in the livestock (Grandin and Shivley, 2015). The high level of job stress, prolonged working hours, and unanticipated animal responses make this job very stressful, consequently leading to poor work quality and improper handling of animals (Coetzee and Klopper, 2010). In the present context of increasing awareness among the general public and consumers, in particular, towards the treatment met out to the animals during meat production, the issue of livestock handlers' training has become paramount for the meat industry (Mathur *et al.*, 2021). Improved attitude, skills, and behavior of livestock handlers with proper training have been well established by several studies (Ceballos *et al.*, 2018).

The muscle injury and inflammation due to preslaughter stress led to increase synthesis of creatine kinase (CK) and lactate dehydrogenase (LDH) enzymes in the liver. Thus, CK and LDH prove good indicators of muscle injury and tissue damage caused under various preslaughter operations such as intense physical activity, rough handling, and trauma (Fuente *et al.*, 2010). In addition, electroencephalography (EEG) spectrum by measuring the electrical activity of cerebrocortical neurons could provide a reliable, sensitive, and real-time status of the brain activity of animals (Kaka *et al.*, 2015) by measuring neural activity during the stress. A detailed analysis of the EEG variables (such as median frequency and total power), and wave patterns (frequencies and amplitude) provides insight into animal stress, pain, and welfare status (Kumar *et al.*, 2022).

There is a lack of studies on the animal handler attitude on the livestock, particularly in between lairage to slaughter point. Thus, the present study was designed to assess the effect of animal handler's attitudes on electroencephalogram (EEG) responses and serum enzymes (LDH and CK). Goats as ideal small ruminants, due to their productivity, availability, distribution throughout the globe, and economic importance, were used in the present study. The outcome of the present study would be helpful in convincing the people involved in the meat industry to follow proper animal handling practices.

MATERIALS AND METHODS

Ethical Approval

The present study was conducted following the animal ethics guidelines of the Research Policy of Universiti Putra Malaysia as per the Institutional Animals Care and Use Committee (Approval No.: UPM/IACUC/AUP-R003/2022 dated 27 May 2022).

Animals and Experiment Design

In the current study, a total of 18 Boer cross bucks aged between 8-12 months and live weights ranging from 24-28 kg were used. The bucks were housed at the small ruminant housing facility at the Institute of Tropical Agriculture and Food Security in Universiti Putra Malaysia (latitude $2^0$59'06.5" N and longitude $101^0$43'40.7" E) for 2 weeks for acclimatization with standard managemental care. During their stay, animals were dewormed before starting the treatment and during the study period, goats were fed twice daily (morning- chopped fresh Napier grass and evening pellets ration), and provided *ad libitum* water. Before commencing the experiment, the bucks were transported to the research slaughterhouse (distance: approx. 2.0 km) of the Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia (258059.000" N; 10144006.400" E). The animals were rested overnight in the lairage with *ad libitum* water.

This study was completed from July to August 2022 in a cross-over design with a one-week resting period. Eighteen bucks were assigned into three groups (n=6) as trained group, contact-trained, and untrained group.

In the present study, six livestock handlers (25-28 years old, education level - matriculation to graduate involved in routine livestock farm operations) were selected. The livestock handlers were divided into three groups viz., trained group (T), untrained group (UT), and contact trained

(CT). The trained handlers were trained for 1 week in animal behavioral principles such as gentle handling, animal flight zone, point of balance, normal movement, and stress responses. The untrained (UT) handlers have not undergone training for these principles. The contact-trained (CT) handlers were not directly trained but interacted with trained handlers and observed the working of trained handlers for a period of one week. After the training session, the attitude and skill of trainees were assessed through interview or oral test and practical examination. The trainees who scored good to excellent grades were categorized as trained handlers. Further, prior to the start of the experiment, the trained handlers were briefed about various handling practices.

