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A PRELIMINARY COMPARISON OF BEEF CARCASES STUNNED USING DTS: DIATHERMIC SYNCOPE® OR CAPTIVE BOLT IN TERMS OF SELECTED MEAT QUALITY ATTRIBUTES AND PLASMA BIOMARKER CONCENTRATIONS

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ABSTRACT

A novel technology for inducing unconsciousness prior to slaughter has been developed (DTS). For commercial reasons meat quality attributes are important, and impacts on endocrine responses provide an indication of animal welfare. We compared endocrine, ultimate pH and meat quality (after 1 and 10 weeks of storage) attributes of cattle carcases processed using DTS with those processed using penetrative captive bolt (CB). There were no significant differences between treatments in the change in plasma cortisol, adrenocorticotropic hormone, β-endorphin and catecholamines between baseline and immediately post-slaughter, and no significant differences in terms of pH at 24 h post slaughter. There were no significant differences in pH, shear force, TBARS or drip loss between DTS and CB samples of round and loin at week 1 and week 10 post slaughter; DTS meat was yellower at quartering (MINOLTA b* $P < 0.05$). At week 1, DTS loins were redder (MINOLTA a*, $P < 0.05$) and yellower (MINOLTA b*, $P < 0.05$), while DTS rounds were lighter (MINOLTA L*, $P < 0.05$), than CB. At week 10, there were no significant meat color differences between treatments. Processing cattle using DTS results in endocrine changes and meat quality attributes that are comparable to CB.

Contribution/Originality: DTS: Diathermic Syncope® is a novel method for inducing unconsciousness in cattle prior to slaughter. This is the first study investigating the impacts of using DTS in cattle processing on meat quality attributes of the carcases and endocrine responses of the animals.

1. INTRODUCTION

To ensure that animal welfare is safeguarded at the point of slaughter, there is an urgent need to develop a means of rendering cattle insensible prior to slaughter that also complies with the requirements of the Halal and Kosher markets: namely that the animal is whole and undamaged at the time of slaughter, and that the animal does not die as a result of the stunning method [1]. Mechanical stunning results in damage to the skull and brain [2, 3] and while head-only electrical stunning does not result in physical damage and the animal can recover, carcase quality problems such as ecchymoses (blood splash) in the muscle [4, 5] pose a commercial obstacle to widespread adoption of electrical stunning in prime beef production. Furthermore, if an animal is mis-stunned and experiences sub-convulsive stimulation rather than Grand mal epilepsy subsequent to electrical stunning, this could be detrimental to welfare [6]. A novel technology that uses microwave energy to selectively raise the temperature of

the brain and thereby induce unconsciousness has been developed (DTS: Diathermic Syncope®; patent WO2011137497-A) and has been shown to induce electro encephalographic (EEG) changes incompatible with sensibility [7, 8].

The purpose of the study reported here was to conduct a preliminary evaluation of the meat quality (pH, shear force, lipid oxidation, water holding capacity and meat color) and endocrine (cortisol, adrenocorticotropic hormone (ACTH), β-endorphin and catecholamines) attributes of cattle carcases stunned prior to slaughter using the DTS: Diathermic Syncope® system (DTS), as compared with cattle stunned prior to slaughter using penetrative captive bolt (CB).

2. MATERIALS AND METHODS

Research ethics: This study was conducted with the approval of the Victorian State Government Wildlife and Small Institutions Animal Ethics Committee, Authority reference 29.13.

