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Effects of Stunning and Thoracic Sticking on Welfare and Meat Quality of Halal-Slaughtered Beef Cattle

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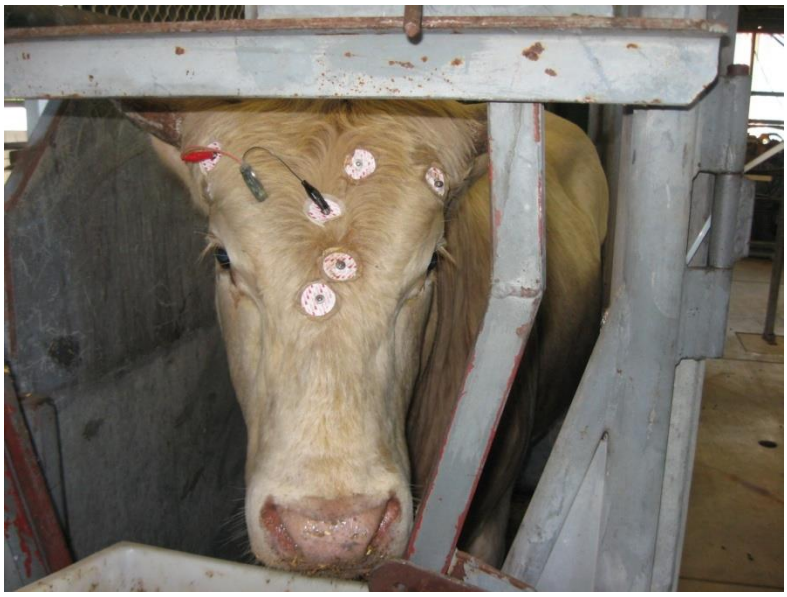
RESEARCH REPORT

EFFECTS OF STUNNING AND THORACIC STICKING ON WELFARE AND MEAT QUALITY OF HALAL-SLAUGHTERED BEEF CATTLE



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EXECUTIVE SUMMARY

It is a requirement of meat production that animals processed are handled and slaughtered in a humane manner. Therefore, most animals are stunned prior to exsanguination, so that they are insensible throughout the process of sticking, and remain insensible until death has ensued. However, animals processed for the Muslim market (Halal meat production) are subject to the requirements of the relevant religious texts (the Al Qu'ran and Hadith), and there is often a conflict in opinion over the suitability of stunning procedures when slaughtering an animal for human consumption. The basic requirements of the religious texts are that the animal is healthy and handled in such a way that it is not injured at the time of carrying out the ritual slaughter cut for exsanguination. Thus, mechanical means of stunning, that cause skull damage, or those that result in the death of the animal, are not approved for the religious markets. Any method used to induce insensibility must not cause injury, and must be fully recoverable. In Australia, all cattle processed for the Halal market are stunned prior to slaughter, using a non-penetrating mechanical stun, referred to as a percussive, or non-penetrative stun. The requirements of the Malaysian Halal market demand that this percussive stun does not result in damage to the skull and low power compressed air systems are therefore used in commercial plants to deliver the stun. There are queries over the efficacy of low power percussive stunning in induction of insensibility, such that some countries have outlawed its use. If the animal is not properly stunned, there is the possibility that it will experience pain and suffering at the time of the neck cut.

Researchers at the Universiti Putra Malaysia approached CSIRO and MLA with a proposal to compare different methods of slaughter in terms of physiological stress, Electroencephalogram (EEG) changes and meat quality. Most of the published work on slaughter using different stunning and sticking methods focuses on individual aspects of animal welfare or meat quality. This study aims to provide a comparative analysis of the effects of penetrative stunning, non-penetrative stunning, post-slaughter stunning and sticking by the 'thoracic' method versus non-thoracic sticking on physiological and biochemical parameters associated with stress in animals, and on meat quality.

CSIRO hosted a visiting researcher from the Universiti Putra Malaysia for a period of 6 months in 2009 in order to facilitate sample collection. The study was carried out in a commercial abattoir. Ten animals were assigned to each treatment group (Unstunned, neck stick only; Penetrative stun with neck stick; Low Power Percussive Stun with neck stick; High Power Percussive Stun with neck stick; Penetrative stun with neck stick followed by thoracic stick 2 minutes later; Low Power Percussive stun with neck stick followed by thoracic stick 2 minutes later). For each animal, blood samples were taken before stun/slaughter; after stun (if applicable); after neck stick; after thoracic stick (if applicable), and tested for a range of plasma metabolites involved in the stress response; EEG traces were taken by telemetric means throughout the slaughter process and for up to 4 minutes after slaughter; and samples of *longissimus dorsi* and *semitendinosus* muscles were taken from the hot carcase, 45 minutes after slaughter, for assessment of meat quality attributes over 2 weeks of storage. The results were analysed by researchers at the Universiti Putra Malaysia.

The results of this study did not indicate significant differences between the slaughter methods in terms of physiological stress or meat quality. The study was carried out at a commercial abattoir and the animals had undergone the normal handling procedures in the lairage. As such, the meat quality and plasma metabolite measurements were confounded by the fact that the animals were already in an 'excited' or 'stressed' state, and therefore the baseline (pre-slaughter) measurements were often already elevated and a further response was not elicited. However, EEG measurements indicated that animals in the unstunned slaughter group were likely to have experienced conscious pain up to 30 seconds post slaughter. They also indicated that Penetrative stunning would maximise the possibility of insensibility (i.e. most likely to be effective). Thoracic sticking carried out two minutes after the neck cut did not impart any further benefit on any of the measurements taken. Queries over the efficacy of Low Power Percussive stunning and the impact of ineffective stuns on meat quality, EEG and blood parameters were not addressed in this study – all stunning procedures used resulted in an effective stun.

The outcomes from this study are to be used by the researchers in Malaysia to produce industry guidelines and documentation suitable for submission to the regulatory and religious authorities on the potential use of other stunning methods for the Halal market. Repeating the

work under more controlled conditions rather than in a commercial establishment may allow better elicitation of differences between the slaughter methods, as efforts can be made to ensure that the animals are less likely to be excited or stressed at the time of baseline sampling. Furthermore, to address the query over efficacy of low power percussive stunning and the impact of ineffective stuns on meat quality, samples and data should be gathered from animals in a situation where the stuns are not effective.

1.0 RESEARCH BACKGROUND

The manner in which livestock are immobilized, slaughtered, and exsanguinated can affect their welfare and final meat quality. From an animal welfare point of view, the practice of slaughtering animals without prior stunning is unacceptable. According to Gregory and Shaw (2000), when stunning is done correctly, the animal feels no pain and become instantly unconscious. The Malaysian Department of Veterinary Services, JAKIM and the National Fatwa Council have approved the application of pre-slaughter electrical stunning (24 November 1988). Stunning procedures include the use of electrical stunning, penetrating and non-penetrating (percussive) captive bolts. One of the adverse effects of electrical stunning in cattle is that it may cause ecchymoses or petechial haemorrhage. JAKIM accepts non-penetrating percussive stunning of cattle but not penetrating captive bolt stunning. The basis for this is that percussive stunning is recognised to be "reversible" while penetrating stunning is considered "non-reversible". Nevertheless, head injuries caused by percussive stunning can be severe. The impact of the heavy knocking head against the relatively thin frontal bone, which forms the roof of the cranium in cattle, can result in severe, well-circumscribed, depressed fracture of the skull with subarachnoid haemorrhage in the sub-adjacent brain. In some countries, the non-penetrative percussive stunning is disallowed because of animal welfare reasons. The prevalence of error in performing the non-penetrative percussive stunning is a major welfare concern. The magnitude of suffering or physiological stress reactions under commercial conditions as a result of the non-penetrative or penetrative percussive stunning itself has yet to be satisfactorily ascertained.

Earlier studies suggested that cutting the throat fails to sever the vertebral arteries supplying the brain. This may prolong duration of sensibility following slaughter (Blackman et al., 1987). On the contrary, Anil et al. (1995) suggested that the blood carried by the vertebral anastomosis would not be sufficient to maintain sensibility. Interruption of the vertebral arteries in cattle may be achieved by severance of the brachiocephalic trunk close to the heart by the use of a `thoracic stick', an incision with a knife through the thoracic inlet. Thoracic sticking which severs the brachiocephalic trunk near the heart is reported to have resulted in greater rate of

blood loss than that following bilateral neck severing (Leigh and Delaney, 1987). The thoracic sticking intervention has been widely practiced by most developed countries and commonly associated with enhanced bleeding and quickened death process. Most research on effects of thoracic sticking has focused on arterial blood flow following the procedure. Little information is available on the neuroendocrinological responses and meat quality of cattle subjected to thoracic sticking. Recently (24 Nov. 2005) Department of Veterinary Services, JAKIM and the National Fatwa Council (NFC) have approved post slaughter thoracic sticking (24 November 2005) procedures as practiced by major meat exporters like Australia and New Zealand. Hence, further studies are needed to provide insights into the physiological stress responses, welfare and meat quality of cattle following stunning, slaughter and sticking.

2.0 GENERAL OBJECTIVE

The objective of the proposed studies was to evaluate the effects of stunning, slaughter and thoracic stick on changes in blood parameters, electroencephalographic activity and meat quality in beef cattle.

3.0 MATERIALS AND METHODS

3.1 Animal data

The work was conducted during the period of July and August 2009 in Queensland (outdoor temperature: 15-20^{o}C). The cattle processed were heifers and steers, of live weights between 268 and 635 kg (mean 446 kg), resulting in hot carcase weights of 138 to 326 kg (mean 233 kg). They had been sourced from one of two feedlots, either 50 km or 160 km distant from the abattoir, and had been lairaged in feedlot pens at the abattoir for up to one week prior to slaughter (Table 1). They were mixed breed animals, about 25-50% Brahman crosses, and were representative of the normal class of animal slaughtered at this abattoir for the Halal market.

The animals were handled using the emergency slaughter area at the abattoir for two main reasons:

- It was not feasible to collect blood samples from animals in the stun-box used regularly by the abattoir, as it is a fully enclosed box with no personnel access. An attempt was made on two animals to collect blood samples prior to slaughter in the regular stun-box, but the exercise was considered to be unsafe for the operator collecting blood, and also highly stressful for the animal concerned.
- The regular stun-box, being fully enclosed, does not allow access to the neck in order to carry out unstunned slaughter. Therefore, the unstunned group would have to be processed using the emergency stun facility. It was considered better overall, in terms of operator safety, animal welfare and study design, to handle all groups through the same system.

Table 1: Distances travelled and times in lairage for cattle processed during this project.

Date of slaughter	Date of transport	Time spent in lairage	Distance travelled	Duration of transport	Ambient temperature during transport and lairage period (°C)
6-May-09	4-May-09	2 days	50km	40 mins	13.2 to 24.3
22-Jul-09	20-Jul-09	2 days	50km	40 mins	5.3 to 23.5
28-Jul-09	27-Jul-09	1 day	160km	2 hours	7.7 to 25.3
30-Jul-09	28-Jul-09	2 days	50km	40 mins	2.8 to 22.9
4-Aug-09	29-Jul-09	6 days	160km	2 hours	5.2 to 22.1
5-Aug-09	29-Jul-09	7 days	160km	2 hours	5.2 to 22.1

The lairage design was such that animals taken from the holding pen could either be placed in the crowd pen, and then enter the single file race to the regular stun box, or be placed into a crowd pen leading to a short race into the emergency slaughter facility. Thus the degree of handling experienced by the trial animals would not be greatly different from that of animals slaughtered under normal conditions.

3.2 Statistical analysis

All data were analysed using the general linear models procedure of SAS® software, and multiple means were separated by Duncan's multiple range test (SAS® institute Inc., 1991). Results were considered statistically significance at $P \leq 0.05$.

3.3 Experiment 1

Experiment 1 was conducted to examine the effects of various pre-slaughter stunning methods (low power non-penetrative stunning, high power non-penetrative stunning and penetrative percussive stunning) on blood parameters, electroencephalogram activity and meat quality in beef cattle. The experiment involved a total of 40 steers. Equal numbers of animals were randomly assigned to one of the four regimens:

- Animals were subjected to traditional Halal slaughter without prior stunning (a clean incision through the structures at the front of the neck – the trachea, esophagus, carotid arteries and jugular veins) and post-cut stun within 10-20 seconds of the Halal cut (after the post-sticking blood sample had been taken) to satisfy the requirements of the Animal Ethics Approval obtained (CSIRO A7/08) (**US**)
- High power percussive stunning using a non-penetrating humane killer (Cash magnum Knocker Concussion Stunner, 0.25 Calibre, 4 grain cartridge) followed by sticking using transverse section of the neck as described above (**HPP**)
- Low power percussive stunning using a non-penetrating humane killer (Cash magnum Knocker Concussion Stunner, 0.25 Calibre, 3 grain cartridge) followed by sticking using transverse section of the neck (as per the AQIS Malaysian Protocol) (**LPP**)
- Penetrative stunning using a captive bolt pistol humane killer (Cash 8000 Model Stunner, 0.22 calibre, 4.5 grain cartridge) followed by sticking using transverse section of the neck (**P**)

3.3.1 Blood parameters

All animals were walked for 5 min from the holding yard to the lairage. The animals arrived at the lairage about 3 h before blood sampling. Blood collection was carried out in the stunning box and the distance between the box and lairage was about 50 m. For each animal, prior to slaughter, a baseline blood sample (10 mL) (T1) was taken from the coccygeal vein using an 18 G needle and EDTA tubes (Becton Dickinson, North Ryde, NSW). During the slaughter process, further blood samples were taken immediately following each action carried out: stunning (from coccygeal vein) (T2); and transverse section of neck (from blood flow) (T3). Thus, from the US animals, only two blood samples were collected – baseline (T1) and post transverse section of neck (T3); from the HPP, LPP and P animals three blood samples were collected – baseline (T1), post-stun (T2) and post transverse section of neck (T3). Once obtained, blood tubes were conserved at 4 °C and immediately centrifuged at 800g for 15 min within the first hour after sampling. The recovered plasma fraction was divided into aliquots and stored at − 80 °C until analysis. Plasma concentrations of ACTH (EIA kit; IBL Hamburg, Germany), cortisol (EIA kit; IBL Hamburg, Germany), adrenaline (EIA kit; IBL Hamburg, Germany), noradrenaline (EIA kit; IBL Hamburg, Germany), beta-endorphin (EIA kit; Phoenix Pharmaceuticals Inc., Burlingam, CA), histamine (EIA kit; IBL Hamburg, Germany) were measured in duplicate. Analyses for plasma creatine kinase and glucose levels were conducted on an Olympus Analyser (Model: AU 400, Olympus America Inc. Diagnostic Systems Division, Melville New York) using standard diagnostic kits.

