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Dielectric induction of temporary insensibility in cattle - animal trials

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Abstract

DTS: Diathermic Syncope® (DTS) is a novel system for rendering animals insensible prior to slaughter. It utilizes electromagnetic energy to induce a rise in temperature in the brain, by 7-8°C, to a point at which the animal loses consciousness, and EEG patterns indicative of insensibility have previously been demonstrated on anaesthetized cattle and sheep. The objective of this study is to provide a proof of concept demonstration of induction of unconsciousness, to determine what signs, comparative to other validated stunning processes, might eventually be examined experimentally for future assessment on the effectiveness of the device in rendering the animal unconscious; and to compare DTS against the industry reference standard (penetrative captive bolt stunning), in terms of, physiological variables (Cortisol, ACTH, β-endorphin and catecholamines) and meat quality (carcase characteristics, pH, Colour, shear force, drip loss and lipid oxidation) at slaughter, one week and ten weeks post slaughter (vacuum packaged primals).

In the current study, ten non-anaesthetized cattle received DTS treatments (high energy <290 kJ, n=3; low energy <200 kJ, n=4; and intermediate, n=3), and seven received captive bolt stunning prior to exsanguination. Based on live observations at the time of stun application, the research team was satisfied that DTS induced a state of insensibility, of a sufficient duration to allow humane slaughter through exsanguination. Live observations indicated that the process was painless, and evidence of distress was not observed. The animals remained unresponsive to stimuli and showed evidence of EEG suppression for 3-4 minutes post DTS. EEG changes for DTS and captive bolt animals did not differ. The clearest indicators of effective induction of insensibility were: Loss of corneal responses; Loss of withdrawal response (pinprick); Eye staring, not following movement. Two animals showed indications of returning reflex responses after 180 seconds post treatment.

There were no significant differences between DTS and captive bolt animals in terms of cortisol, ACTH, β-endorphin and catecholamine responses, and there were no significant differences between DTS and captive bolt carcases in terms of pHu (24 h post slaughter); pH, Warner Bratzler Shear Force and Drip loss at 1or 10 weeks post slaughter. DTS carcases were slightly yellower at quartering (MINOLTA b* 2.71 ± 0.59 DTS; 1.06 ± 0.44 control); DTS loins were slightly redder (MINOLTA a* 23.22 ± 0.92 DTS; 20.89 ± 0.69 control) and slightly yellower (MINOLTA b* 2.79 ± 0.93 DTS; 0.77 ± 0.70 control); and DTS rounds were slightly lighter (MINOLTA L* 43.32 ± 1.05 DTS; 40.94 ± 0.78 control) at week 1, than control samples ($P<0.05$). There were no differences between DTS and captive bolt meat colour measurements at 10 weeks post slaughter.

In conclusion, DTS provides a humane method of inducing insensibility prior to exsanguination, of sufficient duration to allow exsanguination prior to recovery, and produces comparable post slaughter meat quality and physiological responses in treated cattle to those stunned using penetrative captive bolt.

Executive Summary

Some communities require that animals processed for human consumption are healthy, uninjured and normal at the moment of carrying out the slaughter cut. As a result, many methods of stunning used in modern commercial slaughter are not acceptable, because the animals could be considered injured, or because a proportion of animals would not recover from the stun. Head-only electrical stunning is used in sheep, but is not suitable for use in cattle because of concerns over the duration of insensibility being insufficient to allow death through total blood loss to occur prior to recovery from the stun, and also because of problems with blood splash in the meat. Preliminary research has shown that electromagnetic energy technology is likely to induce recoverable insensibility in animals and could result in an effective reversible stunning method that is suitable for religious slaughter.

Background research has shown that electromagnetic energy can lead to unconsciousness in animals. In chickens, Lambooij *et al*. (2011) applied an electromagnetic field, and produced electroencephalogram (EEG) traces that indicated insensibility and the duration of unconsciousness was calculated to be in the range of 15 to 20 s. Guy and Chou (1982) similarly induced unconsciousness in rats that lasted for 4-5 minutes.

The mechanism of action is essentially selectively increasing the temperature slightly in the brain, by only 7° C, to the point that hyperthermic syncope (fainting) occurs, but below the point at which irreversible brain damage and death occurs. Thermal unconsciousness such as that induced by exercise heat stress or fever is reported to occur when core body temperatures reach between 40 and 45°C.