2.1. Sample Collection

Samples were collected during conduct of Trial 1 as described by Small, et al. [8]. Briefly, the study involved 18 Angus cross-bred heifers in the liveweight range 350-400 kg. They were sourced from the normal commercial intake at the abattoir and had been lairaged for 4 days with ad-libitum water and hay provided. The study was conducted over a 2-day period in September 2014, 7 animals processed on the first day (reduced numbers to allow time for discussion with State Regulators and Animal Ethics Committee Inspectors), and 11 on the second. Assigned treatments were: Penetrative Captive Bolt (CB); Cash Magnum .22 caliber penetrative captive bolt (Frontmatec, UK), with a 4-grain cartridge, applied at the frontal position (n = 7); DTS 30 kW incident power (n = 5) and DTS 20 kW incident power (n = 6). During DTS application, technical faults in the system resulted in variable total energy (kJ) being delivered to each animal. DTS energy deliveries ranged from 3.55 to 297.97 kJ. Animals were assigned to treatments randomly as they were presented at the point of slaughter. Each animal was individually brought to the restraint unit by a single lairage staff member, using low-stress animal handling techniques (used of flight-zone pressure-release to move animals within the lairage, and 'flapper' and voice within the race. Electric goads were not used). At the knocking box, a baseline blood sample (Vacutainer brand, anticoagulant Ethylenediaminetetraacetic acid (EDTA), Becton Dickinson (BD) Australia, North Ryde, New South Wales (NSW)) was taken from the tail vein, and the animal's head was captured and restrained for stunning. Restraint was prolonged (2-5 minutes) to allow pre-treatment EEG to be collected (reported in Small, et al. [8]). Following application of the assigned treatment, corneal reflexes, visual function of the eye and response to a painful stimulus of the nose was assessed, and the stunned carcass rolled onto the bleed table. Exsanguination was delayed for 60-120 s to allow EEG to be collected (reported in Small, et al. [8]). A thoracic sticking procedure was used, and a second blood sample collected from the free-flowing exsanguinate. The carcase was then dressed as normal practice, chilled overnight, and de-boned the following day. All blood samples were centrifuged and plasma extracted within one hour of collection. Plasma samples were frozen and stored at -20°C until laboratory processing within 3 months of collection.

Carcase dressing was monitored, with attention paid to evidence of bruises or blood splash. During chilling, pH and temperature measurements were taken at one hour post treatment application until the pH had dropped below pH6 (approximately 4 hours), using a WP-80 digital pH meter (TPS Instruments Pty Ltd., Springwood, Queensland (QLD)), with a combination electrode for temperature compensation. The meter was calibrated using pH4 and pH 7 standards immediately before use, after every hour, and at the end of the session according to the manufacturer's instructions. pH, temperature and meat color, measured using a MINOLTA CR300® colorimeter under light source D65, calibrated immediately before and after the measurement session using a standard white

tile as per the manufacturer's instructions, were recorded from the carcases at quartering, approximately 24 hours post slaughter, prior to de-boning.

During deboning, members of the plant quality assurance (QA) staff and the research team observed the process, with attention paid for any abnormality such as blood splash; ecchymosis or discoloration of the primals. At de-boning, a sample of loin (m. longissimus lumborum) and round (m. semitendinosus) was removed, vacuum packed and refrigerated. One sample of each muscle was randomly assigned to a one-week storage treatment; while the other assigned to a 10-week storage treatment. These samples were transported to the laboratory by refrigerated vehicle, within the first week post slaughter, and placed in a cold room set to 0°C ± 2°C for storage.

2.2. Meat Quality Analysis

2.2.1. Sample Collection and Preparation

At each of 1 week post slaughter, and 10 weeks post slaughter, the muscle samples were unpacked, and sectioned into subsamples for color, pH, shear force, lipid oxidation and drip loss evaluation.

2.2.2. pH

A WP-80 digital pH meter (TPS Instruments, Springwood, QLD), which incorporated a combination electrode to allow temperature compensation, was used to measure carcase pH. The pH meter was calibrated using pH4 and pH 7 standards immediately before use, after every 18 measurements, and at the end of the session according to the manufacturer's instructions. The probes were inserted at least 10 mm into the substance of the muscle from the cross-grain cut surface, and data recorded when the readings had stabilised.

2.2.3. Shear Force

For shear force measurement, samples were first cooked for 60 minutes at 70°C, before being tested using a Lloyd Instruments LRX® Materials testing machine The machine was fitted with a 500 N load cell (Lloyd Instruments Ltd., Hampshire UK). The methodology used for measurement of Warner-Bratzler (WB) shear force followed the procedures described by Bouton, et al. [9] and Bouton and Harris [10].

2.2.4. Lipid Oxidation

Lipid oxidation was determined by the thiobarbituric acid-reactive substances (TBARS) method of Witte, et al. [11]. Duplicate, meat samples (2 ± 0.01 g) were first heated at 75°C for 20 minutes in a water bath and cooled on ice (30 min at 5 °C). A standard curve of malondialdehyde (MDA) was prepared by acidification of TEP (1,1,3,3-tetraethoxypropane), and TBARS, expressed as mg MDA per kg sample were calculated from this curve.