Flowing blood from the transverse section of the neck, and from the thoracic stick where applicable, was collected in buckets, and weighed using an electronic balance (Avery Berkel HL122, England).

3.3.2 Meat quality

All quality measurements were assessed in the *Longissimus dorsi* (LD) and S*emitendinosus* (ST) muscles, which were removed at 45 min post-mortem from the carcasses. The muscles were not controlled for shortening, they were merely bagged as hot cuts. Each muscle sample (1000 g $\pm$ 100 g) was cut into four equal portions and assigned to four different ageing periods: 1) 0 day (no ageing), 2) 1 day, 3) 7 day and 4) 14 days *post mortem.* The samples for each different ageing period were further cut to sub samples for colour (approximately 30g,

10mm thick, 30mm x 50mm), TBARS (approximately 20g) and water holding capacity (approximately 1g) determination. The samples for colour and TBARS were kept in zipper lock plastic bag (40mm x 70 mm) and the samples for water holding capacity were kept in 1.5 mm micro tube (1.5ml PP, Sarstedt, Aktiengeselischaft & Co, D-50588 Nümbrecht, Germany).

All samples for 1, 7 and 14 days post mortem were temporarily kept on ice until transported to CSIRO Food and Nutritional Sciences Laboratory, Cannon Hill, Queensland. On arrival at the laboratory, all samples were vacuum-packaged and stored in a chiller (4^{o}C) according to their ageing period. Samples of day 0 post mortem were immediately transferred to a -80^{o}C freezer (Ultra-low Temperature Freezer, Forma Scientific, Model: 8425) immediately after vacuum packaging, while the samples assigned for 1, 7 and 14 days of ageing were transferred into a cold room (4^{o}C) and aged accordingly.

i. pH

Samples were removed from the chiller and pH was measured using a digital pH meter (WP-80, TPS Instruments, Springwood, QLD) fitted with a combination electrode with temperature compensation by inserting the electrode into the meat. Data were recorded once the readings had stabilised. Prior to measurement, the pH meter was calibrated with pH 7 followed by pH 4.

ii. Colour

Samples were removed from the -80^{o}C freezer and subjected to overnight thawing at 4^{o}C. Meat colour was measured using a MINOLTA CR300 colorimeter under light source D65.

iii. Water Holding Capacity (WHC)

Samples were removed from the -80^{o}C freezer and subjected to overnight thawing at 4^{o}C. Small pieces of muscle (approximately 0.28-0.30g) were sliced along the grain of the muscle fibres, approximately 3mm thick and 15 mm long, weighed and placed in mobicols (LIFM1002, Quantum Scientific, Murrarie, QLD) containing 90µm

filters. The samples were centrifuged (Bench Centrifuge, Model: Eppendorf Minispin Plus) at 4°C at 26G for 1 h and reweighed. The samples were cooked in an oven at 105°C for 24 hours and weight was recorded again. Based on the weights, centrifugation loss (expressed juice), total water content and water holding capacity were calculated.

iv. Cooking loss determination

Samples were removed from the -80^{o}C freezer and thawed overnight at 4^{o}C. The thawed meat samples were then cut (125±5g), weighed (Mettler, Type: PE 3000) and recorded as W1 (raw meat weight). The samples were then placed in plastic bags and cooked at 80^{o}C for 60 min in a water bath (BTC-9090). The cooked samples were removed from their plastic bags, cooled in ice slurry for 20 min and kept in chiller at 4^{o}C overnight. The samples were then re-weighed and recorded as W2 (cooked meat weight). The cooking loss was calculated based on the difference between the weight of raw meat and cooked meat (Dhanda et al., 2003) by using the following equation:

$$\text{Cooking loss (\%)} = \frac{(W1 - W2)}{W1} \times 100$$

v. Texture analysis

Samples for texture analysis were taken from the previous cook loss samples. Assessment of meat texture was made using the Warner-Bratzler (WB) shear force measurement on samples cooked at 70°C for 60 minutes, using a Lloyd Instruments LRX Materials testing machine fitted with a 500 N load cell (Lloyd Instruments Ltd., Hampshire UK). Following overnight storage at 4°C, the cooked samples were cut into sub-samples for textural analysis. The thickness, shape and fibre orientation of samples were cut according to the protocols outlined by Bouton et al. (1971) and Bouton and Harris (1972). Six subsamples of a rectangular cross-section of 15 mm wide by 6.7 mm deep (1 cm² cross-sectional area) were cut from each sample, with fibre orientation parallel to the long axis, and at right angles to the shearing surface.

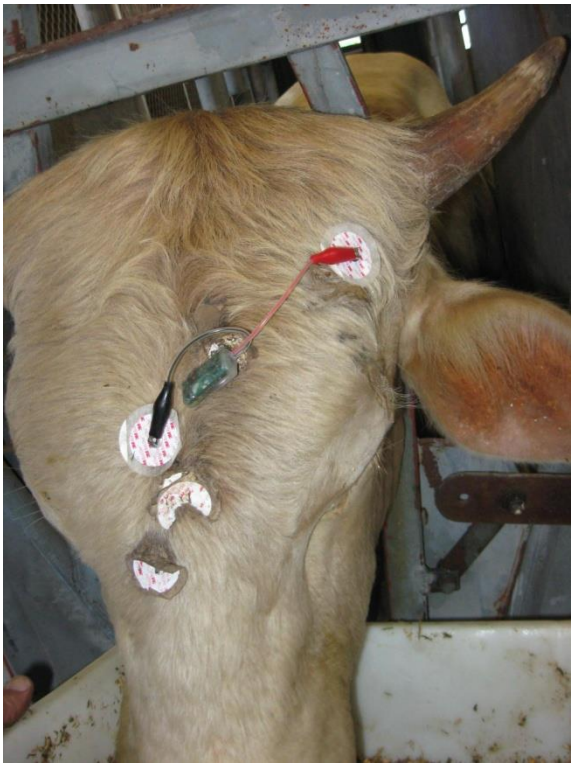
The force required to shear through the clamped sub-sample with a 0.64 mm thick blade pulled upward at a speed of 100 mm/min at right angles to fibre direction was measured as shear force. This allowed the determination of peak force (PF), initial yield (IY), and peak force minus initial yield (PF-IY). Cooking losses were also determined on the first day of the analysis.

vi. Lipid Oxidation

Samples were removed from the -80^{o}C freezer and thawed overnight at 4^{o}C. Lipid oxidation was determined by the thiobarbituric acid-reactive substances (TBARS) method of Witte et al. (1970). All meat samples were heated at 75°C for 20 minutes in a water bath and cooled in ice prior to determination. TBARS were calculated from a standard curve of malondialdehyde (MDA), freshly prepared by acidification of TEP (1,1,3,3-tetraethoxypropane), and calculated as mg MDA per kg sample.

3.3.3 Electroencephalogram (EEG) measurement

Electroencephalogram activities at baseline (T1), immediate post stunning (T2) and 30 seconds post slaughter (T3) were recorded telemetrically with Power Lab^{TM} Biopotential Recordings systems (ADI Instruments, Australia). Low impedance electrodes (less than 5 kOhms) were used. One electrode was attached to the frontal area of the head, and the other to the zygomatic arch, above the eye (Figure 1).



The EEG recordings were sampled again at 1024 Hz and only frequencies between 0.1 to 30 Hz were obtained to minimize presence of artefacts. Signals were processed in blocks of 1-second epochs, yielding 60 epochs per minute. The signal was then filtered into band pass filters to yield delta (0.1 to 4 Hz), theta (4.1 to 8 Hz), alpha (8.1 to 12 Hz) and Beta (12.1 – 20 Hz) waves. The root mean square (RMS) for each of the waveforms at T1, T2 and T3 was calculated. An average of 10 serial epochs with minimal interference were sampled to arrive at the mean values for T1, T2, T3. The RMS value for the lowest or terminal values of alpha, beta, delta and theta waves were also determined. The time taken from point of slaughter to attain the terminal RMS values was also recorded in s. The data were then compared across the various treatment groups. Video recordings of each animal were made in order to assist in defining the exact time of each procedure relative to the EEG traces.

3.4 Experiment 2

Experiment 2 was conducted to examine the effects of two different stunning methods (low power non-penetrative stunning and penetrative percussive stunning) and thoracic sticking on blood parameters, EEG activity and meat quality in beef cattle. The experiment involved a total of 30 steers. Equal number of animals were randomly assigned to one of the three treatments: (i) Animals were subjected to traditional Halal slaughter without prior stunning (a clean incision through the structures at the front of the neck – the trachea, oesophagus, carotid arteries and jugular veins) and post-cut stun within 10-20 seconds of the Halal cut (after the post-sticking blood sample had been taken) to satisfy the requirements of the Animal Ethics Approval obtained (CSIRO A7/08) (**US**); (ii) subjected to low power non-penetrative percussive stunning prior to slaughter [a non-penetrating humane killer (Cash Magnum Knocker Concussion Stunner, 0.25 Calibre, 3 grain cartridge)] (**LPPS**) and thoracic sticking within 2 min after throat cut or (iii) subjected to penetrative percussive stunning prior to slaughter [a captive bolt pistol humane killer (Cash 8000 Model Stunner, 0.22 calibre, 4.5 grain cartridge)] and thoracic sticking within 2 min after throat cut (**PS)**. All throat cuts were made according to the Halal method. The thoracic stick involved a stab rostral to the sternum with a severance of the vessels near the heart at the thoracic inlet.

3.4.1 Blood parameters

Except for frequency of blood sampling, all procedures are as described in Experiment 1. Blood samples were collected prior to stunning (T1), immediately post-stunning (T2), immediately post-slaughter (T3), and immediately post-thoracic sticking (T4).

3.4.2 Meat quality

All procedures are as described in Experiment 1.

3.4.3 Electroencephalogram (EEG) measurement

All procedures are as described in Experiment 1.

3.5 Blood volume collected at slaughter

An attempt was made to collect and measure (by weight) the amount of blood lost at slaughter. This was carried out by holding a bucket under the flow of blood from the sticking wound. However, due to the position of the stunned animals (laying on the floor of the crush) at the time of sticking, and the struggling movements of the unstunned animals, it was very difficult to catch all the blood, and an amount was spilled or splashed out of the bucket in each case. Following thoracic stick, which occurred after the animals were shackled and hoisted, it was much easier to collect the flowing blood.

4.0 RESULTS

4.1 Experiment 1

4.1.1 Blood parameters

Results of plasma cortisol, ACTH, adrenaline, and noradrenaline concentrations are presented in Table 2. Table 3 shows the mean plasma levels of beta-endorphin, histamine, creatine kinase and glucose. Among the groups subjected to stunning (HPP, LPP and P), stunning method had significant effects on plasma levels of ACTH, adrenaline and noradrenaline. Following stunning (T2), the circulating levels of those hormones were significantly higher in HPP cattle when compared to those subjected to P. The plasma levels of ACTH and noradrenaline of LPP animals at T2 were not significantly different from those of HPP and P. However, animals subjected to LPP had significantly higher plasma concentrations of adrenaline than their HPP counterparts at T2. At T2, plasma levels of cortisol, beta-endorphin, histamine, creatine kinase and glucose were neither significantly affected by stunning method nor sampling time.

Irrespective of stunning method, except for plasma levels of noradrenaline, the blood parameter values attained at T1 and T2 were not significantly different. Within the HPP animals, the plasma concentration of noadrenaline was significantly elevated at T2.

Comparison among HPP, LPP, P and US at T3 showed that stunning method had no significant effect on all blood parameters except for plasma levels of adrenaline. The animals

subjected to P had significantly lower plasma adrenaline level than the other three groups following slaughter (T3).