Wagstaff Food Services Pty Ltd and Advanced Microwave technologies have designed a system for delivery of electromagnetic energy to sheep, goats, cattle, buffalo and camel (Patent PCT/AU2011/000527), DTS: Diathermic Syncope®. To date, trails have been carried out on anaesthetised sheep and anaesthetised cattle, and this report describes a proof-of concept study on non-anaesthetised cattle.

The trials on four anaesthetised sheep were highly successful. Application of microwave energy caused rapid increases in brain temperature to a point above which insensibility would be expected to occur (43°C), and below that which damage would be expected to occur (50°C). The sheep breathed regularly and normally throughout the application of energy, and afterward, in contrast to electrically stunned sheep, in which breathing stops until the animal begins to recover from the stun. Two animals were allowed to partially recover from anaesthesia, to the point at which neck tone, jaw movements and swallowing reflexes had returned, indicating that the animals were likely to recover. Full recovery was not permitted under this trial, as a condition of the Australian Animal Ethics Approval.

In the anaesthetised cattle trial, nine cattle were lightly anaesthetised, and electroencephalogram (EEG) traces collected before and after microwave energy application. All applications resulted in EEG traces that indicated unconsciousness (seizure-like activity, similar to that seen when electrical stunning is used). There was a slight slowing of the heart rate during and after application, but the rhythm remained regular. In comparison, when electrical stunning is used, the heart rate drops while the stun is applied, but rises to above normal rates after application, during the unconscious period. Five combinations of microwave power and durations were tested. Higher power led to a longer period of EEG disruption; while shorter application led to more rapid onset of EEG disruption. The EEG traces were analysed for evidence of pain according to the method of Gibson *et al*. (2007), and no evidence of pain was found. These results indicated that microwave application to conscious animals would lead to rapid onset of insensibility.

Methods

Eighteen Aberdeen Angus cross bred heifers (weight range 350-400 kg) with a quiet temperament were selected from the normal commercial intake at Wagstaff Food Services abattoir. They were fed and rested in lairage for 4 days prior to the trial, cared for by an experienced stockperson. On the day of the trial, each animal was individually brought to the restraint box, by the same familiar stockperson, using low-stress animal handling techniques. A baseline blood sample was taken from the tail vein, and the animal was restrained using a head restraint unit, lifting the forehead to be in contact with the DTS energy applicator. After head capture, baseline EEG measurements were recorded. The assigned treatment was then applied. The treatments were:

- Control (captive bolt): animals 1, 2, 8, 9, 12, 13, 15
- High energy DTS, receiving greater than 290 kJ: animals 3, 4, 11
- Low energy DTS, receiving less than 200 kJ: animals 7, 10, 14, 16
- Intermediate and double-application DTS: animals 5, 6 and 18.

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.

Following treatment, insensibility was assessed by corneal reflex, assessment of visual function and response to a painful stimulus of the nose, EEG measurements were repeated, the animal removed from the restraint box, terminal EEG measurements recorded and the animal exsanguinated. A back-up captive bolt stun was delivered in cases where a risk of recovery during exsanguination was perceived, the time elapsed between application of the treatment and exsanguination being prolonged by the need to capture post-treatment EEG recordings and behavioural measures. A second blood sample was collected from the free-flowing exsanguinate. Blood samples were centrifuged and plasma extracted. The entire process was video recorded using six cameras, capturing animal movements and behaviours from above, at the head, and on the roll-out table, and these videos were subsequently annotated against an ethogram designed for the trial.

The carcase was then dressed as normal practice, chilled overnight, and de-boned the following day. pH measurements were taken from the carcases every hour from slaughter till below pH6, and again at 24 hours post slaughter, prior to de-boning. Heads were sections for inspection, and brain samples collected for histological analysis. Colour was measured on the cut surface of the *m. longissimus lumborum* 30 minutes after quartering. At de-boning, two samples each of loin (*m. longissimus lumborum*) and round (*m. semitendinosus*) was removed, vacuum packed and refrigerated. These samples were transported to the laboratory by refrigerated vehicle, within the first week post slaughter. At each of 1 and 10 weeks post slaughter, muscle samples were unpacked, and sectioned into subsamples for colour, pH, shear force, lipid oxidation and drip loss evaluation.