2.2.5. Meat Color

Meat color was measured using a MINOLTA CR400® chromameter (Minolta Pty Ltd., Japan, light source D65, observer angle 2°, Φ11 mm illumination area) calibrated immediately before and after each measurement session using a standard white tile as per the manufacturer's instructions.

2.2.6. Water Holding Capacity

Drip loss was measured using the method outlined by Honikel, et al. [12] and Warner, et al. [13]. Briefly, blocks of muscle were cut to a target weight of approximately 50 g, measuring 2-3 cm thick and 3-4 cm across. Each block was weighed to the nearest 0.1g on a laboratory balance (SPE6001, Ohaus Corporation, USA), and suspended using suture material (3.5 metric braided silk, Ethicon Inc, USA) from a frame housed in a cold room set to 4 ± 2 °C. After 48 h storage, the muscle blocks were removed from the frame and reweighed. Drip loss was calculated as the percentage reduction in weight over 48 hours.

2.3. Plasma Sample Analysis

Plasma samples were tested for cortisol concentration using Radio-Immuno-Assay (Cortisol CT Kit, MP Biomedical, Eschwege, Germany), adapted and validated for bovine plasma according to the method described by Paull, et al. [14]. Plasma concentrations of ACTH were determined using commercial Enzyme-Immuno-Assay (EIA) kits (EK-001-01; Phoenix Pharmaceuticals Inc., Burlingame, CA, USA). Plasma β-endorphin was determined using commercial EIA kits (EK-022-14; Phoenix Pharmaceuticals Inc., Burlingame, California (CA), USA). Plasma concentrations of the catecholamines (epinephrine and norepinephrine) were determined using the Cat-Combi Enzyme-Linked Immunosorbent Assay (ELISA) kit (RE59242; IBL Hamburg, Germany). All commercial kits were used in accordance with the manufacturers' instructions.

2.4. Statistical Analysis

Data were analysed using the nlme package within Core and Team [15]. Plasma biomarker data were analysed within timepoint (baseline, T1, and post treatment, T2), and as the change in concentration between T1 and T2. Differences between treatments for each parameter measured were assessed using a mixed model, fitting day (2 levels) and treatment (3 levels), with animal as the random variable and were considered significant at the $P<0.05$ level. The distribution of the residuals from the model were checked for normality using the Shapiro-Wilkes test, and transformations were used when required. pH declines were analysed using a repeated measures procedure to account for non-independent data points. Animal 17 was excluded from analysis: it received a very low dose of energy (35.55 kJ), which did not render the animal insensible, and was euthanased by captive bolt stun and exsanguination. Initial analysis indicated that there was no significant effect of day, and that there were no significant differences between DTS 30 kW and DTS 20 kW treatments. Thus, DTS data were pooled leading to a simple regression with treatment (CB or DTS) as the only fixed variable.

3. RESULTS

3.1. Carcase Characteristics

Superficial bruising was evident on the bony protuberance of the wing of the ilium (pelvis) on one, or both sides of 12 carcases, 6 of which were captive bolt animals. The right side was most often affected, and where the left side was affected, the bruise was smaller than that on the right side. These bruises are most likely to be associated with pre-treatment contact with the rigid structure of the raceway and restraint box. On deboning, no evidence of blood splash, ecchymosis or discoloration of primal cuts was noted.

3.2. pH

Values below pH 6 while the carcase temperature remained above 35°C were recorded for animals 7 (DTS 20 kW, 191.28 kJ: pH 5.87 at 36.0°C, 1 h post slaughter); 14 (DTS 30 kW, 45.87 kJ: pH 5.84 at 37.6°C, 1 h post slaughter) and 16 (DTS 20 kW, 184.68 kJ: pH 5.91 at 38.2°C, 1 h post slaughter). In all carcases, pH had dropped below 6 before a temperature of 15°C was recorded. There were no significant differences between treatments ($P > 0.05$) in terms of pH at 24 h post slaughter (pHu, CB 5.69 ± 0.05, DTS 5.68 ± 0.06). Similarly, there were no significant differences in pH between DTS and CB samples of round and loin at week 1 and week 10 post slaughter Table 1.