4.1.2 Meat quality

Results of pH, cooking loss, water holding capacity, TBARS and peak force of LD and ST are presented in Table 4 and 5, whilst, Table 6 and 7 show the results of meat color values (L, a*, b*, enhanced redness, Hue and Chroma) of LD and ST muscles, respectively.

i) Muscle pH

The pH of LD and ST muscles were not significantly affected by stunning method. Irrespective of the stunning method, significant declines in pH occurred at Day 1 of ageing and these were consistently present in both muscles.

ii) Cooking loss

Neither stunning method nor days of ageing had a significant effect on cooking loss in both muscles.

iii) Water holding capacity (WHC)

The effects of stunning method on WHC in LD and ST depended on the days of post mortem ageing. In LD muscle, the effects of stunning on WHC were only present in the 7 days ageed samples. Similar to the US group (control), the applications of HPP and P stunning methods have also resulted in significantly lower WHC than the LPP group. Among the stunning groups, the differences in WHC were only seen in the LPP samples whereby, higher WHC was found in the 7 days ageed samples. In the ST muscle, significant effects of stunning methods on WHC were present at Day 1 and Day 7 of ageing. At day 1 post mortem the WHC of the P group was significantly higher than those of HPP and US groups. However, both LPP and P had similar values. In the 7 days ageed samples, LPP resulted in higher WHC compared to HPP, P and US samples.

iv) Muscle lipid oxidation (TBARS)

The effects of stunning method on TBARS values were noticed in LD and ST samples aged for 1, 7 and 4 days post mortem. Significant differences were noted among the stunning methods in the unaged or pre rigor (Day 0) samples. In LD muscle, without ageing (Day 0), the highest and lowest TBARS values were shown by the HPP and US samples, respectively. However, no differences in TBARS values were seen between HPP and P and between LPP and P groups. At Day 1, the values indicated by US samples remained significantly lower compared to the HPP, LPP and P groups. Similarly, at Day 7, the US group showed the lowest TBARS values compared to those indicated by the HPP, LPP and P. In the 14 days aged LD samples, although not significantly different to the HPP, the LPP method has resulted in a significantly higher TBARS values than the P and US samples. As for the ST, the highest TBARS values were consistently found in the HPP group across the 0 day, 1 day, 7 days and 14 days aged samples. The application of LPP resulted in lower TBARS values in the ST muscle samples subjected to 0 day, 1 day, 7 days and 14 days of ageing. In general, compared to the other methods employed in this study, HPP has significantly increased muscle lipid oxidation as indicated by the TBARS.

v) Meat toughness (Peak force)

In LD, stunning had no significant effect on meat toughness expressed as peak force. Unlike the LD, the peak force values presented by the ST differed significantly among the stunning groups particularly at Day 0, Day 7 and Day 14 of ageing. The results indicate that the effects of stunning method on the peak force values of the ST were influenced by the days of ageing. The highest peak force values were consistently noted in the HPP group over the 14 days of ageing. At day 0, HPP stunning resulted in significantly higher peak force values than the LPP and US groups. Significant difference was also seen between P and US groups. In the 7 days aged samples, the values presented by the HPP group were found to be significantly higher than those from the P and US stunning methods. Besides, the values shown by the LPP and P stunning groups were also significantly different. Similarly, the highest peak force values at day 14 of ageing were also indicated by the HPP stunning group. However, there were no differences in the peak force values between the LPP, P and US groups. In general, cattle in the HPP

produced significantly tougher meat than cattle in the LPP, P and US group and these were only present in the ST muscle.

vi) Meat colour characteristics (Lightness (L*), redness (a*), yellowness (b*), hue and chroma)

The lightness (L*) values shown by LD muscle were not affected by the stunning methods. However, stunning has resulted in significant differences in the L* values of the ST particularly at 0, 1, and 7 days post mortem. At Day 0, significant differences in L* of the ST were seen between HPP and US, and between LPP and US group. At day 1, significant differences in L* were only noticed between LPP and P group. As at day 7 post mortem, the L* values of the P and US group were significantly higher than the HPP and LPP stunning group. In general, the brightest meat colour were produced by the US and P stunning group. The values of redness (a*) of LD and ST muscles did not differ between the stunning groups and these were consistently seen over the 14 days of post mortem conditioning. However, a* values in LD and ST were significantly affected by the days of ageing. In general, there were significant increases in a* with the increasing period of ageing and these were encountered in both muscles. The presence of interactions between stunning methods and days of ageing indicate that the effects of stunning on yellowness values (b*) of LD and ST depend on the days of ageing. In LD, the effects of stunning method on b* values appeared to be significant at day 0, 1, 7 and 14 of ageing. Interestingly, LPP stunning resulted in significantly higher b* values than the other stunning methods at day 1, 7 and 14 of ageing. Unlike the LD, the influence of days of ageing on the significant effects of stunning on b* values in ST muscle were only noticed at days 1 and 7. At day 1, significant differences in yellowness were seen between HPP and P, and between P and US (control) groups. Compared to the HPP and US groups, the highest yellowness values were indicated by the P stunning group. A day 14, b* values differed significantly between HPP and LPP, HPP and P, and between LPP and US group. In general, the highest yellowness values in the 14 days aged ST were indicated by the LPP stunning group. In this study, the significant effects of stunning on

hue values were only present in the LD at days 0, 1 and 14 of conditioning. At day 0, the hue values presented by the LPP and US were significantly higher than those in the HPP and P groups. At day 1, the hue values indicated by the LPP samples remained significantly the highest compared to the other stunning groups. At day 7 of ageing, the hue values were unaffected by the stunning methods employed in the study. Consistent with day 0 and day 1, the highest hue values at Day 14 of ageing was also indicated by the LPP group. In general, the application of LPP stunning method resulted in higher hue values of the LD at days 0, 1 and 14 of ageing. However, stunning had no effect on the hue values of ST at Days 0, 1, 7 and 14 of ageing. The chroma values or saturation index of both LD and ST were not affected by the stunning methods.

4.1.3 Electroencephalogram (EEG) measurement

Generalizing from the results shown in Tables 8 and 9, the US animals generally showed increased levels of alpha and beta wave activities at T3, compared to T1. The RMS for alpha waves among US animals increased by almost 3 fold from 11.77 ± 2.70 microvolts to 31.44 ± 8.94 microvolts within 30 s post slaughter. Whereas the RMS value for beta waves had doubled after 30 s from the initial throat cut for US animals. In contrast, all stunned animals (P, LPP and HPP) did not experience significant elevation of brain electrical activity. At T2, the P animals consistently showed significantly lower alpha and beta wave activities than those of HPP and LPP.

4.2 Experiment 2

4.2.1 Blood parameters

Data on plasma cortisol, ACTH, adrenaline, and noradrenaline concentrations are shown in Table 10. Table 11 shows the mean plasma levels of beta-endorphin, histamine, creatine kinase and glucose. Following stunning (T2), all the blood parameters values of both LPPS and PS were not significantly different. Comparison among LPPS, PS and US during slaughter showed significant effect of stunning on plasma noradrenaline and creatine kinase concentrations. Neck cutting resulted in a significantly higher noradrenaline in LPPS animals when compared to their PS and US counterparts. US had significantly higher plasma creatine

kinase level than those of LPPS and PS. After thoracic sticking (T4), both LPPS and PS had similar plasma levels of ACTH, cortisol, adrenaline, noradrenaline, beta-endorphin, histamine, creatine kinase, and glucose. Comparison at T1, T2, T3 and T4 showed that all blood parameters were not significantly affected by sampling time.

4.2.2 Meat quality

Results of pH, cooking loss, water holding capacity, TBARS and peak force of LD and ST are presented in Tables 12 and 13, whilst, Tables 14 and 15 show the results of meat color values (L, a*, b*, Hue and Chroma) of LD and ST muscles, respectively.

i) Meat pH

Consistent with Experiment 1, pH in either muscle did not significantly differ between the stunning methods. Irrespective of stunning method, a significant decline in pH occurred at Day 1 of ageing and these were consistently present in both muscles.

ii) Cooking loss

Cooking loss was significantly affected by the stunning and sticking method. The significant effects appeared at day 0 and day 7 (LD) and day 0 only (ST). In both muscles, the highest cooking losses were indicated by the US group.

iii) Water holding capacity

In both muscles, significant effects of stunning and sticking on WHC were consistently seen at day 1 and day 7 post mortem. The highest WHC were noted in the PS group and these appeared in both muscles.

iv) Muscle lipid oxidation (TBARS)

There were significant differences in the level of TBARS among the stunning and sticking groups. In LD, LPPS had significantly higher TBARS level at 0, 1 and 7 days post mortem. Similar results were also seen in the ST at 1 and 7 days post mortem.

v) Meat toughness (peak force)

In LD, comparison among LPPS, PS and US at 0, 1, 7 and 14 days post mortem showed that stunning and sticking had significant effects on meat toughness expressed as peak force. However, the significant effects in ST were only seen at 7 and 14 days post mortem. In both muscles, the highest peak force values were noted in meat samples of the US group.

vi) Meat colour characteristics (Lightness (L*), redness (a*), yellowness (b*), hue and chroma)

Lightness in both muscles was not affected by the stunning and sticking method. However, the other colour characteristics like a*, b*, hue and chroma were found to be significantly affected by the stunning and sticking treatments. In both muscles, the lowest a* values were found in US group. The PS group had significantly lower b* values in the LD and ST. The hue values at 7 days post mortem of LD was significantly elevated following LPPS. In ST, the hue values of US were significantly higher than those in the LPPS and PS groups. The chroma values of LD and ST were significantly affected by the stunning and sticking at 0 day and 1 day post mortem, respectively. In both cases, US resulted in significantly lower chroma values compared to those of LPPS and PS.

4.2.3 Electroencephalogram (EEG) measurement

Results from Experiment 2 are depicted in Tables 16 and 17. In general, RMS waveforms at T1 and T2 among the LPPS and PS animals were not significantly different which are consistent with those in Experiment 1. Table 16 also shows that LPPS and PS animals had significantly lower RMS readings for all waveforms at T3, both when compared to the US animals and against their respective values at T2. However, the duration (in seconds) to arrive at the lowest possible RMS values for a waveform was similar across all treatment groups (Table 17).

4.3 Blood volume collected at slaughter

In general, blood collected at slaughter tended to be greater in stunned animals than unstunned, and substantially greater from animals that received a thoracic stick (figure 2). Statistical analysis was, however, not carried out as the data were considered to be inaccurate due to the difficulty in collecting all the blood expressed by the carcase. Much was spilled during bleeding.

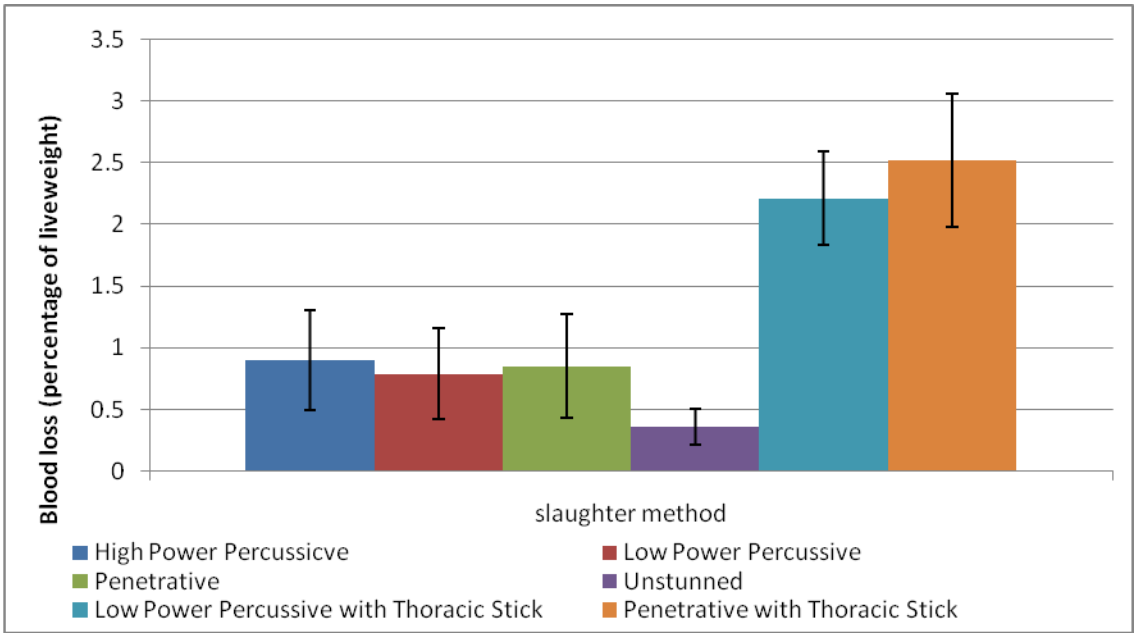


Figure 2: Blood weight collected at sticking (as a percentage of liveweight).

5.0 DISCUSSION

5.1 Experiment 1

5.1.1 Blood parameters

Although the main purpose of stunning is to eliminate animal suffering during slaughter, earlier studies showed that the stunning procedure itself was physiologically stressful (Nowak et al., 2007; Linares et al., 2008; Micera et a., 2010). The effect of mechanical stunning on plasma cortisol, cathecolamines, and beta-endorphin concentrations have been reported in horses (Micera et al., 2010). Similarly, Linares et al. (2008) noted significant elevation in cortisol and cathecolamines following electrical stunning in sheep. In the present study, all the blood

parameters values (except for plasma levels of noradrenaline) attained prior to (T1) and following stunning (T2) were not significantly different. It appears, from the current study, that LPP, HPP and P are not stressful to the animals. However, there is a possibility that maximum secretions of cortisol and cathecolamines were reached prior to stunning in the present study. The mean plasma cortisol concentrations of 73.55 ng/mL attained prior to stunning is not within the "normal" range (Henricks et al., 1984; Mitchell et al., 1988). The higher plasma concentrations of cathecolamines and cortisol prior to slaughter suggested that the animals were excited prior to stunning. The excitement could be attributed to distractions that impede forward movement of animals from lairage to stun box, noise, prolonged head restraint or the animals were not accustomed to human contact (Grandin, 1996). It is important to identify, quantify and manage the potential stressors prior to stunning in order to optimise animal welfare and meat quality.