Goats were moved from the lairage to the slaughter point by animal handlers covering the same distance and same floor conditions. The welfare status of goats after being handled by trained staff (T), contact trained (CT), and untrained staff (UT) were assessed by recording encephalogram variables (Sabow *et al.*, 2016), CK and LDH concentrations before handling (control value in lairage) and after handling (at slaughter point). The preslaughter handling of goats was recorded by carefully observing the human-animal interactions by tactile (handling of goats by ear, legs, tail, hitting, auditory (whistling, shouting, and banging of pen fittings), and visuals means (waving of hands or objects in front of goats, blocking, and hand raising).

Electroencephalogram Recording

The electrical activity of cerebrocortical neurons was measured in the form of EEG recording at the lairage before handling (Control) and at the slaughter point after handling using a Powerlab 4/20 data recording system (Powerlab data acquisition system, ADInstruments Ltd. Sydney, Australia) with the help of Chart 5.0 (PowerlabTM data acquisition system, Sydney, Australia). The EEG was recorded by placing two conductive electrode patches attached to the zygomatic process of the frontal bone and the mastoid areas of the goat. Two 4-6 cm skin areas in between the mastoid process and medial canthi of eyes (for non-inverting/positive electrode patch) and on the right side at the zygomatic process of the frontal bone approx. 1.5 cm below eye level (for inverting/ negative electrode) were finely shaved, cleaned, and degreased with 70% ethyl alcohol for close and smooth attachment of conductive adhesive disposable electrodes hydrogel (Covidien LLC, Mansfield, MA, USA) and to improve the quality of electrical signals (Kumar *et al.*, 2023b). The EEG was recorded immediately within 30 s upon the placement of the electrodes for 3-4 min. The animals were gently handled minimally by the experienced technical staff during EEG recording.

EEG recordings were analysed offline using LabChart 5.0 software (ADInstruments Ltd. Sydney, Australia). The individual power spectrum of alpha, beta, delta, theta, and gamma waves/ band oscillation was interpreted using fast Fourier transformation analysis. The amplitude and frequency of the EEG signals were sampled at a sampling rate of 1 kHz. In addition to minimizing artifacts, the raw EEG was resampled below 200 Hz (Sabow *et al.*, 2019). The EEG recordings were cleaned, deleting artifacts from the spectrum caused due to physical and physiological movements and other disturbances. Total power (Ptot, the total area under the curve), root mean square (RMS) of alpha, beta, delta, theta and gamma waves, and median Frequency (F50, frequency below which 50% of Ptot lies) were calculated repeatedly for non-overlapping of one -second epochs, yielding 60 epochs per minute (Murrell and Johnson, 2006). A 60-second block EEG data were collected before and after treatments. Further, each block was calculated for consecutive non-overlapping 1-s epochs, and for each animal, 10 values were sampled to arrive at a mean value.

Blood Collection

During the present study, blood samples were collected by the trained technical staff from the jugular vein of goats two times, viz., before handling in lairage (Control) and at the slaughter point after handling by T, CT, and UT groups. A 21-gauge needle connected to a 10 mL clot activator (BD Vacutainer®, Plymouth, UK) ethylenediaminetetraacetic acid tube was used to collect blood samples. These tubes with blood were held in a box containing crushed ice for 1 h. The tubes were centrifuged (Eppendorf Centrifuge 5810) at 3500 g for 15 min at 4°C. The supernatant (plasma) was separated and kept in a

deep freezer (Sanyo Electric Co Ltd, UK) at -80° C, for further LDH and CK analysis.

Creatine Kinase (CK) and Lactate Dehydrogenase (LDH) Analysis

Plasma concentrations of LDH and CK were assessed using LDH kit (QuantiChrome™ Lactate Dehydrogenase Kit, Bioassay System, D2DH-100, CA, USA), and CK kit (EnzyChrom™ creatine kinase assay kit ECPK-100, Bioassay System, CA, USA) following the manufacturer's instructions.