3.3. Shear Force, Lipid Oxidation and Water Holding Capacity

Shear force in both loin and in round did not differ significantly between treatments ($P \geq 0.05$) at either week 1 or week 10 (Table 1). There were no significant differences in TBARs measurements by treatment ($P > 0.05$) at week 1 or week 10, in either muscle Table 1. There were no significant treatment differences in drip loss ($P > 0.05$) from either round or loin at week 1 or week 10 of storage Table 1.

3.4. Meat Color

Meat color measurements taken at quartering, on the day following slaughter; one week post slaughter and 10 weeks post slaughter are shown in Table 1. DTS meat was slightly yellower at quartering (MINOLTA b* 2.71 ± 0.59 DTS; 1.06 ± 0.44 CB, $P < 0.05$); DTS loins were slightly redder (MINOLTA a* 23.22 ± 0.92 DTS; 20.89 ± 0.69 CB, $P < 0.05$) and slightly yellower (MINOLTA b* 2.79 ± 0.93 DTS; 0.77 ± 0.70 CB, $P < 0.05$) at week 1; and DTS rounds were slightly lighter (MINOLTA L* 43.32 ± 1.05 DTS; 40.94 ± 0.78 CB, $P < 0.05$) at week 1, than CB samples. These values in turn affected the Hue and Chroma results at these time points; Hue and Chroma being calculated from the MINOLTA a* and b* values. There were no significant differences between treatments in terms of meat color attributes of loin and round at week 10.

Table 1. Meat quality attributes of CB and DTS carcases (Mean ± standard deviation).

Analysis	Captive Bolt	DTS	Significance#
Ultimate pH (pHu) 24 hrs post slaughter	5.69 ± 0.05	5.68 ± 0.06	n/s
MINOLTA L* at quartering	33.45 ± 0.50	33.71 ± 0.67	n/s
MINOLTA a* at quartering	23.16 ± 1.39	23.74 ± 1.86	n/s
MINOLTA b* at quartering	1.06 ± 0.44	2.71 ± 0.59	P < 0.05
Hue at quartering	0.045 ± 0.02	0.11 ± 0.02	P < 0.05
Chroma at quartering	24.10 ± 4.38	38.27 ± 5.87	P < 0.05
After 1 week of storage			
Week 1 loin pH	5.50 ± 0.03	5.50 ± 0.04	n/s
Week 1 loin Shear Force (N)	45.31 ± 3.33	39.32 ± 4.41	n/s
Week 1 loin TBARS (mg/kg)	2.01 ± 0.26	2.31 ± 0.35	n/s
Week 1 loin MINOLTA L*	34.73 ± 0.82	36.05 ± 1.11	n/s
Week 1 loin MINOLTA a*	20.89 ± 0.69	23.22 ± 0.92	P < 0.05
Week 1 loin MINOLTA b*	0.77 ± 0.70	2.79 ± 0.93	P < 0.05
Week 1 loin Hue	0 03 ± 0.03	0.113 ± 0.04	n/s
Week 1 loin Chroma	22.51 ± 4.71	40.29 ± 6.31	P < 0.05
Week 1 loin water holding capacity (% drip lost)	15.08 ±0.79	16.33 ±1.06	n/s
Week 1 round pH	5.45 ± 0.02	5.48 ± 0.02	n/s
Week 1 round Shear Force (N)	53.45 ± 2.45	47.46 ± 3.24	n/s
Week 1 round TBARS (mg/kg)	3.17 ± 0.19	2.56 ± 0.26	n/s
Week 1 round MINOLTA L*	40.94 ± 0.78	43.32 ± 1.05	P < 0.05
Week 1 round MINOLTA a*	21.93 ± 0.49	22.47 ± 0.65	n/s
Week 1 round MINOLTA b*	4.12 ± 0.56	5.25 ± 0.75	n/s
Week 1 round Hue	0.19 ± 0.02	0.23 ± 0.03	n/s
Week 1 round Chroma	48.21 ± 4.56	57.08 ± 6.12	n/s
Week 1 round water holding capacity (% drip lost)	13.38 ±0.34	12.91 ±0.45	n/s
After 10 weeks' storage			
Week 10 loin pH	5.51 ± 0.03	5.51 ± 0.04	n/s
Week 10 loin Shear Force (N)	34.72 ± 2.84	32.17 ± 3.82	n/s
Week 10 loin TBARs (mg/kg)	3.43 ± 0.38	3.17 ± 0.51	n/s
Week 10 loin MINOLTA L*	35.14 ± 0.67	36.06 ± 0.90	n/s
Week 10 loin MINOLTA a*	22.16 ± 0.52	23.14 ± 0.69	n/s
Week 10 loin MINOLTA b*	2.00 ± 0.33	2.77 ± 0.44	n/s
Week 10 loin Hue	0.09 ± 0.01	0.12 ± 1.57	n/s
Week 10 loin Chroma	32.59 ± 3.18	40.27 ± 4.26	n/s
Week 10 loin water holding capacity (% drip lost)	21.43 ± 0.80	21.95 ± 1.07	n/s
Week 10 round pH	5.44 ± 0.02	5.48 ± 0.03	n/s
Week 10 round Shear Force (N)	53.54 ± 2.55	46.19 ± 3.53	P = 0.05
Week 10 round TBARs (mg/kg)	2.62 ± 0.13	2.45 ± 0.18	n/s
Week 10 round MINOLTA L*	41.04 ± 0.89	42.75 ± 1.19	n/s
Week 10 round MINOLTA a*	21.92 ± 0.46	22.31 ± 0.61	n/s
Week 10 round MINOLTA b*	5.85 ± 0.39	6.47 ± 0.52	n/s
Week 10 round Hue	0.26 ± 0.02	0.28 ± 0.02	n/s
Week 10 round Chroma	59.49 ± 2.90	64.45 ± 3.89	n/s
Week 10 round water holding capacity (% drip lost)	19.60 ± 1.00	21.00 ±1.35	n/s