It has been observed that plasma concentrations of adrenaline and nordarenaline increased in horses (Micera et al., 2010) and lambs (Linnares et al., 2008) in response to mechanical and electrical stunning, respectively. In the present work, circulating noadrenaline but not adrenaline rose following HPP. Work in humans has shown that the longer-lasting noradrenaline release may be a useful an index of postsurgical trauma (Wilmore et al., 1976). Dramatic elevation in cathecolamines may increase post-mortem glycolysis through the activation of phosphorylase, and thus adversely affect meat quality.

One of the main objectives of the present study was to compare the stressfulness of various stunning methods. There are no previous studies in cattle that compare physiological stress reactions to non-penetrative and penetrative percussive stunning. As measured by plasma levels of adrenaline, noradrenaline and ACTH in the current study, HPP resulted in a greater magnitude of physiological response when compared to P. Increase in cathecolamines indicates stimulation of the adrenal medulla and suggests that animals were experiencing some emotional or physical distress and presumably damage to tissues (Mellor et al., 2002; De la Fuente, 2006; Nowak et al., 2007). It is interesting to note that despite the lack of a significant difference in plasma cortisol concentration between LPP, HPP and P, the circulating levels of ACTH were higher in HPP than P. Thus, it appears that ACTH may be a more sensitive indicator of stunning stress than cortisol.

There is the question of why HPP was more stressful to cattle than those of P. Working with lambs, Finnie et al. (2000) demonstratedthat generally both penetrative and non-penetrative percussive stunning, carried out properly, resulted in similar structural tissue damage. However, the authors reported that while focal injury was more severe in the former, the latter caused more widely distributed damage. Grandin (2004) suggested that penetrative captive bolt stunning was more effective and the likelihood of error was lower than non-penetrative stunning. The present findings, as measured by plasma adrenaline concentration, also suggest that the power of the non-penetrative percussive stunning is crucial in determining the magnitude of the physiological response following stunning. Although the plasma concentrations of ACTH and noradrenaline of LPP and P were not significantly different, the former had smaller increases in circulating adrenaline response following stunning.

Slaughter by throat or neck cut without prior stunning is a major welfare issue in some countries. Pain caused by the neck or throat cut has been the subject of much debate. It has been suggested that the use of a very sharp knife produces little behavioural reaction in non-stunned cattle and hence such a neck cut is not perceived by the animal as painful (Grandin, 1994). Based on behavioural and neurophysiological reactions, Rosen (2004) concluded that slaughter without prior stunning as in *shechita* is painless. Based on EEG responses, Gibson et al. (2009a) reported otherwise Mitchell et al. (1988) found that slaughter following mechanical stunning elevated plasma levels of cathecolamines, lactate and glucose, suggesting elicitation of the physiological stress responses. Within the limits of this study, there was no indication that US was more stressful than LPP or HPP at T3 (post neck cut). Stunning within 5 s after the throat cut, would be expected to render the animals insensible to further pain and distress (Gibson et al., 2009d). Hence, these findings suggest that whether the animals are subjected to prior stunning or post stunning, the magnitude of physiological stress experienced following slaughter is similar. It is unknown whether a clean incision through the structures at the front of the neck (trachea, oesophagus, carotid arteries and jugular veins) with a very sharp knife without any form of stunning as in Halal or Shechita method will produce similar results as those that were subjected to post-slaughter stunning. This merits further investigation.

Animals subjected to P had significantly lower circulating levels of adrenaline than the other groups following slaughter. Shaw and Tume (1992) suggested that the measurement of

cathecolamines in post-slaughter blood samples is of limited value as mechanical stunning itself elevated the hormones. In the present study, the plasma adrenaline and noradrenaline concentrations of P animals at T1 and T2 were not significantly different.

5.1.2 Meat quality

In this experiment, the pH at 0, 1, 7 and 14 days post mortem in either muscle were not affected by the stunning treatments. The results are in agreement with the findings of Petersen and Blackmore (1982), Vergara and Gallego (2000), and Velarde et al. (2003) who found no significant differences in pH between non-stunned and electrically stunned lambs. A previous study in cattle using percussive captive bolt stunning reported a significantly higher muscle pH at 15 min post mortem than the non-stunned animals (Önenc and Kaya, 2004). In their study, the use of percussive stunning has resulted in a significantly faster rate of pH decline than those without stunning. However, pH at 15 min post mortem (nearest to at death muscle pH) was not measured in this study and thus has limited the determination of pH decline rate in both muscles. Stunning method had no effect on meat cooking loss which agrees with previous studies in lamb (Vergara et al., 2005), pig (Channon et al., 2002), cattle (Önenc and Kaya, 2004), broiler chicken (Mohan Raj et al., 1990) and turkey (Northcutt et al., 1998).

A significant increase in WHC was shown in both LD and ST following the LPP stunning method. Although not different with the US and P groups, HPP has resulted in a significantly lower WHC than the LPP group. The results suggest that HPP stunning could have adversely affected meat WHC in both muscles. The noted lower WHC following HPP stunning compared to the others could be explained by earlier onset of rigor due to more rapid glycolytic changes as a result of more stressful slaughtering condition subjected to the animals. In an earlier study in lamb, a higher drip loss was found in stunned animals than in the un-stunned group (Linares et al., 2007) and this could be due to earlier onset of rigor development (Vergara and Gallego, 2000) and myofibrillar proteolysis in the stunned muscles (Rosenvold et al., 2002; Melody et al., 2004).

Lipid oxidation in muscle starts immediately after death, following failure of circulatory system and cessation of metabolic activities. It has been associated with deterioration in the quality of meat (Buckley et al., 1995). Stress and handling of animals during slaughter influences

the degree of lipid oxidation in meat (Juncher et al., 2003). In the present study, the use of HPP has resulted in a higher level of TBARS which indicates greater lipid oxidation in both muscles. The results suggest that the HPP stunning employed in the present experiment could have resulted in more stressful condition to the animals compared to those subjected to the US, LPP and P methods. The higher levels of adrenaline, noradrenaline and ACTH in the plasma of HPP animals in this study support these findings. In general, cattle subjected to HPP produced significantly tougher meat than those assigned to the LPP, P and US groups, but these were only present in the ST. A recent study in lamb reported significant effects of stunning method on meat lipid oxidation only after 7 days of post mortem storage (Bornez et al., 2009). However, in the present study, the significant effects of stunning on the level of TBARS were detected in both muscles as early as at day 0 post mortem and continued to present at day 1, 7 and 14 of post mortem conditioning.

In studies in lambs and cattle, no differences in colour values were found between stunning methods (Vergara and Gallego, 2000; Velarde et al., 2003; Önenc and Kaya, 2004). In the present study, except for the b* (yellowness) and hue values, the other colour characteristics of the LD were all not affected by the stunning method. However, in the case of ST, L* (Lightness) and b* values were significantly affected by the stunning methods. Brighter meat colour (as indicated by higher L* values) were shown by the P and US samples at 0, 1 and 7 days post mortem compared to those in the HPP and LPP groups. The results indicate that the application of HPP has not only resulted in lower b* values in both muscles at all time points post mortem but also decreased lightness (L*) values. Besides, the lowest hue values (colour tone) were also found in the LD from the HPP stunned animals. Based on the present findings, the HPP stunning has also resulted in inferior meat colour characteristics compared to the other stunning treatments employed. In general, cattle subjected to the HPP stunning method produced significantly tougher meat than those assigned to the LPP, P and US procedures and these were only present in the ST. The reason why only the ST and not the LD was affected by the stunning could be explained by the differences in metabolic and contractile properties between both muscles. It is well accepted that ST is mainly involved in locomotion and exercise during pre-slaughter handling. Thus, the response given by both muscles as a result of different pre slaughter and slaughter conditions could also be influenced by their activities. In this experiment,

as supported by the plasma results, the HPP stunning method as the most physiologically stressful to the animals could have resulted in a more sudden and rapid glycogen depletion in the affected muscle which in turn, may have caused rapid pH decline. It has been well documented that low muscle pH deactivates the calpain enzymes role in post mortem proteolysis which has been closely linked with early meat tenderization (Huff-Lonergan et al., 1996). Earlier work in lamb has implicated decreased calpain activity due to differences in muscle pH as a possible factor causing increased toughness in the stunned animals (Vergara and Gallego, 2000). So, in the case of HPP stunned animals, myofibrillar proteolysis could have been suppressed by the acidic muscle pH and this could have resulted in the tougher meat products as seen in the HPP stunned animals.

5.1.3 Electroencephalogram (EEG) measurement

The objectives of pre-slaughter stunning are to induce rapid desensitization of animals to pain of slaughtering and to minimize bodily injury risks to abattoir personnel. This is crucial as the neck region is innervated by nociceptive fibres that are capable of unleashing massive firing of somatosensory signals upon throat cut (Mellor et al., 2009). Therefore, stunning should be done effectively, thereby minimizing the possibilities of animals regaining consciousness and rendering the animal in a period of insensibility during throat-cut to the point of total cessation of vital signs. Previous work reported by Gibson et al. (2009 d) clearly illustrated the benefits of stunning in ameliorating the noxious stimuli associated with the throat cut. However, in religious slaughter where only certain modes of stunning are acceptable, it is mandatory that stunning should be reversible, and should not be the cause of death other than the throat cut itself. Newhook and Blackmore (1982) proposed a window of sensibility, indicated by EEG recordings between 10 to 35 microvolts. However, these may not be applicable under all stunning conditions as shown by Devine et al. (1986). Furthermore, the emergence of newer EEG analytical methods and evidence may suggest that the determination of a window of sensibility in cattle may be more complex than first thought. For instance, following non-penetrative stunning, periods of a rapid and spiking barrage of transitional EEG waveforms are often seen, none of which are necessarily an indication of pain being perceived by the animal (Gibson et al., 2009c).

Results from Tables 8 and 9 indicated that alpha and beta waves spiked rapidly post stunning, but declined gradually to their respective terminal values among the HPP and LPP animals. Coupled with the appearance of slow frequency waves within the frequency range of theta and delta waves in all stunned animals, we concluded that stunning did render the animal unconscious, and less able to perceive noxious stimuli compared to the US animals. This finding was similar to that reported by Lambooy and Spanjaard, (1981), and consistent with trauma-induced unconsciousness of the brain as described by Shaw (2002) in human patients. It should be noted that all animals started with similar alpha, beta, delta and theta RMS values at baseline (T1), and thus the increase in brain electrical activity in the US animals at T3 could be attributed to possible conscious pain even at 30 s post slaughter. All the HPP, LPP and P groups demonstrated immediate prominence of slow frequency delta and theta waves at T2 although it was not reflected in the RMS values. The appearance of slow frequency delta and theta waves probably pointed to the possible loss of consciousness, although in the case of LPP and HPP animals, these wave forms were also accompanied by significant increase in alpha and beta wave RMS values at T2, which could be linked to post stunning conscious pain.

The time post slaughter to attain terminal RMS values or Terminal Time for all waveforms has been used in conjunction with the absence of vital signs such as corneal reflex to determine the point of cessation of brain electrical activity. However, the Terminal Time was not significantly different across treatment groups (Table 9), indicating that stunning method was not a significant contributor in hastening Terminal Time, or cessation of all visible vital signs and reflexes.

However, based on the present results, P seemed to be the best method for maximizing the possibility of post stunning insensibility, while US animals seemed to demonstrate increases in EEG activities that are consistent with the presence of post slaughter noxious stimuli associated with tissue cut and injury as proposed by Gibson et al., (2009b). It should be noted that the US animals in the present study were subjected to penetrative percussive stunning shortly after throat cut. Hence, the spike in EEG activities at T3 could be attributed to the additive effects of both throat cut and stunning. If post-slaughter stunning resulted in more "suffering" to the animals there is the question of whether the procedure is necessary.

5.2 Experiment 2

5.2.1 Blood parameters

The aim of thoracic sticking is to improve bleeding and mitigate the effects of any occlusion of the carotid artery. When carotid occlusion occurs there is a delay in the onset of brain failure (Anil et al., 1995). Gregory and Shaw (2000) indicated that with effective stunning, thoracic sticking was not a welfare concern. The present findings showed that the thoracic sticking procedure had negligible effects on all blood parameters measured. Hence, it appears that the all the animals in the present study have been stunned effectively prior to thoracic sticking.

5.2.2 Meat quality

In this experiment, the applications of LPPS, PS and US did not result in any significant difference in LD and ST pH. However, in both muscles, the highest cooking losses were found in the US group and this is not in agreement with Onenc and Kaya (2004) who reported higher cooking losses in muscle from the electrically and percussively stunned animals. The highest WHC were produced by the PS group and these appeared to be consistent in both muscles. The penetrative stunning employed in this experiment could have eventually resulted in enhanced myofibrillar protein degradation. The improved WHC could be due to the enhanced proteolytic degradation of cytoskeletal proteins in the affected muscles, which has subsequently caused swelling of the myofibrils and allowed the meat to retain water (Kristensen and Purslow, 2001). It has been well accepted that degradation of the cytoskeletal proteins during ageing would increase WHC of meat by removing inter-myofibrillar and costameric connections and thereby reduce or remove the linkage between the rigor-induced lateral shrinkage of myofibrils and shrinkage of the whole muscle fibre (Huff-Lonergan and Lonergan, 2005). The explanation could be confirmed through the results of myofibrillar protein degradation patterns using SDS-PAGE. Apart from the earlier explanation, rate of pH decline in skeletal muscle may also play a major role in determining water holding capacity and cooking loss of meat (Lyon and Buhr, 1999). However, the present results did not indicate any significant effects of stunning on muscle pH. The results of TBARS in both experiments demonstrated the benefits of PS in reducing lipid

oxidation of both muscles. This could be explained by differences in the amount of blood removed following LPP, P and US. Residual blood in the carcass and meat has been associated with decreased colour stability and shelf life. However, bleeding efficiency and carcass blood residue were not determined in this experiment and this limits further discussion in relation to lipid oxidation and bleeding efficiency. The level of TBARS in both muscles of animals subjected to LPPS was significantly higher especially when compared to those of US. In comparison with PS and US, the results suggest that the application of LPPS could have hastened lipid oxidation in both muscles. This highlights the potential benefits of penetrative stunning application for a better stability and shelf life of meat products.