Statistical Analysis

The normality of the data was assessed using Shapiro–Wilk test using SPSS Statistics Version 27 software (IBM Corporation, New York, USA). The changes in the mean values of electroencephalogram spectrum variables and blood enzymes in goats in lairage before handling and at slaughter point after handling were compared with Duncan's multiple-range test through a One -way Analysis of Variance (ANOVA, n=18). A p -value of less than 0.05 was deemed statistically significant in the present study.

RESULTS AND DISCUSSION

EEG Variables

In the present study, the activity of alpha, beta, delta, and gamma waves increased non-significantly ($p>0.05$) and theta waves decreased non-significantly ($p>0.05$) after preslaughter handling (Table 1).

Alpha Wave

The alpha wave activity of goats recorded a non-significant ($p>0.05$) increase after handling as compared to control values measured in the lairage before moving to the slaughter point. The alpha wave activity was recorded as the highest in the CT group. The control value of the alpha wave was recorded as the lowest among all groups. The handling of animals and moving to a different place could attributed to the increase in the alpha wave activity. In previous studies, the increase in the alpha band oscillation was recorded in the lairage as compared to their baseline value at the farm prior to the start of the journey in goats (Othman *et al.*, 2021; Raghazli *et al.*, 2021). Similarly, an increased alpha wave activity was noticed after electrical stunning in lambs and goats by Llonch *et al.* (2015).

Beta Wave

The beta wave activity in goats after handling was reported to be non-significantly higher ($p>0.05$) as compared to control. The beta waves were recorded as the highest for the T group and followed the following trend T> CT > UT > Control**.** In humans, an increased beta wave activity correlates to stressful conditions (Seo and Lee, 2010). An increase in beta wave activity in goats during preslaughter handling was observed under stressful conditions such as transportation (Othman *et al.*, 2021; Raghazli *et al.*, 2021). A non-significant increase in the beta waves was reported by Raghazli *et al.* (2021) after 2 h transportation in goats, whereas increasing the transportation duration decreased the beta waves due to stress adaptation. Increased beta wave activity of the EEG spectrum was observed with increased brain activity in panic conditions and fear perceptions (Wigham *et al.*, 2018). Similarly, a significant increase in the beta wave was observed in goats due to exposure to slaughter ambient (Kumar *et al.*, 2023c).

Delta Wave

The delta wave (1- 4 Hz) was observed to follow an increasing trend with the training of the livestock handler. The control values for delta waves were the lowest, and the UT had the highest ($p>0.05$) delta wave intensity. The lower delta wave activity could be due to the improvement in the attitude of the trained handlers, leading to positive human-animal interactions causing lower stress responses in the T goats. The delta waves are associated with the brain's default mode network (Siegel *et al.*, 2000), associated with past experiences and imagining upcoming events/ consequences (Buckner, 2013). A higher delta wave activity was observed in goats during slaughtering as compared to their respective delta waves at farms was also reported by Raghazli *et al.* (2021).

Theta Wave

There were no statistically significant differences ($p>0.05$) observed among the theta wave activity of various groups in the present study. The highest theta waves were recorded in the Control, followed by the T group. The lowest

theta waves were recorded for the UT group. Further, a non-significantly higher ($p>0.05$) gamma wave activity (30-80 Hz) was observed in the Control group as compared to the treatment groups (T, CT, and UT). In the present study, higher theta waves during the control stage prior to handling indicate that goats were relaxed and alert. In humans, the presence of higher theta waves (4-7 Hz) correlates with creativity, deep relaxation, intuition, daydreaming, memory, and dominance during drowsiness and early stages of sleep. However, dominant theta waves indicate arousal and alertness (Bell, 2018). With the handling of these animals, the theta waves were decreased, and the UT group recorded a higher suppression of these waves. An improved human-animal interaction (HAI) in the T and CT group in the present study was observed to have an effect on the theta waves in the goats. In livestock, theta waves activity was also recorded as statistically non-significant ($p>0.05$) under transport stress by Raghazli *et al.* (2021) however, these values increased significantly during slaughtering stress.