Note: # n/s: not significant, *P > 0.05.

3.5. Plasma Biomarkers

Both treatments resulted in an increase in cortisol from baseline (DTS 33.19 ± 16.89 nmol/L; CB 61.43 ± 12.59 nmol/L, $P > 0.05$) to post-treatment levels (DTS 150.38 ± 17.79 nmol/L; CB 160.64 ± 13.26 nmol/L, $P > 0.05$). The increase in plasma cortisol concentration was not significantly different between treatments ($P > 0.05$).

ACTH levels required inverse transformation for statistical analysis, therefore data presented in Table 2 are backtransformed. There were no significant differences in ACTH levels ($P > 0.05$) between DTS and CB animals at T1 (0.097 and 0.053 ng/mL respectively; backtransformed data) or T2 (0.033 and 0.063 ng/mL respectively; backtransformed data). The change in ACTH levels was not significantly different between treatments ($P > 0.05$).

There were no significant differences in β- endorphin levels between DTS and CB animals at T1 (2.75 ± 0.68 and 2.38 ± 0.5 ng/mL respectively, $P > 0.05$) or T2 (2.24 ± 0.61 and 2.13 ± 0.46 ng/mL respectively, $P > 0.05$). The reduction in β- endorphin levels was not significantly different between treatments ($P > 0.05$).

Epinephrine concentrations required inverse transformation for statistical analysis, therefore data presented in Table 2 are backtransformed. There were no significant differences in epinephrine concentrations between DTS and CB animals at T1 (8.37 and 8.44 ng/mL respectively, $P > 0.05$; backtransformed data) or T2 (16.22 and 8.96 ng/mL respectively, $P > 0.05$; backtransformed data). The change in epinephrine levels was not significantly different between treatments ($P > 0.05$).

Plasma norepinephrine levels were below detection limit (0.02 ng/mL) at T1 (baseline) in ten samples (55%). The data were therefore not normally distributed, and assessment of change in norepinephrine as a result of the treatment was not possible. At T1, the highest norepinephrine concentration in the CB group was 18.75 ng/mL; and in the DTS group 9.54 ng/mL. Data at T2 were square root transformed to achieve normality. There were no significant differences between the CB and the DTS group at T2 (mean 9.70 ng/mL and 11.34 ng/mL respectively, $P > 0.05$; backtransformed data).