Meat Colour was measured using a MINOLTA CR300® colorimeter under light source D65; pH was measured using a WP-80 digital pH meter (TPS instruments, Springwood, QLD), with a combination electrode for temperature compensation; Warner-Bratzler (WB) shear force was measured according to the protocols outlined by Bouton *et al*. (1971) and Bouton and Harris (1972a). Drip loss was measured using the method outlined by Honikel *et al*. (1986); Lipid oxidation was determined by the thiobarbituric acid-reactive substances (TBARS) method of Witte *et al*. (1970). Plasma samples were tested for cortisol (RIA), ACTH

(EIA), β-endorphin (EIA) and catecholamines (ELISA) concentrations. Data were analysed using R Studio (R Core Team 2014).

Results

Under live observation of the animals, the clearest indicators of effective induction of insensibility were:

- Loss of corneal responses
- Loss of withdrawal response (pinprick)
- Eye staring, not following movement

Unreliable indicators are:

- Loss of posture: there is an initial loss of posture, but by the time the DTS cycle is complete, the legs have re-extended, and paddling or walking movements of the hind limbs in particular, are seen. This pushes the shoulders of the animal hard into the neck bail, jamming it into an uptight position. When the animal is removed from this jammed position, it is in lateral recumbency, entering a convulsive or clonic phase, after which reflexes do appear to begin to return.
- Breathing characteristics: breathing and heart function is maintained – the brain stem appears to be unaffected by the DTS application. Breathing tends to be fast and shallow.
- Vocalisation: it appears that some animals do vocalize, even when there are no responses from the eye (following movement, corneal response, palpebral response) nor withdrawal reflexes. This vocalization, when it occurs, is continuous, occurring on every exhalation, and is not associated with any external stimulus.
- Blinking: as in electric stun, random movements of the facial muscles and eyelids can occur, particularly as the animal enters the clonic (convulsive) phase. Therefore, it is difficult to interpret a blink as to whether it is a response, or a random movement.

From the video footage it was confirmed that on application of a captive bolt, all four limbs immediately tucked into the body, and the animal fell to the floor of the restraint box. As it was rolled out of the box, the forelegs first became rigidly extended, followed by the hind limbs. No rhythmic breathing was observed, and the animal's eye was fixed and staring. There was no corneal response, no pupillary response to light, and no response to nose prick. No vocalisation and no righting reflex was observed.

For DTS animals, application of energy resulted in rapid blinking and flickering of the third eyelid, with nystagmus. In some animals the back arched and the muscles of the neck contracted, pulling the chin down into the chin lift. In the low energy applications, this was followed by a period of convulsive movements, lasting 10-20 seconds, then ataxia and loss of posture, particularly in the hindquarters. Following this ataxic phase, the body became tense and tetanic, with the four limbs slightly splayed. There was no response to efforts to topple the animal: the tail hung limp and flaccid, and the limbs remained motionless. The animal then progressed into slow walking and paddling movements, pushing forward into the head restraint unit. There was no vocalisation, and rhythmic breathing continued throughout. The eyes became fixed and staring, with no ocular following of movement, and no pupillary response to light. There was no withdrawal response to nose prick.

Head inspection of captive bolt animals revealed one or two (in the instances where the animal was re-stunned) circular penetrations of the frontal bone. Haemorrhage was evident

within the skull cavity, with clotted blood pooling around the lower parts and brainstem. This contusive damage was also noticeable in histological sections from deeper regions of the brain. Heads from high-energy DTS showed varying degrees of heating damage. On the forehead, an extensive area of scorched, dried skin was easily sloughed, showing brown discolouration of the bone below. On splitting the skull, the mucous lining of the sinus cavity and nostrils was noted to be dried and discoloured. The brain was discoloured and firm immediately below the application site, with the appearance of cooked brain tissue. On histological examination, marked evidence of vascular congestion and thrombosis, with loss of neuropil structure and malacia was found in upper sections, and less markedly in middle sections. Low energy DTS heads similarly showed some degree of heating damage to the forehead, but this was much reduced compared to those of High energy DTS. The skin at centre point of waveguide application was dry and leathery, with loss of hair. Brain tissues were essentially normal, although there was some malacia and hyperaemia of the upper 2mm of the surface of the cerebral cortex.