In both muscles, the toughest meat, as indicated by the highest peak force values, were observed in the US animals. The present findings further support the earlier report in cattle by Onenc and Kaya (2004). The most tender meat was produced by the animals subjected to PS and this was consistently exhibited in both muscles. The improved tenderness seen in PS group could be explained by a possible enhancement in myofibrillar protein degradation process involving the calpain proteolytic system (Melody et al., 2004). Furthermore, the improved tenderness in PS group is consistent with the enhanced WHC described earlier of which could also had involved similar mechanism.

In this experiment, L* values (lightness) in both muscles were not affected by the stunning and sticking method and this is in agreement with the earlier report on cattle by Onenc and Kaya (2004). However, the other colour characteristics like a*, b*, hue and chroma were all significantly different between the stunning and sticking treatments employed. In both muscles, the lowest a* values (redness) were found in US group. Meanwhile, animals subjected to penetrative stunning with sticking (PS) had significantly produced the lowest b* values (yellowness) in both muscles. Our findings disagree with Vergara and Gallego (2000) and Velarde et al. (2003) who reported no difference in a* and b* values between stunned and un-stunned lambs. In addition, the present results also contradict previous findings in pork that muscle a* and b* values were not influenced by stunning method (Channon et al., 2002). The results of hue values indicate color tone or paleness in muscle whereby lower hue values indicate paler meat. In this experiment, penetrative stunning followed by sticking resulted in a significantly paler colour of the LD at 7 days post mortem. In contrast, darker meat colour was

found in the muscle from the low-powered percussive stunning with sticking (LPPS). In ST, compared to the US and LPPS groups, the hue values were significantly reduced (pale colour) by the use of penetrative stunning with sticking (PS). In both muscles, LPPS and PS have significantly increased the chroma values (more vivid) compared to those of the US animals. The present enhancement in chroma values (vividness) in both groups of stunning is in agreement with those reported earlier by Onenc and Kaya (2004).

5.2.3 Electroencephalogram (EEG) measurement

Based on the window of sensibility concept put forth earlier by Newhook and Blackmore (1982), it is clear that all terminal RMS values of alpha, beta, delta and theta waveforms were very much below 10 microvolts. This indicated deep unconsciousness or even death itself in the case of this experiment. At this stage, most of the terminal RMS values were associated with isoelectric EEG traces. The LPPS and PS animals had lower RMS values for most waveforms 30 seconds post slaughter, where most readings were very near to their terminal values. However, some of these values should be interpreted with care as their coefficient of variation normally ranges between 30 to greater than 60 %. These results suggested that stunning resulted in a period of insensibility and thoracic sticking played a role in hastening the onset of terminal RMS, but not eventual death itself. This is because the terminal RMS values of LPPS and PS were similar to that of US. Note that the duration (in seconds) to arrive at the lowest possible RMS values for a waveform was similar across all treatment groups. This suggested that sticking was not a significant contributor in hastening the disappearance of vital signs and reflexes, but merely responsible for the rapid suppression of brain activity which resulted in the animal being in a state of deep unconsciousness prior to the cessation of vital signs. It was also noted that the terminal RMS values of LPPS, PS and US animals for all waveforms were not significantly different.

In discussing thoracic sticking, Gregory and Shaw (2000) indicated that effective stunning was more important in improving animal welfare as sticking only serves at promoting rapid exsanguination in a state where the animal may already be insensible. Based on the present findings in Experiments 1 and 2, the similar degree of EEG changes between P versus PS animals, as well as between LPP versus LPPS animals, suggested a degree of unconsciousness

and evidence of insensibility post-stunning prior to slaughter. This further reinforces the fact that sticking is more crucial as a carcass management approach rather than to improve animal welfare, in the case of properly stunned animals.

6.0 GENERAL DISCUSSION

"Humane slaughter" in many countries requires that an animal becomes unconscious and does not regain consciousness until death. Percussive stunning using a penetrating or non-penetrating captive bolt gun is a common method used to render animals unconscious rapidly and effectively. Based on the EEG reactions in the current study, LPP, HPP and P were effective in rendering animals unconscious. However, both blood parameters and EEG data suggested that P was less stressful than LPP and HPP. These findings could be associated with differences in brain injury inflicted (Finnie et al., 2000). The significant differences in plasma adrenaline concentration and marked numerical differences in plasma levels of ACTH and noradrenaline suggested that HPP was more physiologically noxious than LPP. These findings could be associated with the inferior meat quality noted in the former. The elevated circulating adrenaline may lead to increase in the rate of post-mortem glycolysis through the activation of phosporylase, and thus affect meat quality (Shaw and Tume, 1992).

Although we did not notice marked differences in blood parameters between US and other groups, EEG reaction clearly showed that US was more stressful to the animals following throat cut. Because the US animals were stunned shortly after throat cut, it is unclear whether the dramatic EEG changes could be attributed to the throat cut, stunning or both procedures. The effect of throat cut on EEG response in unstunned animals warrants further investigation. In discussing neurophysiological and behavioural data, Rosen (2004) concluded that *Shechita* which does not involve stunning is a painless and humane method of slaughtering.

Anil et al. (1995) concluded that thoracic sticking can mitigate the effects of carotid occlusion and thus time to onset of brain failure may be hastened. Unexpectedly, in the present study, the time from post-slaughter to attain terminal RMS values of LPPS, PS and US animals did not differ. There is no clear explanation to the phenomenon because bleeding efficiency and carcass blood residue were not determined in this experiment. On the contrary, the noted

markedly beneficial effects of thoracic sticking on meat quality suggest that the procedure can improve bleeding rate. Further studies are necessary to ascertain these discrepancies.

7.0 CONCLUSIONS

7.1 Experiment 1

Blood parameters

- As measured by plasma adrenaline, noradrenaline and ACTH concentrations, HPP was more stressful to cattle when compared to LPP and P
- Except for changes in plasma adrenaline concentration, there was little indication that US animals were more stressed than those of HPP and LPP following throat cut.

Meat quality

- As indicated by the low water holding capacity, higher level of TBARS, higher peak force values and lowering of some of the colour values, it can be concluded that HPP resulted in inferior meat quality in cattle.

Electroencephalogram (EEG) measurement

- It was evident that P was less noxious and more effective in inducing insensibility when compared to HPP and LPP.
- Subjecting animals to post-slaughter penetrative percussive stunning (US) was more stressful than HPP and LPP following throat cut.

7.2 Experiment 2

Blood parameters

- With effective stunning, thoracic sticking has negligible effect on physiological stress responses in cattle.

Meat quality

- Animals subjected to LPPS and PS had better meat colour (redness and chroma).
- The application of PS improved cooking loss, water holding capacity, meat stability, shelf life (through the reduction of muscle lipid oxidation) and tenderness.

Electroencephalogram (EEG) measurement

- Although thoracic sticking did impede brain function considerably post slaughter, it had minimal impact on hastening the eventual cessation of vital signs of life when compared to US animals.

8.0 GENERAL CONCLUSIONS

In this study, there were few differences in plasma metabolites and meat quality measurements between the different slaughter methods. These measurements may well have been confounded by the animals already being in a state of excitement or 'stress' as a result of the handling inherent in commercial slaughter systems. However, EEG readings indicated that unstunned animals experienced increased electrical activity in the brain following slaughter, and this may have been indicative of conscious pain. Penetrative stunning overall seemed to be the best method to ensure insensibility.

Thoracic sticking did not appear to have any influence on the measurements taken. However, in these experiments, the thoracic stick was carried out 2 minutes after the neck cut, so any impact may be confounded by the fact that at this point, the animal was almost certainly fully unconscious, if not dead – the brain activity as recorded by the EEG was ceasing at around 2 minutes post neck cut.

One of the concerns in industry is that low power percussive stunning, as used to ensure that skulls are not cracked by stunning, is likely to result in a greater number of animals that are not properly stunned, and therefore likely to suffer pain at sticking. In this study, although a lower grain cartridge was used to deliver the low power percussive stun than the high power

percussive stun, no attempt was made to assess the skulls for damage, so it is unknown as to whether the power used would comply with these requirements. What is evident from the data is that the cartridge used did cause proper stunning in the test animals. No conclusion can therefore be drawn as to the humaneness of improper stunning resulting from insufficient power to the percussive stun.

REFERENCES

Anil, M.H., McKinstry, J.L., Gregory, N.G., Wotton, S.B. and Symonds, H. 1995. Meat Sci., 113-123.
Blackman, N.L., Blackman, K. Cheetham and D.K. Blackmore, 1986. Res. Vet. Sci,. 40:252-254.
Bornez, R., Linares, M.B. and Vergara, H. 2009. Meat Sci., 83: 383-389.
Bouton, P.E. and Harris, P.V., 1972. J. Fd. Sci. 37:218.
Bouton, P.E., Harris, P.V. and Shorthose, W.R., 1971. J. Fd Sci. 36:435.
Buckley, D.J., MOrissey, P.A. and Gray, J.I. 1995. J. Anim. Sci., 73: 3122-3130.
Channon, H.A., Payne, A.M. and Warner, R.D. 2002. Meat Sci., 60: 63-68.
De la Fuente, J., Sánchez, M., Pérez, C., Lauzurica, S., Vieira, C., González de Chávarri, E. and Díaz, M.T. 2009. Animal, 4:250-258.
Devine, C.E., Gilbert, K.V., Graafhuis, A.E., Tavener, A., Reed, H. and Leigh P. 1986. Meat Sci., 17:267-281.
Dhanda, J.S., Taylor, D.G. and Murray, P.J., 2003. Small Ruminant Res., 50:57-66.
Finnie, J.W., Blumberg, P.C., Manavis, J., Summersides, G.E. and Davies, R.A. 2000. Aust. Vet. J., 78:775-778.
Gibson, T.J., Johnson, C.B., Murrell, J.C., Hulls, C.M., Mitchinson, S.L., Stafford, K.J., Johnstone, A.C. and Mellor, D.J. 2009a. New Zealand Vet J., 57(2):77-83.
Gibson, T.J., Johnson, C.B., Murrell, J.C., Chambers, J.P., Stafford, K.J. and Mellor, D.J. 2009b. New Zealand Vet. J., 57(2):84-89.
Gibson, T.J., Johnson, C.B., Murrell, J.C., Mitchinson, S.L., Stafford, K.J. and Mellor, D.J. 2009c. New Zealand Vet. J., 57(2):90-95.
Gibson, T.J., Johnson, C.B., Murrell, J.C., Mitchinson, S.L., Stafford, K.J. and Mellor, D.J. 2009d. New Zealand Vet. J., 57(2):*96-101*.
Grandin, T., 2006. Proc. of the 29th Annual Conf. Am. Assoc. Bovine Practitioners, pp. 22-26.
Gregory, N and Shaw, F. 2000. J. Appl. Anim. Welfare Sci., 3:215-230.
Henricks, D.M., Cooper, J.W., Spitzer, J.C. and Grimes, L.W. 1984. J. Anim. Sci.,59:376-383.
Huff-Lonergan, E. and Lonergan, S. M. 2005. Mechanisms of water holding capacity of meat: the role of post mortem biochemical and structural changes. Meat Sci., 71: 194-204.
Huff-Lonergan, E., Mitsuhashi, T., Beekman, D., Parrish, F.C., Jr. Olson, D. and Robson, R. 1996. J. Anim. Sci., 74: 993-1003.