Table 2. Change in plasma biomarker concentrations between pre- and post-treatment samples (Mean ±standard deviation).

Parameter	Captive Bolt	DTS	Significance#
Change in Cortisol between pre- and post-treatment samples (nmol/L)	99.21 ± 17.19	117.19 ± 23.07	n/s
Change in ACTH between pre- and post-treatment samples (ng/mL)	-0.141*	-0.051*	n/s
Change in β-endorphin between pre- and post-treatment samples (ng/mL)	-0.25 ± 0.56	-0.51 ± 0.76	n/s
Change in epinephrine between pre- and post-treatment samples (ng/mL)	146.16*	17.28*	n/s

Note: # n/s: not significant, $P > 0.05$; *backtransformed data.

4. DISCUSSION

Visual inspection of the carcases showed no abnormality. Bruises were present on the bony tip of the pelvis on some animals, from both treatment groups, and were deemed to be caused pre-slaughter during handling and restraint. No abnormalities of color were noted at quartering. In general, there were no significant differences between meat from DTS animals and meat from CB animals.

Three carcases showed a very rapid initial drop in pH post slaughter, reaching values below pH 6, while the carcase temperature was still above 35°C: Animals 7 (DTS 20 kW, 191.28 kJ); 14 (DTS 30 kW, 45.87 kJ) and 16 (DTS 20 kW, 184.68 kJ). This rapid fall in pH may be indicative of a raised metabolic rate [16] and may suggest a tendency towards development of heat toughening, although this was not borne out by the meat quality analyses undertaken on loin and round at 1 and 10 weeks post slaughter. The raised metabolic rate could be a result of the prolonged restraint prior to stunning (collection of EEG data), the stunning treatment, or the prolonged stun-stick interval (collection of EEG data). The current data set is insufficient to assess which of these parameters was most

influential, and ideally the study would be repeated under more commercial conditions that did not involve prolonged restraint or delayed exsanguination.

After death, anaerobic metabolism utilises stored glycogen reserves, facilitating an accumulation of lactate, prolonging glycolysis and utilising 'free' hydrogen ions, which is associated with a decline in muscle pH post-mortem. Well-fed and well-rested animals normally have sufficient muscle glycogen energy reserves at processing to reduce the pH of muscles to near to 5.5. If an animal has lowered glycogen <0.6 % (usually as a result of fatigue, that could be due to long-distance transport, fighting, mustering or prolonged periods of raised metabolic rate immediately prior to slaughter) and has had insufficient time to re-establish the muscle energy reserves, the pH will not fall to below 5.8, and will yield what is known as 'dark-cutting' meat [16, 17]. In the current study, pHu in both groups was below pH 5.8, indicating that dark cutting was not induced, and pH values of loin and round at weeks 1 and 10 of storage lay within normal ranges (pH4 – pH 6), suggestive that glycogen reserves were sufficient (1-1.5 %) [17].

There were some slight differences in meat color at quartering, and in the first week after slaughter. Marbling was not objectively measured but was considered similar across all carcases in the study. DTS quarters were slightly yellower (greater MINOLTA b*) at quartering; and at 1 week post slaughter DTS loins were slightly redder (greater MINOLTA a*) and slightly yellower (greater MINOLTA b*); and DTS rounds were slightly lighter (greater MINOLTA L*) than CB samples. However, these differences were marginal, and the values align with published data on MINOLTA color attributes of loin (m. longissimus lumborum) and round (m. semitendinosus) [18-20]. In light of the small sample size the current findings should be interpreted with caution.