The EEG responses of both Captive bolt and DTS animals were similar. Qualitative assessment of the EEG traces indicated that immediate post treatment EEG traces showed a reduction in amplitude with intermittent activity (trace not compatible with sensibility), and post-rollout traces tended towards the isoelectric state (flat-line, trace not compatible with sensibility). There were no significant treatment differences between baseline, post-treatment and terminal values of Mean Power; Root Mean Square power (RMS); Amplitude; Median Power Frequency (F50); or 95% Spectral Edge Frequency (F95).

There were no significant differences between DTS and captive bolt animals in terms of cortisol, ACTH, β-endorphin and catecholamine responses. Both treatments resulted in an increase in cortisol from baseline (DTS 33.19 ±16.89 nmol/L; Captive bolt 61.43 ±12.59 nmol/L) to post-stun levels (DTS 150.38 ±17.79 nmol/L; Captive bolt 160.64 ±13.26 nmol/L), indicating physiological stress. However, it is unclear if this stress is due to the stunning methods; or to the head capture and restraint, which was longer than in a commercial situation due to the need to take pre-stun EEG recordings, or to a combination of both restrain and stun.

There were no significant differences between DTS and captive bolt carcases in terms of pHu (24 h post slaughter); pH, Warner Bratzler Shear Force and Drip loss at 1or 10 weeks post slaughter. DTS carcases were slightly yellower at quartering (MINOLTA b* 2.71 ± 0.59 DTS; 1.06 ± 0.44 control); DTS loins were slightly redder (MINOLTA a* 23.22 ± 0.92 DTS; 20.89 ± 0.69 control) and slightly yellower (MINOLTA b* 2.79 ± 0.93 DTS; 0.77 ± 0.70 control); and DTS rounds were slightly lighter (MINOLTA L* 43.32 ± 1.05 DTS; 40.94 ± 0.78 control) at week 1, than control samples ($P<0.05$). There were no differences between DTS and captive bolt meat colour measurements at 10 weeks post slaughter.

Conclusions

Based on live observations at the time of stun application, the research team was satisfied that DTS induced a state of insensibility, of a sufficient duration to allow humane slaughter through exsanguination. Live observations indicated that the process was painless, and evidence of distress was not observed.

EEG data indicated that DTS induced insensibility. DTS animals in the current study remained unresponsive to stimuli and showed evidence of EEG suppression for 3-4 minutes post energy application. In a commercial situation, it would be expected that exsanguination would be carried out within 30-60 seconds of energy application.

Video footage demonstrated a convulsive phase during low energy DTS application, but it is unclear as to whether this is involuntary convulsion, or an attempt to escape. Facial expression seems to indicate that it is involuntary. A further convulsive or clonic phase occurred between 60 and 90 seconds post energy application.

Endocrine data indicated no differences between DTS and captive bolt. Meat quality parameters in DTS carcases did not differ from captive bolt carcases. pH declines suggested that there may be a potential for increased metabolic rate, which would be predicted to result in a PSE-like condition, but this was not corroborated by the meat quality analyses.

Brain lesions suggest that 300 kJ would result in a non-recoverable state, while behavioural observations suggest that less than 100 kJ gives a short-duration insensibility.

DTS animals maintained rhythmic breathing and a strong heart beat throughout the period of insensibility, which lasted for at least 3-4 minutes post application of energy. During this time, there was no corneal reflex, no response to a painful stimulus of the nose, and no evidence of the eye beginning to focus and follow movement.

Two animals showed evidence of return to consciousness:

1. Animal 16 received 184.68 kJ. Return of blink and corneal reflexes were noted prior to captive bolt application, 228 seconds after DTS application.
2. Animal 18 received 217.62 kJ. It was unresponsive to stimuli for the first 90 seconds post treatment, and then appeared to go into a clonic or convulsive phase. Following this, it lay quietly for a further 90 seconds, showing no response to stimuli. Towards the end of this period the eye was beginning to regain focus, followed closely by corneal reflex, and within 15 seconds, return of righting reflex.

It is evident that the duration of insensibility achieved in DTS animals is sufficient to allow exsanguination prior to recovery, but in a commercial situation it would be strongly recommended to maintain a back-up captive bolt instrument on the bleed rail, as the return from focusing of the eye, through corneal reflex to return of righting reflex is rapid.

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