Juncher, D., Ronn, B., Hansen, T.B., Henckel, P., Karlsson, A. and Skibsted, L.H. 2003. Meat Sci., 63(2): 151-159.
Kristensen, L. and Purslow, P. P. 2001. The effect of ageing on the water holding capacity of pork: role of cytoskeletal proteins. Meat Sci., 58:17-23.
Lambooy, E. and Spanjaard, W. 1981. Vet. Rec., 109:359-361.
Leigh, P. and Delany, M. 1987. New Zealand Vet. J., 38:124-125.
Linares, M.B., Bórnez, R. and Vergara, H. 2007. Meat Sci., 76: 675-681.
Linares, M.B., Bornez, R. and Vergara, H. 2008. Livestock Sci., 115:53-61.
Lyon, C.E. and Buhr, R.J. 1999. In Poultry Meat Science. Poultry Science Symposium Series (Vol. 25), 99-127.
Mellor, D.J., Gibson, T.J. and Johnson, C.B. 2009. New Zealand Vet. J. 57(2):74-76.
Mellor, D.J., Stafford, K.J., Todd, S.E., Lowe, T.E., Gregory, N.G., Bruce, R.A. and Ward, M. 2002. Aust. Vet. J., 80:228-233.
Melody, J.L., Lonergan, S.M., Rowe, l.J., Huiatt, T.W, Mayes, M.S. and Huff-Lonergan, E. 2004. J. Anim.Sci., 82: 1195-1205.
Micera, E., M. Albrizio, N.C. Surdo, A.M. Moramarco and A. Zarrilli, 2010. Meat Sci., 84:634-637.
Mitchell, G., J. Hattingh and M. Ganhao, 1988. Vet. Rec., 123:201-205.
Mohan Raj, A.B., Grey, T.C., Audsely, A.R. and Gregory, N.G. (1990). Poultry Sci., 31:725-733.
Newhook, J.C. and Blackmore, D.K. 1982. Meat Sci., 7:19-28.
Northcutt, J.K., Buhr, R.J. and Yung, L.L. 1998. Poultry Sci., 77: 487-492.
Nowak, B., Mueffling, T.V. and Hartung, J. 2007. Meat Sci., 75:290-298.
Önenc, A. and Kaya, A. 2004. Meat Sci., 66: 809-815.
Petersen, G.V. and Blackmore, D.K. 1982. New Zealand Vet. J., 30: 195-198.
Rosen S.D., 2004. Vet. Rec., 154:759-765.
Rosenvold, K., Laerke, H.N., Jensen, S.K., Karlsson, A.H., Lundstrom, K. and Andersen, H.J. 2002. Meat Sci., 62: 485-496.
SAS Institute. 1991. SAS/STAT User's Guide, release 6.03: SAS Institute; Cary, NC, USA.
Shaw, F.D. and Tume, R.K. 1992. Meat Sci., 32:311-329.
Shaw, N.A. 2002. Prog. Neurobiol., 67:281-344.
Short, C.E. and A.V. Poznak, 1992. Animal Pain, Churchill Livingstone, London.
Velarde, A., Gispert, M., Diestre, A. and Manteca, X. 2003. Meat Sci., 63: 35-38.
Vergara, H. and Gallego, L. 2000. Meat Sci., 56: 345-349.
Vergara, H., Linares, M.B., Berruga, M.I. and Gallego, L. 2005. Meat Sci., 69:473-478.
Wilmore, D.W., Long, J.M., Mason, A.D., Pruitt, Jr., B.A. 1976. Surg. Gyecol. Obstet, 142:257-269.

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Table 2. Plasma cortisol, ACTH, adrenaline, and noradrenaline concentrations by stunning method and blood sampling time (Mean ± SEM)

	Sampling time		
Plasma cortisol level (ng/mL)	T1	T2	T3
HPP	77.11±5.91	74.24±5.30	80.89±6.76
LPP	81.59±12.42	73.73±12.65	88.67±8.20
P	69.75±6.28	76.21±6.10	79.67±5.68
US	88.17±11.17	NA	101.53±14.67
Plasma ACTH level (pg/mL)			
HPP	13.54±2.94	18.64±7.38^{a}	15.60±5.07
LPP	12.09±3.07	10.00±2.82^{ab}	22.44±8.19
P	5.60±1.45^{y}	3.87±0.89^{by}	19.75±4.35^{x}
US	14.93±3.49	NA	15.62±3.43
Plasma adrenaline level (pg/mL)			
HPP	74.08±21.13	67.62±13.65^{a}	55.98±7.91^{a}
LPP	56.27±26.84	32.78±13.09^{b}	63.49±12.68^{a}
P	38.90±20.61	15.34±2.54^{c}	29.85±9.65^{b}
US	68.14±8.38	NA	72.08±5.88^{a}
Plasma noradrenaline level (pg/mL)			
HPP	103.27±21.82^{y}	179.60±28.87^{ax}	106.43±9.96^{y}
LPP	62.05±16.16	105.02±62.22^{ab}	80.48±19.39
P	69.07±12.48	40.51±13.52^{b}	102.20±33.94
US	108.01±19.63	NA	139.78±16.99

^{a,b}Means within a column with no common letters differ at P<0.05.
^{x,y}Means within a row with no common letters differ at P<0.05.
HPP = high power non-penetrative percussive stunning prior to slaughter.
LPP = low power non-penetrative percussive stunning prior to slaughter.
P = penetrative percussive stunning prior to slaughter.
US = penetrative percussive stunning after slaughter.
NA=not available.
T1=prior to stunning; T2=post stunning; T3=post slaughter

Table 3. Plasma beta-endorphin, histamine, creatine kinase and glucose concentrations by stunning method and blood sampling time (Mean ± SEM)

	Sampling time		
Plasma beta-endorphin level (ng/mL)	T1	T2	T3
HPP	1.06±0.11	1.27±0.14	1.15±0.11
LPP	1.16±0.15	1.14±0.19	1.27±0.13
P	1.04±0.14	1.13±0.16	1.12±0.17
US	1.35±0.18	NA	1.43±0.18
Plasma histamine level (ng/mL)			
HPP	2.04±0.41	2.09±0.29	1.64±0.23
LPP	2.31±0.22	2.25±0.70	1.70±0.31
P	1.49±0.32	0.97±0.34	1.17±0.37
US	2.29±0.48	NA	1.94±0.36
Plasma creatine kinase level (U/L)			
HPP	465.92±132.75	560.11±129.35	483.57±67.92
LPP	454.59±128.89	581.60±127.03	624.91±121.89
P	847.67±390.50	568.47±326.35	748.47±376.52
US	596.25±149.45	NA	937.82±169.14
Plasma glucose level (mmol/L)			
HPP	6.16±0.39	6.17±0.47	6.65±0.44
LPP	7.66±0.86	7.75±1.51	8.30±0.93
P	6.00±0.40	6.90±1.04	7.18±0.61
US	6.35±0.26	NA	7.03±0.36

Means are not significantly different ($P>0.05$).
HPP = high power non-penetrative percussive stunning prior to slaughter.
LPP = low power non-penetrative percussive stunning prior to slaughter.
P = penetrative percussive stunning prior to slaughter.
US = penetrative percussive stunning after slaughter.
NA = not available.
T1=prior to stunning; T2=post stunning; T3=post slaughter

Table 4. pH value, cook loss, water holding capacity, tbars, and peak force of *longissimus dorsi* muscle by stunning method and day(s) of conditioning (Mean±SEM)

	Day 0	Day 1	Day 7	Day 14
Ph				
HPP	6.14±0.09^{x}	5.46±0.05^{y}	5.47±0.03^{y}	5.48±0.04^{y}
LPP	6.06±0.11^{x}	5.53±0.04^{y}	5.52±0.04^{y}	5.49±0.05^{y}
P	5.92±0.07^{x}	5.53±0.04^{y}	5.55±0.03^{y}	5.54±0.05^{y}
US	6.13±0.11^{x}	5.58±0.05^{y}	5.53±0.04^{y}	5.52±0.02^{y}
Cook loss (%)				
HPP	34.91±0.51	35.07±0.50	35.40±0.65	34.74±0.32
LPP	34.57±0.39	34.94±0.46	34.62±0.42	33.55±0.66
P	34.71±0.43	35.35±0.35	35.33±0.59	34.78±0.46
US	35.20±0.27	35.80±0.59	36.13±0.61	35.34±0.53
Water holding capacity				
HPP	0.90±0.01	0.85±0.02	0.89±0.01^{b}	0.94±0.04
LPP	0.90±0.02^{y}	0.89±0.01^{y}	0.96±0.01^{ax}	0.89±0.03^{y}
P	0.90±0.01	0.89±0.01	0.88±0.01^{b}	0.91±0.01
US	0.90±0.02	0.88±0.01	0.90±0.01^{b}	0.87±0.03
Tbars (mg/kg)				
HPP	0.28±0.02^{ay}	0.25±0.02^{ay}	0.29±0.02^{by}	0.38±0.04^{abx}
LPP	0.19±0.02^{bz}	0.28±0.03^{ay}	0.42±0.02^{ax}	0.44±0.03^{ax}
P	0.24±0.05^{aby}	0.31±0.04^{axy}	0.43±0.04^{ax}	0.30±0.04^{bxy}
US	0.06±0.01^{cz}	0.11±0.01^{bz}	0.20±0.02^{cy}	0.31±0.02^{bx}
Peak Force(kgf)				
HPP	12.15±1.37^{x}	11.88±1.00^{x}	9.58±0.70^{xy}	8.22±1.02^{y}
LPP	10.59±0.95^{y}	14.20±1.50^{x}	9.19±1.37^{y}	7.30±0.81^{y}
P	9.75±0.88^{x}	11.80±0.84^{x}	9.91±0.97^{x}	7.11±0.75^{y}
US	12.22±1.10^{xy}	12.99±0.97^{x}	9.96±0.72^{yz}	8.69±0.91^{z}

^{ab} Means within a column with no common superscripts differ at $P<0.05$.
^{xyz} Means within a row with no common superscripts differ at $P<0.05$.
HPP = high power non-penetrative percussive stunning prior to slaughter.
LPP = low power non-penetrative percussive stunning prior to slaughter.
P = penetrative percussive stunning prior to slaughter.
US = penetrative percussive stunning after slaughter.

Table 5. pH value, cook loss, water holding capacity, tbars, and peak force of *semitendinosus* muscle by stunning method and day(s) of conditioning (Mean±SEM)

	Day 0	Day 1	Day 7	Day 14
pH				
HPP	5.94±0.06^{x}	5.61±0.05^{y}	5.63±0.03^{y}	5.63±0.04^{y}
LPP	5.94±0.04^{x}	5.61±0.06^{y}	5.70±0.05^{y}	5.68±0.03^{y}
P	5.92±0.07^{x}	5.50±0.04^{y}	5.62±0.04^{y}	5.61±0.05^{y}
US	5.92±0.06^{x}	5.59±0.07^{y}	5.62±0.04^{y}	5.72±0.05^{y}
Cook loss (%)				
HPP	38.85±0.79	39.07±0.64	38.77±0.61	39.27±0.53
LPP	37.49±0.59	37.30±0.44	37.83±0.76	38.28±0.66
P	37.52±0.80	37.91±0.90	35.84±0.87	38.39±0.56
US	39.43±0.66	38.98±0.32	38.78±0.39	38.41±0.58
Water holding capacity				
HPP	0.92±0.01	0.87±0.01^{cy}	0.90±0.01^{bxy}	0.94±0.03^{x}
LPP	0.92±0.01	0.91±0.01^{ab}	0.93±0.01^{a}	0.95±0.01
P	0.91±0.02^{xy}	0.95±0.02^{ax}	0.90±0.01^{by}	0.90±0.01^{y}
US	0.90±0.01	0.90±0.01^{bc}	0.89±0.01^{b}	0.91±0.01
Tbars(mg/kg)				
HPP	0.28±0.03^{az}	0.26±0.02^{az}	0.41±0.02^{ay}	0.52±0.02^{ax}
LPP	0.14±0.02^{byz}	0.09±0.02^{bz}	0.18±0.02^{cxy}	0.21±0.02^{cx}
P	0.17±0.02^{by}	0.22±0.02^{axy}	0.30±0.05^{bx}	0.20±0.02^{cy}
US	0.17±0.03^{bz}	0.21±0.02^{ayz}	0.27±0.02^{bcxy}	0.28±0.02^{bx}
Peak Force(kgf)				
HPP	14.49±0.99^{a}	13.08±1.43	13.07±0.78^{a}	12.73±1.03^{a}
LPP	10.99±0.88^{bcxy}	9.83±0.71^{y}	12.54±1.17^{abx}	8.47±0.72^{by}
P	12.43±0.92^{ab}	10.72±1.17	9.47±0.92^{c}	8.62±1.07^{b}
US	9.51±0.87^{c}	10.37±0.82	10.19±0.84^{bc}	9.85±0.55^{b}

^{ab} Means within a column with no common superscripts differ at $P<0.05$.
^{xyz} Means within a row with no common superscripts differ at $P<0.05$.
HPP = high power non-penetrative percussive stunning prior to slaughter.
LPP = low power non-penetrative percussive stunning prior to slaughter.
P = penetrative percussive stunning prior to slaughter.
US = penetrative percussive stunning after slaughter.