There was also a trend that DTS samples were more tender than CB samples, but similarly, this trend should be interpreted with caution, in light of the small sample size. When calculating from newtons to kg/ force, shear force values for loins at one week post slaughter in the current study were 4.62 ± 0.34 kg and 4.01 ± 0.45 kg in CB and DTS respectively; while at 10 weeks post slaughter these values were 3.45 ± 0.29 kg and 3.28 ± 0.39 respectively. These results are indicative of intermediate (week 1) and tender (week 10) classifications and lie within normal ranges Sullivan and Calkins [21]. Warner, et al. [20] report values of 7.0 kg at 6 days post slaughter, and 4.8 kg at 21 days post slaughter; Gruber, et al. [22] report a range of 3.5 to 5.11 kg measured over a range of ageing periods from 3 to 28 days; while Sazili, et al. [19] report 9.19 ± 0.97 to 9.96 ± 0.72 kg at one week post slaughter. For rounds, the current study measured 5.54 ± 0.25 kg for CB and 4.84 ± 0.33 kg for DTS at one week post slaughter, and 5.46 ± 0.26 and 4.71 ± 0.36 kg respectively at week 10. These values again align with previously published ranges, for example 4 – 18 kg [23], 4.12 ± 0.16– 6.63 ± 0.2 kg [24] and 4.6 – 9.5 kg [25].

The TBARs results are higher than would be expected from fresh meat. This may not be reflective of the product or treatment but due to a loss of temperature control during sample storage, after sample collection. The samples in week 1 underwent an unforeseen delay between collection (held under refrigeration + 2°C and storage at - 80°C prior to analysis, which may have resulted in excess lipid oxidation. For consistency, at week 10, we replicated the storage conditions encountered at week 1. Nevertheless, there were no significant differences in TBARs levels between the DTS and the CB muscles.

The endocrine stress responses measured indicate activation of two physiological response systems [26]. One is the 'fight – or – flight' response initiated by all animals when a threatening or 'emergency' situation is perceived: activation of this response leads to increases in catecholamines (epinephrine and norepinephrine). The catecholamines speed blood circulation and divert blood, and therefore oxygen, from internal organs to the brain and muscle, readying the animal for action. The second response is the HPA axis (Hypothalamic-Pituitary-Adrenal axis): activation of this response leads to the release of Adrenocorticotrophic hormone (ACTH) which leads to increased cortisol levels. This in turn increases the rate of production of glucose from glycogen reserves, so that the energy required for action is available. This increase in rate of glycogen breakdown, circulation, and metabolism generally can have detrimental effects on meat quality: very rapid metabolism at the point of slaughter

can lead to the pale, soft, exudative condition; prolonged raised metabolism leads to reductions in glycogen stores, which in turn lead to dark cutting [16].

In a commercial slaughterhouse situation, comparison against published reference ranges, based on resting animals, is inappropriate, as there will always be some underlying effect of the pre-slaughter transport and handling phase. Even differences between abattoirs can strongly influence the endocrine stress response [27]. For example, Zulkifli, et al. [28] working in a large commercial slaughterhouse, report higher, and a wider range in, cortisol concentrations than do Tume and Shaw [29], from a small experimental facility. Thus, baseline samples are taken prior to treatment, against which the post-treatment samples are compared. The values obtained in the current study fall with the ranges published for pre and post slaughter samples collected from cattle [28-33].

In the current study, baseline cortisol in the DTS group was significantly lower than post-treatment cortisol in that group, but not significantly different from baseline CB, and post-treatment cortisol in either group was not significantly different from the aggregate of all baseline cortisol results. Therefore, the fact that post-treatment cortisol was significantly different from baseline cortisol in the DTS group, and not in the CB group, is likely to be an artefact of small sample size.

Both methods resulted in an increase in cortisol from baseline (DTS 33.19 ±1 6.89 nmol/L; CB 61.43 ± 12.59 nmol/L) to post-treatment levels (DTS 150.38 ± 17.79 nmol/L; CB 160.64 ± 13.26 nmol/L), indicating a physiological response. However, it is unclear if this response is due to the stunning method; or to the head capture and prolonged restraint, which was longer than in a commercial situation due to the need to take baseline EEG recordings; or to a combination of both restraint and treatment. Shaw and Tume [30] and Zulkifli, et al. [28] both report that cortisol levels in cattle are not affected by captive bolt stunning, however, the former carried out their study in a highly controlled research abattoir environment, in which external stimuli are likely to have been minimised, while the latter carried out their study in a commercial slaughterhouse, and baseline cortisol levels prior to slaughter were already high, as a result of the preslaughter handling and environment. Both Dunn [33] and Zulkifli, et al. [28] report post stun cortisol levels greater than those measured in the current study, but it is important to note that these both relate to the case of slaughter without prior stunning. Indeed, the latter authors demonstrated an increase in cortisol levels associated with slaughter without prior stunning, but not in the case of captive bolt stunned slaughter. Mitchell, et al. [32] did find that captive bolt stunned slaughter resulted in an increase in cortisol levels (+ 61.2 nmol/L), but this increase was less than in cattle that were handled through a race (+ 151.7 nmol/L). By inference, it is likely that the cortisol response seen in the current study is predominantly due to the head capture and restraint. A tightly restrained head is required to ensure delivery of the DTS energy; however, prolonged tight head capture is likely to be very stressful to the animal, and struggling while restrained is considered to be an indication of excessive pressure [34]. The European Food safety Authority recommends that "all restraining devices should use the concept of optimal pressure" [35] however, the parameters that constitute 'optimal pressure' have not been determined.