Gamma Wave

The gamma wave is a high-frequency wave correlated with information processing, cognitive learning, and memory (Abhang *et al.*, 2016). Gamma waves are characterized by rapid rhythm, thus causing excitation in the network without inhibition. This helps in effective and precise communication between multiple regions (Tiesinga *et al.*, 2004). In humans, a dominant gamma indicates anxiety, stress, and high arousal, whereas depression correlates with depression and learning disability (Abhang *et al.*, 2016). Gamma waves could be a potential biomarker of depressive state and therapeutic response in humans (Fitzgerald and Watson, 2018). A behavioral disruption was also observed to increase the gamma wave by Leung and MA (2016). In addition, gamma wave in optimal conditions facilitates focus, attention to senses (optic, olfactory, and auditory), mental condition, consciousness, and perceptions (Abhang *et al.*, 2016). Thus a higher gamma waves in the treatment groups, as compared to the Control, could be correlated with the preslaughter handling stress in goats.

Total Power and Median Frequency

The total power (Ptot) and median frequency (MF) of the EEG spectrum increased upon the handling of goats (Table 2). The highest Ptot value was recorded in the goats handled by untrained handlers (UT group), followed by goats handled by contact-trained handlers (CT group) and trained handlers (T group). The Ptot of the UT group was significantly ($p<0.05$) higher than those of the control group. No significant difference was observed in the median frequency (MF or F50) of the EEG spectrum for the control and T groups, whereas, MF increased significantly ($p<0.05$) in CT and UT groups compared to in the control. Overall, the MF value of CT and UT groups was significantly ($p<0.05$) higher (with approx. 3-fold increase) as compared to those of the Control and T groups.

The total power of the EEG spectrum is correlated with the stress levels with a higher Ptot in stressful conditions and a lower Ptot in the relaxed phase (Kumar *et al.*, 2022; Tilbrook and Ralph, 2018). An increased MF of the EEG spectrum was reported to be associated with increased reactivity to sensory stimuli (Murrell and Johnson, 2006). Raghazli *et al.* (2021) observed an increase in the MF of the EEG spectrum was

Table 1. Effect of Training of Livestock Handlers on Alpha, Beta, Delta, Theta, and Gamma Waves Activity of Electroencephalogram Spectrum During Preslaughter Handling in Goats

Group	Control	T	CT	UT
Alpha (μV)	0.888±0.047	0.970±0.066	1.021±0.082	0.960±0.055
Beta(μV)	1.285±0.064	1.881±0.342	1.782±0.352	1.471±0.094
Delta(μV)	7.850±0.985	8.058±0.987	9.158±0.813	11.105±2.166
Theta (μV)	1.969±0.163	1.765±0.182	1.741±0.235	1.658±0.116
Gamma(μV)	2.625±0.183	3.179±0.224	2.831±0.232	2.988±0.235

Values are means ± standard error with no superscript within a row-wise did not differ significantly ($p>0.05$; $n=18$). Control- values recorded at lairage before handling, T- goats handled by trained handlers, CT-goat handled by handler not trained but interacted and saw the handling of trained handlers, UT- goats handled by the untrained handler.

Table 2. Effect of Training of Livestock Handlers on Total Power (Ptot) and Median Frequency (MF) Variables of Electroencephalogram Spectrum During Preslaughter Handling in Goats

Group	Control	T	CT	UT
Ptot (V^2/Hz)	12.615±0.712^{a}	14.691±1.160^{ab}	16.507±1.528^{ab}	18.059±2.313^{b}
MF (Hz)	6.838±0.571^{a}	8.320±0.660^{a}	35.170±3.038^{b}	32.478±2.528^{b}