Table 6. Colour characteristics (L*, a*, b*, Hue and Chroma) of *longissimus dorsi* muscle by stunning method and day(s) of conditioning Mean (± SEM)

	Day 0	Day 1	Day 7	Day 14
L				
HPP	33.38±0.47^{z}	34.70±0.68^{yz}	35.11±0.36^{y}	37.14±0.53^{x}
LPP	34.41±0.53	35.54±0.79	35.74±0.82	36.57±1.12
P	33.83±0.70^{y}	34.35±0.93^{y}	36.04±0.54^{xy}	36.93±0.98^{x}
US	34.57±0.84	35.71±0.97	36.71±0.80	37.02±0.84
a				
HPP	14.69±0.39^{y}	14.83±0.57^{y}	16.70±0.52^{x}	18.06±0.73^{x}
LPP	14.85±0.40^{y}	16.26±0.65^{y}	18.32±0.47^{x}	18.70±0.66^{x}
P	13.99±0.20^{z}	14.77±0.44^{z}	17.50±0.66^{y}	18.85±0.24^{x}
US	14.23±0.58^{y}	15.15±0.25^{y}	16.78±0.26^{x}	17.20±0.50^{x}
b				
HPP	-1.62±0.48^{by}	0.13±0.57^{bxy}	0.93±0.79^{bx}	0.08±0.47^{bxy}
LPP	0.57±0.45^{ay}	2.56±0.53^{ax}	3.61±0.41^{ax}	2.67±0.63^{ax}
P	-1.51±0.40^{by}	0.25±0.63^{bx}	1.51±0.69^{bx}	0.59±0.39^{bx}
US	-0.73±0.59^{ab}	0.32±0.51^{b}	1.22±0.77^{b}	-0.72±0.40^{b}
Enhanced redness				
HPP	-29.98±23.43	0.22±7.27	3.67±13.72	-14.24±10.58
LPP	16.24±12.52	14.55±14.15	6.23±0.65	20.27±15.96
P	-25.50±10.51	0.57±11.82	1.55±8.13	-33.07±23.65
US	-40.89±38.22	-26.42±34.30	-33.37±35.9	-14.66±8.01
Hue				
HPP	-6.43±1.81^{by}	0.13±2.10^{bx}	2.77±2.71^{x}	-0.09±1.42^{bx}
LPP	2.13±1.70^{ay}	8.69±1.77^{ax}	10.94±1.05^{x}	7.65±1.83^{ax}
P	-6.16±1.65^{by}	0.69±2.32^{bx}	4.63±2.11^{x}	1.74±1.18^{bx}
US	-3.49±2.81^{ab}	1.16±1.94^{b}	3.96±2.63	-2.62±1.37^{b}
Chroma				
HPP	14.85±0.38^{y}	14.93±0.59^{y}	16.89±0.53^{x}	18.11±0.74^{x}
LPP	14.92±0.41^{y}	16.53±0.68^{y}	18.70±0.53^{x}	18.97±0.71^{x}
P	14.12±0.19^{y}	14.88±0.46^{y}	17.67±0.69^{x}	18.90±0.24^{x}
US	14.38±0.50^{y}	15.23±0.24^{y}	16.98±0.26^{x}	17.26±0.49^{x}

^{ab} Means within a column with no common superscripts differ at $P<0.05$.
^{xyz} Means within a row with no common superscripts differ at $P<0.05$.

HPP = high power non-penetrative percussive stunning prior to slaughter.
LPP = low power non-penetrative percussive stunning prior to slaughter.
P = penetrative percussive stunning prior to slaughter.

Table 7. Colour characteristics (L*, a*, b*, Hue and Chroma) of *semitendinosus* muscle stunning method and day(s) of conditioning (Mean±SEM)

	Day 0	Day 1	Day 7	Day 14
L				
HPP	36.29±0.72 ^{bcy}	38.40±0.93 ^{abxy}	37.08±0.67^{by}	40.01±0.88^{x}
LPP	35.84±0.53^{cy}	37.13±0.69 ^{bxy}	37.15±0.91 ^{bxy}	39.40±0.86^{x}
P	38.14±0.73^{ab}	41.05±0.84^{a}	40.19±0.72^{a}	40.66±0.98
US	38.76±0.70^{a}	39.44±1.05 ^{ab}	40.27±1.15 ^{a}	41.59±1.03
A				
HPP	14.99±0.40	14.08±0.41	15.85±0.54	15.95±1.43
LPP	14.82±0.41 ^{z}	15.31±0.30 ^{yz}	16.34±0.67 ^{xy}	17.66±0.51 ^{x}
P	14.55±0.63^{y}	14.68±0.63^{y}	16.26±0.70 ^{y}	18.05±0.39^{x}
US	14.94±0.40 ^{xy}	13.81±0.47 ^{y}	15.43±0.26 ^{x}	16.17±0.83^{x}
B				
HPP	-0.11±0.46	0.94±0.51^{b}	1.26±0.73	0.46±0.24^{c}
LPP	-0.12±0.53^{y}	1.79±0.21^{abx}	2.2±0.71^{x}	1.92±0.42^{ax}
P	-0.07±0.47^{y}	2.53±0.60^{ax}	2.80±0.70^{x}	1.74±0.42^{abx}
US	-0.99±0.64	0.92±0.39^{b}	2.10±0.59	0.66±0.41^{bc}
Enhanced redness				
HPP	-5.74±6.7	19.95±20.37	1.70±7.42	0.55±12.01
LPP	-9.10±5.22 ^{y}	10.3±1.28 ^{x}	3.2±4.16 ^{x}	7.00±4.26 ^{x}
P	-42.58±35.77	1.9±8.62	18.37±9.24	104.92±84.88
US	25.32±23.70	5.78±13.69	2.39±2.99	5.97±6.87
Hue				
HPP	-0.42±1.76	3.77±1.95	3.92±2.57	2.37±1.32
LPP	-0.69±1.98^{y}	6.61±0.75^{x}	6.97±2.14^{x}	5.93±1.27^{x}
P	-0.09±1.85^{y}	9.4±2.11^{x}	9.71±2.42^{x}	5.32±1.19^{xy}
US	3.58±2.40	3.79±1.53	7.75±2.22	2.01±1.32
Chroma				
HPP	15.05±0.40	14.18±0.43	16.04±0.56	15.98±1.43
LPP	14.91±0.42^{z}	15.42±0.31^{yz}	16.59±0.75^{xy}	17.80±0.54^{x}
P	14.62±0.63^{z}	14.99±0.68^{yz}	16.63±0.71^{xy}	18.17±0.42^{x}
US	15.09±0.41^{xy}	13.89±0.48^{y}	15.67±0.23^{x}	16.22±0.84^{x}

^{ab} Means within a column with no common superscripts differ at $P<0.05$.
^{xyz} Means within a row with no common superscripts differ at $P<0.05$.
HPP = high power non-penetrative percussive stunning prior to slaughter.
LPP = low power non-penetrative percussive stunning prior to slaughter.

P = penetrative percussive stunning prior to slaughter.

Alpha Waves	T1	T2	T3
HPP	9.52 ± 2.33^{axy}	16.74 ± 4.55^{ax}	5.12 ± 1.34^{ay}
LPP	9.03 ± 1.16^{ax}	17.65 ± 4.11^{ax}	7.79 ± 2.36^{ax}
P	7.34 ± 1.54^{ax}	9.06 ± 2.42^{bx}	4.93 ± 1.81^{ax}
US	11.77 ± 2.70^{ax}	NA	31.44 ± 8.94^{by}
Beta Waves			
HPP	14.10 ± 3.42^{ax}	33.26 ± 13.45^{ax}	16.50 ± 6.10^{ax}
LPP	10.67 ± 1.48^{ax}	21.71 ± 6.93^{ax}	11.52 ± 3.53^{ax}
P	8.51 ± 1.34^{ax}	10.58 ± 2.49^{bx}	6.30 ± 1.90^{bx}
US	17.43 ± 3.80^{ax}	NA	37.53 ± 16.58^{ay}
Delta Waves			
HPP	28.39 ± 6.07^{ax}	43.04 ± 7.47^{ax}	21.96 ± 7.92^{ax}
LPP	42.96 ± 5.03^{ax}	95.65 ± 15.66^{by}	24.48 ± 5.68^{ax}
P	33.26 ± 6.38^{ax}	42.08 ± 14.11^{ax}	17.01 ± 6.70^{ax}
US	48.91 ± 10.33^{ax}	NA	51.77 ± 15.78^{ax}
Theta Waves			
HPP	12.26 ± 2.34^{axy}	18.97 ± 4.58^{abx}	5.76 ± 1.41^{ay}
LPP	17.14 ± 2.31^{ax}	24.94 ± 5.66^{ax}	11.36 ± 4.40^{ax}
P	11.02 ± 2.07^{ax}	11.57 ± 2.74^{bx}	5.17 ± 1.94^{ax}
US	19.93 ± 5.09^{ax}	NA	33.06 ± 16.32^{ax}

US = penetrative percussive stunning after slaughter.

Table 8. Electroencephalogram Root Mean Square (RMS) values (microvolts) by stunning method and time point (Mean ± SEM)

[ab] Means within a column with no common superscripts differ at $P<0.05$.
[xyz] Means within a row with no common superscripts differ at $P<0.05$.
HPP = high power non-penetrative percussive stunning prior to slaughter.
LPP = low power non-penetrative percussive stunning prior to slaughter.

P = penetrative percussive stunning prior to slaughter.
US = penetrative percussive stunning after slaughter.
NA=not available.
T1=prior to stunning; T2=post stunning; T3=post slaughter

Table 9. Terminal alpha, beta, delta and theta RMS values and time post slaughter to achieve the terminal values by stunning method (Mean ± SE)

Alpha Waves	Terminal RMS values (microvolts)
HPP	1.55 ± 0.27^{ab}
LPP	2.01 ± 0.24^{ab}
P	1.49 ± 0.30^{a}
US	0.97 ± 0.16^{b}
Beta Waves	Terminal RMS values (microvolts)
HPP	2.93 ± 0.85^{a}
LPP	2.48 ± 0.16^{a}
P	1.79 ± 0.29^{a}
US	1.42 ± 0.26^{a}
Delta Waves	Terminal RMS values (microvolts)
HPP	3.72 ± 0.70^{a}
LPP	7.60 ± 1.21^{a}
P	5.56 ± 1.84^{a}
US	5.03 ± 1.93^{a}
Theta Waves	Terminal RMS values (microvolts)
HPP	1.94 ± 0.31^{a}
LPP	3.00 ± 0.32^{b}
P	1.89 ± 0.44^{a}
US	1.43 ± 0.26^{a}
	Time from post slaughter (s) to attain terminal RMS values
HPP	173.40 ± 15.43^{a}
LPP	214.00 ± 11.71^{a}
P	194.50 ± 16.61^{a}
US	178.50 ± 26.49^{a}

[ab] Means within a column with no common superscripts differ at $P<0.05$.
HPP = high power non-penetrative percussive stunning prior to slaughter.
LPP = low power non-penetrative percussive stunning prior to slaughter.
P = penetrative percussive stunning prior to slaughter.
US = penetrative percussive stunning after slaughter.

Table 10. Plasma cortisol, ACTH, adrenaline, and noradrenaline, concentrations by stunning-thoracic sticking method and blood sampling time (Mean ± SEM)

	Sampling time			
Plasma cortisol level (ng/mL)	T1	T2	T3	T4
LPPS	63.62±10.37	66.75±8.29	74.42±4.87	72.33±6.24
PS	87.62±8.44	68.22±6.06	72.14±6.15	82.06±9.08
US	88.17±11.17	NA	101.53±14.67	NA
Plasma ACTH level (pg/mL)				
LPPS	9.06±2.64	15.59±7.27	17.86±7.44	15.68±6.49
PS	15.60±5.12	12.86±5.46	31.94±12.51	25.06±9.58
US	14.93±3.49	NA	15.62±3.43	NA
Plasma adrenaline level (pg/mL)				
LPPS	97.01±15.22	102.00±24.19	78.16±9.37	92.33±13.02
PS	96.65±21.25	82.05±27.40	61.51±15.98	80.03±13.81
US	68.14±8.37	NA	72.08±5.88	NA
Plasma noradrenaline level (pg/mL)				
LPPS	208.67±34.35[a]	238.86±64.62	240.26±39.71[a]	175.54±22.45
PS	116.23±17.57[b]	190.54±25.43	136.73±28.36[b]	164.79±26.41
US	108.01±19.63[b]	NA	139.78±16.99[b]	NA

[a,b]Means within a column with no common letters differ at P<0.05.
LPPS = low power non-penetrative percussive stunning prior to slaughter and thoracic sticking.
PS = penetrative percussive stunning prior to slaughter and thoracic sticking
US = penetrative percussive stunning after slaughter and no thoracic sticking
NA= not available.
T1=prior to stunning; T2=post stunning; T3=post slaughter; T4=post thoracic sticking

Table 11. Plasma beta-endorphin, histamine, creatine kinase and glucose concentrations by stunning-thoracic sticking method and blood sampling time (Mean±SEM)

	Sampling time			
Plasma beta-endorphin level (ng/mL)	T1	T2	T3	T4
LPPS	0.70±0.09[b]	1.01±0.16	0.91±0.13	0.89±0.16
PS	0.70±0.05[bxy]	0.57±0.11[y]	0.88±0.05[x]	0.77±0.05[x]
US	1.35± 0.18[a]	NA	1.43±0.18	NA
Plasma histamine level (ng/mL)				
LPPS	2.93±0.35	2.65±0.32	2.94±0.58	2.45±0.47
PS	2.37±0.46	2.14±0.27	1.91±0.29	1.89±0.23
US	2.29±0.48	NA	1.94±0.36	NA
Plasma creatine kinase level (U/L)				
LPPS	666.01±242.56	747.17±209.30	340.74±39.23[b]	387.74±72.96
PS	411.46±117.90	1054.48±439.58	443.46±71.61[b]	381.91±62.65
US	596.29±149.45	NA	937.82±169.14[a]	NA
Plasma glucose level (mmol/L)				
LPPS	5.83±0.24	6.20±0.46	6.71±0.45	7.35±0.52
PS	6.83±0.60	6.48±1.05	7.52±0.87	8.43±0.97
US	6.35±0.26	NA	7.03±0.36	NA

[a,b]Means within a column with no common letters differ at P<0.05.
LPPS = low power non-penetrative percussive stunning prior to slaughter and thoracic sticking.
PS = penetrative percussive stunning prior to slaughter and thoracic sticking
US = penetrative percussive stunning after slaughter and no thoracic sticking

NA= not available.
T1=prior to stunning; T2=post stunning; T3=post slaughter; T4=post thoracic sticking

Table 12. pH value, cook loss, water holding capacity, tbars, and texture of *longissimus dorsi* muscle by stunning-thoracic sticking method and day(s) of conditioning (Mean±SEM)