In the current study, there was neither a difference in ACTH levels between baseline and post-treatment samples, nor was there a treatment effect. The values generated in the current study were lower than those reported by Zulkifli, et al. [28] but similar to those reported by Mitchell, et al. [32] who found that ACTH was, like cortisol, affected more by transportation and handling than by stunning alone.

Catecholamines (epinephrine and/or norepinephrine) have been reported to increase as a result of stunning Zulkifli, et al. [28]; Rulofson, et al. [31]; Mitchell, et al. [32]. Zulkifli, et al. [28] report no significant changes in epinephrine levels as a result of penetrative captive bolt stunning, or high powered percussive stunning, but an increase as a result of low powered percussive stunning and slaughter without prior stunning, while norepinephrine levels increased in all treatment groups; findings that concur with the current study. However, Mitchell, et al. [32] and Rulofson, et al. [31] both report an increase in both epinephrine and norepinephrine associated with captive

bolt slaughter. In the current study, both epinephrine and norepinephrine levels tended to increase between the pre-slaughter and post-slaughter samples, and there were no significant differences between stunning treatments.

β-endorphins have a role in modulating the physiological stress response, and increases are associated with painful stimuli, fear and excitement. In the current study, there was no significant effect of treatment on β-endorphin concentrations in either group, although the mean value appeared to decrease slightly. This finding concurs with that of Zulkifli, et al. [28] who demonstrated no significant change in concentrations as a result of penetrative captive bolt, percussive captive bolt, and slaughter without prior stunning at a commercial abattoir. The slight decline in β-endorphin concentrations also reflects the findings of Shaw and Tume [30] who demonstrated a significantly lower concentration in captive bolt slaughtered cattle than in the live animals. Those authors however, carried out their study in a small research abattoir, and the live animal blood samples were collected on a separate occasion within a month prior to slaughter. Thus, the live animal values they report may not relate well to the baseline samples collected in the current study and that of Zulkifli, et al. [28] which were collected immediately prior to stunning, in the restraint box.

To summarise, this study provides a preliminary assessment of the meat quality attributes and plasma biomarker concentrations in cattle stunned prior to slaughter using the DTS: Diathermic Syncope® system, demonstrating comparable outcomes to cattle stunned using penetrative captive bolt. Key limitations of this study are (i) a prolonged period in restraint, which may have increased arousal and metabolic rate in the animals; (ii) a prolonged stun-stick interval, which could have resulted in returning sensibility and thus alterations in metabolic rate (although any animal showing return of brainstem reflexes was immediately re-stunned using a penetrative captive bolt; (iii) technical faults during energy delivery resulting in a wide range of total energy applied to individual animals – the DTS application system has undergone further development since this study was conducted, and these issues have been corrected (J. Ralph, Wagstaff Food Services, personal communication); and (iv) a small sample size, such that there is a risk that any differences identified may not be true findings: indeed application of a Bonferroni adjustment to the analysis would render all observed differences as not statistically significant.

5. CONCLUSIONS

This preliminary study indicates that stunning of cattle prior to slaughter using the DTS: Diathermic Syncope system results in no, or negligible impacts on changes in plasma biomarker concentrations or meat quality attributes, the outcomes being comparable to those associated with stunning using penetrative captive bolt. These results should be validated in a larger sample size under more commercial processing conditions.

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