Values are mean ± standard error with different superscripts within a row-wise differ significantly ($p<0.05$), Control- values recorded at lairage before handling, T- goats handled by trained handlers, CT-goat handled by handler not trained but interacted and saw the handling of trained handlers, UT- goats handled by the untrained handler n=18, level of significance $p<0.05$

correlated with pain and stressful conditions. An increased MF value was recorded due to stress caused by transportation and stocking density in cattle by Abubakar *et al.* (2021). Similar to the present findings, Kumar *et al.* (2023b) reported significantly higher Ptot and MF values of the EEG spectrum were observed in goats under exposure to the slaughter ambient. In the present study, the higher Ptot and MF values in the goats in T groups as compared to the Control could be attributed to the novelty of the environment and the visual and physical separation of animals away from the herd. This could cause fear and anxiety in gregarious animals such as goats handled by trained handlers (Forkman *et al.*, 2007).

Creatine Kinase and Lactate Dehydrogenase

Animal handler attitude has a significant effect on the blood plasma creatine kinase (CK) concentration (Figure 1). The plasma CK concentrations of the C and T groups were comparable, with the T group exhibiting a non-significantly higher ($p>0.05$) than the C group. The UT group had the highest CK concentration, and the C group had the lowest CK concentration. The CT group had significantly ($p<0.05$) lower CK concentration than the UT group. Plasma LDH concentration followed a similar pattern to plasma

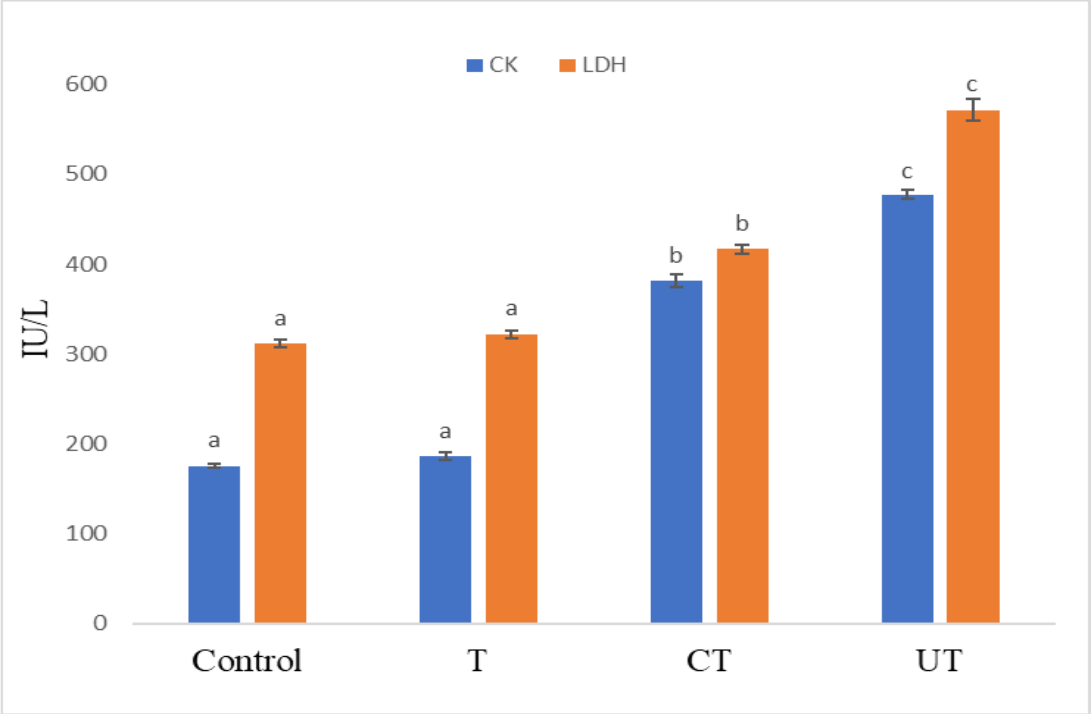

Figure 1. Effect of training of livestock handlers on CK and LDH concentrations during preslaughter handling in goats. Different superscripts (a, b, and c) within an enzyme concentration differ significantly ($p<0.05$); CK-creatine kinase, LDH-lactate dehydrogenase, Control- values recorded at lairage before handling, T- goats handled by trained handlers, CT-goat handled by handler not trained but interacted and saw the handling of trained handlers, UT- goats handled by the untrained handler, IU-international unit, n=18

CK concentration, with the UT group exhibiting the highest LDH concentration (UT>CT>T>C) (Figure 1). The LDH concentration in the control group recorded at the slaughter point after handling was observed to be lower than those in the trained group.