	Day 0	Day 1	Day 7	Day 14
pH				
LPPS	6.19±0.08^{x}	5.70±0.12^{y}	5.48±0.04^{y}	5.57±0.04^{y}
PS	6.05±0.07^{x}	5.65±0.10^{y}	5.62±0.07^{y}	5.67±0.07^{y}
US	6.13±0.11^{x}	5.58±0.05^{y}	5.53±0.04^{y}	5.52±0.02^{y}
Cook loss (%)				
LPPS	33.48±0.49^{b}	35.29±0.67	34.39±0.54^{ab}	33.20±0.70
PS	32.99±0.57^{b}	33.70±0.64	33.48±0.93^{b}	33.15±0.95
US	35.20±0.27^{a}	35.80±0.59	36.13±0.61^{a}	35.34±0.53
Water holding capacity				
LPPS	0.92±0.01^{x}	0.89±0.01^{by}	0.87±0.01^{by}	0.92±0.01^{x}
PS	0.91±0.01	0.94±0.01^{a}	0.93±0.01^{a}	0.92±0.01
US	0.90±0.02	0.88±0.01^{b}	0.90±0.01^{ab}	0.87±0.03
Tbars (mg/kg)				
LPPS	0.21±0.03^{by}	0.26±0.03^{ay}	0.37±0.05^{ax}	0.20±0.02^{cy}
PS	0.12±0.04^{a}	0.12±0.04^{a}	0.16±0.06^{a}	0.22±0.03^{a}
US	0.17±0.03^{cz}	0.21±0.02^{bz}	0.27±0.02^{by}	0.28±0.02^{bx}
Peak Force (kgf)				
LPPS	9.67±0.91^{abxy}	10.19±0.62^{bx}	7.80±0.93^{aby}	5.35±0.43^{bz}
PS	8.50±0.85^{bx}	8.59±1.14^{bx}	5.67±0.66^{by}	4.38±0.31^{by}
US	12.22±1.10^{axy}	12.99±0.97^{ax}	9.96±0.72^{ayz}	8.69±0.91^{az}

^{ab} Means within a column with no common superscripts differ at $P<0.05$.
^{xyz} Means within a row with no common superscripts differ at $P<0.05$.
LPPS = low power non-penetrative percussive stunning prior to slaughter and thoracic sticking.
PS = penetrative percussive stunning prior to slaughter and thoracic sticking.
US = penetrative percussive stunning after slaughter and no thoracic sticking.

Table 13. pH value, cook loss, water holding capacity, tbars and texture of *semitendinosus* muscle by day(s) stunning method and day(s) of conditioning (Mean±SEM)

	Day 0	Day 1	Day 7	Day 14
pH				
LPPS	6.04±0.04^{x}	5.67±0.06^{yz}	5.59±0.04^{z}	5.74±0.04^{y}
PS	5.94±0.09	5.69±0.12	5.59±0.09	5.84±0.11
US	5.92±0.06^{x}	5.59±0.07^{y}	5.62±0.04^{y}	5.72±0.05^{y}
Cook loss (%)				
LPPS	39.63±0.46^{a}	40.04±0.56	38.81±0.59	39.00±0.63
PS	36.40±1.25^{b}	38.28±1.6	39.23±3.19	38.09±1.21
US	39.43±0.66 ^{a}	38.98±0.32	38.78±0.39	38.41±0.58
Water holding capacity				
LPPS	0.93±0.01^{x}	0.87±0.01^{bz}	0.90±0.01^{by}	0.92±0.01^{x}
PS	0.92±0.01	0.91±0.02^{a}	0.93±0.01^{a}	0.92±0.01
US	0.90±0.01	0.90±0.01 ^{ab}	0.89±0.01^{b}	0.91±0.01
Tbars (mg/kg)				
LPPS	0.21±0.03^{y}	0.26±0.03^{ay}	0.37±0.05^{ax}	0.20±0.02^{y}
PS	0.12±0.04	0.12±0.04 ^{b}	0.16±0.06^{b}	0.22±0.03
US	0.17±0.03 ^{z}	0.21±0.02 ^{abyz}	0.27±0.02 ^{abxy}	0.28±0.02 ^{x}
Peak Force				
LPPS	10.65±0.92^{x}	10.62±0.67^{x}	8.35±1.01^{abxy}	7.19±0.9^{by}
PS	8.77±0.92	8.09±1.03	6.23±0.57 ^{b}	6.64±0.83 ^{b}
US	9.51±0.87	10.37±0.82	10.19±0.84^{a}	9.85±0.55^{a}

^{ab} Means within a column with no common superscripts differ at $P<0.05$.
^{xyz} Means within a row with no common superscripts differ at $P<0.05$.
LPPS = low power non-penetrative percussive stunning prior to slaughter and thoracic sticking.
PS = penetrative percussive stunning prior to slaughter and thoracic sticking.
US = penetrative percussive stunning after slaughter and no thoracic sticking.

Table 14. Colour characteristics (L, a*, b*, Hue and Chroma) of *longissimus dorsi* by stunning-thoracic sticking method and by day(s) of conditioning (Mean ± SEM)

	Day 0	Day 1	Day 7	Day 14
L				
LPPS	33.37±0.53^{y}	33.80±0.57^{xy}	35.27±0.75^{xy}	35.81±0.81^{x}
PS	34.76±0.52	36.08±1.08	35.43±0.97	37.09±0.94
US	34.57±0.84	35.71±0.97	36.71±0.80	37.02±0.84
A				
LPPS	15.99±0.20^{ay}	15.87±0.36^{y}	17.98±0.35^{x}	18.00±0.48^{x}
PS	16.70±0.51^{a}	16.21±0.41	17.33±0.56	17.64±0.56
US	14.23±0.58^{by}	15.15±0.25^{y}	16.78±0.26^{x}	17.20±0.50^{x}
b				
LPPS	0.32±0.41^{a}	0.59±0.47	1.75±0.43^{a}	0.45±0.35
PS	-1.54±0.38^{b}	-1.00±0.66	0.54±0.61^{b}	-0.82±0.55
US	-0.73±0.59^{ab}	0.32±0.51	1.22±0.77^{ab}	-0.72±0.40
Enhanced redness				
LPPS 0.3297	-15.13±16.73	-2.49±11.31	-5.14±11.94	20.89±15.33^{a}
PS 0.5719	-10.82±10.43	4.35±16.76	-56.92±58.74	-28.04±15.57^{b}
US 0.9454	-40.89±38.22	-26.42±34.30	-33.37±35.9	-14.66±8.01
Hue				
LPPS 0.1234	1.06±1.43	1.86±1.68	5.4±1.35^{a}	1.32±1.14
PS 0.7060	-5.59±1.51	-3.95±2.41	-2.25±2.33^{b}	-2.92±2.14
US 0.0900	-3.49±2.81	1.16±1.94	3.96±2.63^{ab}	-2.62±1.37
Chroma				
LPPS	16.04±0.21^{ay}	15.94±0.37^{y}	18.11±0.37^{x}	18.03±0.48^{x}
PS	16.82±0.47^{a}	16.37±0.37	17.46±0.49	17.75±0.49
US	14.38±0.50^{by}	15.23±0.24^{y}	16.98±0.26^{x}	17.26±0.49^{x}

^{ab} Means within a column with no common superscripts differ at $P<0.05$.
^{xyz} Means within a row with no common superscripts differ at $P<0.05$.
LPPS = low power non-penetrative percussive stunning prior to slaughter and thoracic sticking.
PS = penetrative percussive stunning prior to slaughter and thoracic sticking.
US = penetrative percussive stunning after slaughter and no thoracic sticking.

Table 15. Mean (± SEM) colour characteristics (L, a*, b*, Hue and Chroma) of *semitendinosus* samples by day(s) of conditioning and stunning method

	Day 0	Day 1	Day 7	Day 14
L				
LPPS	37.32±0.25^{y}	37.42±0.62^{y}	38.42±0.58^{xy}	39.56±0.84^{x}
PS	38.64±1.05^{y}	37.93±1.11^{y}	39.08±0.97^{y}	42.06±0.87^{x}
US	38.76±0.70	39.44±1.05	40.27±1.15	41.59±1.03
a				
LPPS	16.11±0.37^{ay}	16.37±0.24^{ay}	18.11±0.45^{ax}	17.70±0.41^{x}
PS	15.16±0.17^{aby}	15.44±0.32^{ay}	16.88±0.57^{ax}	17.15±0.68^{x}
US	14.94±0.40^{bxy}	13.81±0.47^{by}	15.43±0.26^{bx}	16.17±0.83^{x}
b				
LPPS	-0.14±0.30^{aby}	0.38±0.30^{y}	1.57±0.38^{x}	0.53±0.36^{y}
PS	-0.99±0.49^{b}	-0.37±0.61	0.74±0.72	0.44±0.48
US	-0.99±0.64^{a}	0.92±0.39	2.10±0.59	0.66±0.41
Enhanced redness				
LPPS	-38.34±14.45^{b}	14.76±35.46	-25.91±41.61	6.32±6.94
PS	-7.08±3.33^{ab}	-30.68±20.81	30.51±27.35	-79.18±60.50
US	25.32±23.70^{b}	5.78±13.69	2.39±2.99	5.97±6.87
Hue				
LPPS	-0.68±0.99^{aby}	1.26±1.04^{y}	4.74±1.06^{x}	1.63±1.18^{y}
PS	-3.67±1.85^{b}	-1.58±2.37	1.81±2.48	1.00±1.73
US	3.58±2.40^{a}	3.79±1.53	7.75±2.22	2.01±1.32
Chroma				
LPPS	16.13±0.38^{y}	16.40±0.24^{ay}	18.21±0.48^{x}	17.74±0.40^{x}
PS	15.26±0.18^{y}	15.56±0.29^{ay}	17.03±0.57^{x}	17.22±0.67^{x}
US	15.09±0.41^{xy}	13.89±0.48^{by}	15.67±0.23^{x}	16.22±0.84^{x}

^{ab} Means within a column with no common superscripts differ at $P<0.05$.
^{xyz} Means within a row with no common superscripts differ at $P<0.05$.
LPPS = low power non-penetrative percussive stunning prior to slaughter and thoracic sticking.
PS = penetrative percussive stunning prior to slaughter and thoracic sticking.
US = penetrative percussive stunning after slaughter and no thoracic sticking.

Table 16. Electroencephalogram RMS values (microvolts) by stunning method and time point (Mean ± SEM)

Alpha Waves	T1	T2	T3
LPPS	16.22 ± 3.70^{ax}	20.85 ± 5.51^{ax}	2.73 ± 0.64^{ay}
PS	10.83 ± 2.59^{ax}	13.91 ± 5.74^{ax}	2.24 ± 0.49^{ay}
US	11.77 ± 2.70^{ax}	NA	31.44 ± 8.94^{by}
Beta Waves			
LPPS	20.65 ± 4.59^{ax}	26.88 ± 7.62^{ax}	4.12 ± 1.04^{ay}
PS	12.93 ± 2.79^{ax}	12.61 ± 4.59^{bx}	2.90 ± 0.45^{ay}
US	17.43 ± 3.80^{ax}	NA	37.53 ± 16.58^{bx}
Delta Waves			
LPPS	40.53 ± 10.49^{ax}	48.51 ± 11.75^{ax}	5.85 ± 1.59^{ay}
PS	28.53 ± 10.10^{ax}	29.83 ± 11.98^{ax}	9.09 ± 5.05^{ax}
US	48.91 ± 10.33^{ax}	NA	51.77 ±15.78^{bx}
Theta Waves			
LPPS	23.81 ± 6.09^{ax}	25.36 ± 6.24^{ax}	3.98 ± 0.94^{ay}
PS	11.71 ± 3.22^{ax}	14.71 ± 5.55^{ax}	3.36 ± 0.98^{ax}
US	19.93 ± 5.09^{ax}	NA	33.06 ± 16.32^{bx}

^{ab} Means within a column with no common superscripts differ at $P<0.05$.
^{xyz} Means within a row with no common superscripts differ at $P<0.05$.
LPPS = low power non-penetrative percussive stunning prior to slaughter and thoracic sticking.
PS = penetrative percussive stunning prior to slaughter and thoracic sticking
US = penetrative percussive stunning after slaughter and no thoracic sticking
NA= not available.
T1=prior to stunning; T2=post stunning; T3=post slaughter

Table 17. Terminal alpha, beta, delta and theta RMS values and time post slaughter to achieve the terminal values by stunning-thoracic sticking method (Mean ± SE)

Alpha Waves	Terminal RMS values (microvolts)
LPPS	2.35 ± 0.68
PS	2.24 ± 0.78
US	0.97 ± 0.16
Beta Waves	Terminal RMS values (microvolts)
LPPS	3.81 ± 0.93
PS	2.74 ± 0.88
US	1.42 ± 0.26
Delta Waves	Terminal RMS values (microvolts)
LPPS	5.15 ± 1.26
PS	10.02 ± 6.37
US	5.03 ± 1.93
Theta Waves	Terminal RMS values (microvolts)
LPPS	2.37 ± 0.64
PS	2.54 ± 0.80
US	1.43 ± 0.26
	Time from post slaughter (s) to attain terminal RMS values
LPPS	183.10 ± 13.67
PS	179.30 ± 7.69
US	178.50 ± 26.49

All means are not significantly different (P>0.05).
LPPS = low power non-penetrative percussive stunning prior to slaughter and thoracic sticking.
PS = penetrative percussive stunning prior to slaughter and thoracic sticking
US = penetrative percussive stunning after slaughter and no thoracic sticking