The higher CK and LDH concentrations in the UT group could be associated with poor attitude and improper handling of goats by untrained handlers. The marginal increase in plasma CK and LDH concentrations of the T group over the Control group could be due to the improved attitude and gentle handling by the trained handlers. All the animals used in the present study were male and, due to their aggressive nature, had higher intensity of stress reactivity and muscle damage, thereby leading to higher CK concentration. Similar findings of higher CK in boars as compared to gilts were also reported by Merlot *et al.* (2011). A significant increase in the CK concentration was recorded in goats in lairage after transportation stress (Othman *et al.*, 2021) and after being slaughtered (Sabow *et al.*, 2016, 2019). The plasma LDH concentration of goats was also reported to have a significant ($p<0.05$) increase due to slaughter stress in goats (Sabow *et al.*, 2016, 2019). Various preslaughter operations, such as improper handling, cause stress, and muscle injury in animals, which lead to an increase in the CK and LDH content in the blood (Ekiz *et al.*, 2012; Fuente *et al.*, 2010).

CONCLUSION

The present study highlighted the importance of training to the livestock handlers in mitigating stress during preslaughter handling. The goats handled by trained handlers with improved attitudes and following positive human-animal interactions exhibited a lower electroencephalogram response and lower plasma concentration of creatine kinase and lactate dehydrogenase, thereby indicating lower preslaughter stress and improved animal welfare status.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Geran Putra-IPS (Vote No.: 9737800) funded by the Universiti Putra Malaysia (UPM) for Project No.: GP-IPS/2022/9737800 as the financial grant for the research work.

The first author, Pavan Kumar, is thankful to the Indian Council of Agricultural Research, New Delhi, India, for providing the Netaji Subhas ICAR International Fellowship for pursuing his doctoral study at Universiti Putra Malaysia.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abhang, P.A., B.W. Gawali and S.C. Mehrotra. 2016. Technical aspects of brain rhythms and speech parameters. In: Introduction to EEG- and Speech-Based Emotion Recognition (P.A. Abhang, B.W. Gawali, and S.C. Mehrotra, eds.). Academic Press. San Diego. P. 51-79. https://doi.org/10.1016/B978-0-12-804490-2.00003-8

Abubakar, A.A., I. Zulkifli, Y.M. Goh, U. Kaka, A.B. Sabow, E.A. Awad, J.C. Imlan, A.H. Othman, R. Raghazli, H. Mitin and A.Q. Sazili. 2021. The effects of stocking density and distances on electroencephalographic changes and cortisol as welfare indicators in Brahman crossbred cattle. Animals. 11(10): 2895. https://doi.org/10.3390/ani11102895

Bell, A. 2018. The neurobiology of acute pain. Vet. J. 237: 55–62. https://doi.org/10.1016/j.tvjl.2018.05.004

Buckner, R.L. 2013. The brain's default network: origins and implications for the study of psychosis. Dialogues Clin. Neurosci. 15(3): 351–358. https://doi.org/10.31887/DCNS.2013.15.3/rbuckner

Ceballos, M.C., A.C. Santanna, X. Boivin, F. De Oliveira Costa, M.V.De L. Carvalhal and M.J.R.P. Da Costa. 2018. Impact of good practices of handling training on beef cattle welfare and stockpeople attitudes and behaviors. Livest. Sci. 216: 24–31. https://doi.org/10.1016/j.livsci.2018.06.019

Coetzee, S.K. and H.C. Klopper. 2010. Compassion fatigue within nursing practice: A concept analysis. Nurs. Health Sci. 12(2): 235–243. https://doi.org/10.1111/j.1442-2018.2010.00526.x

Ekiz, B., E.E. Ekiz, O. Kocak, H. Yalcintan and A. Yilmaz. 2012. Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters,

carcass and meat quality characteristics in Kivircik lambs. Meat Sci. 90(4): 967–976. https://doi.org/10.1016/J.MEATSCI.2011.11.042

Fitzgerald, P. J. and B.O. Watson. 2018. Gamma oscillations as a biomarker for major depression: An emerging topic. Transl. Psychiatry. 8(1): 177. https://doi.org/10.1038/s41398-018-0239-y

Forkman, B., A. Boissy, M.-C. Meunier-Salaün, E. Canali and R.B. Jones. 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav. 92(3): 340–374. https://doi.org/10.1016/j.physbeh.2007.03.016

Fuente, J. D. La, Snchez, M., C. Prez, S. Lauzurica, C. Vieira, E.G. De, Chvarri and M.T. Daz. 2010. Physiological response and carcass and meat quality of suckling lambs in relation to transport time and stocking density during transport by road. Animal. 4(2): 250–258. https://doi.org/10.1017/S1751731109991108

Grandin, T. and C. Shivley. 2015. How farm animals react and perceive stressful situations such as handling, restraint, and transport. Animals. 5(4): 1233–1251. https://doi.org/10.3390/ani5040409

Kaka, U., C.H. Cheng, G.Y. Meng, S. Fakurazi, A. Kaka, A.A. Behan and M. Ebrahimi. 2015. Electroencephalographic changes associated with antinociceptive actions of lidocaine, ketamine, meloxicam, and morphine administration in minimally anaesthetized dogs. BioMed Res. Int. 2015: 1–10. https://doi.org/10.1155/2015/305367

Kumar, P., A.A. Abubakar, M.A. Ahmed, M.N. Hayat, U. Kaka, M. Pateiro, A.Q. Sazili, L.C. Hoffman and J.M. Lorenzo (2023a): Pre-slaughter stress mitigation in goats: Prospects and challenges. Meat Sci. 195: 109010. https://doi.org/10.1016/j.meatsci.2022.109010

Kumar, P., A.A. Abubakar, M.A. Ahmed, M.N. Hayat, M. Ajat, U. Kaka, Y.M. Goh and A.Q. Sazili. 2023b. Electroencephalogram and physiological responses as affected by slaughter empathy in goats. Animals. 13(6): 1100. https://doi.org/10.3390/ani13061100

Kumar, P., M.A. Ahmed, A.A. Abubakar, M.N. Hayat, U. Kaka, M. Ajat, Y.M. Goh and A.Q. Sazili. 2023c. Improving animal welfare status and meat quality through assessment of stress biomarkers: a critical review. Meat Sci. 197: 109048. https://doi.org/10.1016/j.meatsci.2022.109048

Kumar, P., A.A. Abubakar, A.Q. Sazili, U. Kaka and Y.-M. Goh. 2022. Application of electroencephalography in preslaughter management: A review. Animals. 12(20): 2857. https://doi.org/10.3390/ani12202857

Leung, L.S. and J. MA. 2016. Ketamine mediates psychosis through the medial septum, hippocampus, and nucleus accumbens. In: Neuropathology of Drug Addictions and Substance Misuse (V.R. Preedy, ed). Academic Press. San Diego. P. 661–671. https://doi.org/10.1016/B978-0-12-800212-4.00061-3

Llonch, P., P. Rodríguez, N. Casal, R. Carreras, I. Muñoz, A. Dalmau and A. Velarde. 2015. Electrical stunning effectiveness with current levels lower than 1 A in lambs and kid goats. Res. Vet. Sci. 98: 154–161. https://doi.org/10.1016/j.rvsc.2014.12.009

Mathur, M.B., J. Peacock, D.B. Reichling, J. Nadler, P.A. Bain, C.D. Gardner and T.N. Robinson. 2021. Interventions to reduce meat consumption by appealing to animal welfare: Meta-analysis and evidence-based recommendations. Appetite. 164: 105277. https://doi.org/10.1016/J.APPET.2021.105277

Merlot, E., A.M. Mounier and A. Prunier. 2011. Endocrine response of gilts to various common stressors: A comparison of indicators and methods of analysis. Physiol. Behav. 102(3–4): 259–265. https://doi.org/10.1016/J.PHYSBEH.2010.11.009

Murrell, J.C. and C.B. Johnson. 2006. Neurophysiological techniques to assess pain in animals. J. Vet. Pharmacol. Ther. 29(5): 325–335. https://doi.org/10.1111/j.1365-2885.2006.00758.x

Othman, A., Y.M. Goh, N. Mohamed Mustapha, R. Raghazli, U. Kaka, J.C. Imlan, A.A. Abubakar and R. Abdullah. 2021. Physiological and electroencephalographic changes in goats subjected to transportation, lairage, and slaughter. Anim. Sci. J. 92(1). https://doi.org/10.1111/asj.13610

Raghazli, R., A.H. Othman, U. Kaka, A.A. Abu-

bakar, J.C. Imlan, H. Hamzah, A.Q. Sazili and Y.M. Goh. 2021. Physiological and electroencephalogram responses in goats subjected to pre-and during slaughter stress. Saudi J. Biol. Sci. 28(11): 6396–6407. https://doi.org/10.1016/J.SJBS.2021.07.013

Sabow, A.B., Y.M. Goh, I. Zulkifli, M.Z.A. Kadir, U. Kaka, K.D. Adeyemi, A.A. Abubakar, J.C. Imlan, M. Ebrahimi and A.Q. Sazili. 2019. Electroencephalographic and blood parameters changes in anaesthetised goats subjected to slaughter without stunning and slaughter following different electrical stunning methods. Anim. Prod. Sci. 59 (5): 849–860. https://doi.org/10.1071/AN17486

Sabow, A.B., Y.M. Goh, I. Zulkifli, A.Q. Sazili, U. Kaka, M.Z.A.A. Kadi, M. Ebrahimi, K. Nakyinsige and K.D. Adeyemi. 2016. Blood parameters and electroencephalographic responses of goats to slaughter without stunning. Meat Sci. 121: 148–155. https://doi.org/10.1016/j.meatsci.2016.05.009

Seo, S.-H. and J.-T. Lee. 2010. Stress and EEG. In: Convergence and Hybrid Information Technologies (M. Crisan, ed)). InTechOpen, Rijeka, Croatia. P. 413-426. https://doi.org/10.5772/9651

Siegel, P.B. and W.B. Gross. 2000. General principles of stress and well-being. Livestock Handling and Transport. (T. Grandin, ed). CABI Books. Oxford, UK. P. 27-42. https://doi.org/10.1079/9781845932190.0019

Tiesinga, P. H., J.-M. Fellous, E. Salinas, J.V. José and T.J. Sejnowski. 2004. Inhibitory synchrony as a mechanism for attentional gain modulation. J. Physiol.-Paris. 98(4–6): 296–314. https://doi.org/10.1016/j.jphysparis.2005.09.002

Tilbrook, A. J. and C. R. Ralph. 2018. Hormones, stress and the welfare of animals. Anim. Prod. Sci. 58(3): 408. https://doi.org/10.1071/AN16808

Wigham, E. E., A. Butterworth and S. Wotton. 2018. Assessing cattle welfare at slaughter – Why is it important and what challenges are faced? Meat Sci. 145: 171–177. https://doi.org/10.1016/j.meatsci.2018.06.010