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Quality Assessment of *Longissimus* and *Semitendinosus* Muscles from Beef Cattle Subjected to Non-penetrative and Penetrative Percussive Stunning Methods

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ABSTRACT: This study provides a comparative analysis of the effects of pre-slaughter penetrative and non-penetrative stunning and post-slaughter stunning on meat quality attributes in *longissimus lumborum* (LL) and *semitendinosus* (ST) muscles in heifers. Ten animals were assigned to each of four treatment groups: i) animals were subjected to conventional Halal slaughter (a clean incision through the structures at the front of the upper neck - the trachea, oesophagus, carotid arteries and jugular veins) and post-cut penetrating mechanical stun within 10 to 20 s of the neck cut (Unstunned; US); ii) high power non-penetrating mechanical stunning followed by the neck cut (HPNP); iii) low power non-penetrating mechanical stunning followed by the neck cut (LPNP); and iv) penetrative stunning using a captive bolt pistol followed by the neck cut (P). For each carcass, muscle samples were removed within 45 min of slaughter, portioned and analysed for pH, cooking loss, water holding capacity (WHC), tenderness (WBS), lipid oxidation (TBARS) and color, over a two week storage period. Stunning did not affect pH and cooking loss. Significant differences in water holding capacity, tenderness, lipid oxidation and color were present at different storage time points. HPNP stunning resulted in lower WHC and color values, particularly lightness (L*), higher TBARS values and peak force values compared with those stunned using LPNP, P and US. These adverse effects on quality were mostly encountered in the ST muscle. In conclusion, the meat quality achieved using P, LPNP and US treatments was comparable, and no treatment stood out as considerably better than another. (**Key Words:** Stunning, Slaughter, Welfare, Cattle, Meat Quality)

INTRODUCTION

What happens to the animals prior to slaughter usually influences the physiological state, particularly energy metabolism within skeletal muscle (Petersen and Blackmore, 1982), which in turn, affects post-mortem muscle metabolism. Stress associated with improper pre-slaughter handling of livestock has been associated with poor meat quality attributes such as toughness, undesirable ultimate pH, water holding capacity, cooking loss and color (Bray et al., 1989; Simmons et al., 1997; Lensink et al., 2001; Bond et al., 2004; Mournier et al., 2006; Bond and Warner, 2007). These effects differ between muscles within the same carcass as a result of differences in metabolic and contractile characteristics between different groups of skeletal muscles (Melody et al., 2004).

Welfare requirements dictate that animals should be insensible to noxious, potentially painful stimuli during slaughter. In Australia, cattle are stunned prior to slaughter using a penetrative captive bolt, non-penetrative (percussive) captive bolt, or electrical methods, and, according to Gregory and Shaw (2000), when stunning is done correctly, the animal feels no pain and becomes instantly unconscious. With mechanical stunning, the intent is to cause concussion with or without penetration.

Non-penetrating (percussive) captive bolt stunners may or may not fracture the skull. According to Grandin (2009), non-penetrating captive bolt stunning that does not fracture

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the skull could be less effective than stunning that does fracture the skull. Head injuries caused by non-penetrating stunning that fractures the skull can be severe. The impact of the heavy mushroom head against the relatively thin frontal bone can result in a severe, well-circumscribed, and depressed fracture of the skull with subarachnoid haemorrhage in the sub-adjacent brain (Finnie, 1995). In some countries, non-penetrative mechanical stunning is disallowed because of a risk that insufficient power could result in an ineffective stun and, hence, compromise animal welfare. The prevalence of error in performing non-penetrative mechanical stunning is a major welfare concern.

Although previous researchers have compared the meat quality over 14 d of ageing from unstunned animals against electrically stunned (Petersen and Blackmore, 1982; Vergara and Gallego, 2000; Velarde et al., 2003) or percussively stunned (Ö nenc and Kaya, 2004) animals, no single piece of work compares meat quality attributes from unstunned, and mechanically (penetrative and non-penetrative) stunned cattle. The aim of this study was to compare meat quality attributes in cattle subjected to different mechanical stunning methods - high power non-penetrating mechanical stun (HPNP), low power non-penetrating stun (LPNP), penetrative mechanical stunning (P) and slaughter followed by penetrative mechanical stun (US) in cattle.

MATERIAL AND METHODS

This project was carried out under CSIRO Animal Ethics Committee Authorisation A7/08.

Animals

The work was conducted during the period of July 2009 in Queensland (outdoor temperature: 15 to 20°C) at a commercial abattoir. Three cohorts of animals were processed across three separate slaughter days, over a period of 2 wk. Within each cohort, animals were randomly assigned to treatment group by selection of a card from an envelope, as the animal was presented for slaughter. Cohorts 1 and 2 each contained three animals of each treatment group, while cohort 3 contained four. The cattle processed were heifers, with live weights between 268 and 635 kg (mean 446 kg), resulting in hot carcass weights of 138 to 326 kg (mean 233 kg). There were no significant differences between live weights and hot carcass weights between treatment groups. 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 2 d prior to slaughter. While in holding pens they were provided with grass hay and water *ad libitum* until 4 pm the day before slaughter. They were *Bos taurus*×*Bos indicus* crossbreds, and were representative of the normal class of animal slaughtered at this abattoir for the Halal export market. The animals were handled using the emergency slaughter area at the abattoir as the regular stun-box, being fully enclosed, does not allow access to the neck in order to carry out unstunned slaughter. 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 experimental animals was not greatly different from that of animals slaughtered under normal conditions.

Stunning and slaughtering procedure

The experiment involved a total of 40 heifers. Equal numbers of animals were randomly assigned to one of the four slaughter treatments:

US (un-stunned): Animals were restrained in a crush, with a neck extender apparatus fitted to the yoke, and 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). A post-cut penetrative stun (as described under 'P' below) was delivered within 10 to 20 s of the Halal cut (after the post-sticking blood sample had been taken). The post-cut stun was applied to satisfy the requirements of the Animal Ethics Approval obtained (CSIRO A7/08).

HPNP (high power non-penetrative percussive): High power percussive stunning using a non-penetrating humane killer (Cash magnum Knocker Concussion Stunner, 0.25 Calibre, 4 grain cartridge) followed by exsanguination using transverse incision of the neck as described above.

LPNP (low power non-penetrative percussive): Low power percussive stunning using a non-penetrating humane killer (Cash magnum Knocker Concussion Stunner, 0.25 Calibre, 3 grain cartridge) followed by exsanguination using transverse incision of the neck as described above. This treatment was included, because there is a desire within industry to use low power non-penetrative stun in an attempt to maintain skull integrity as described in the Australian Quarantine and Inspection Service (AQIS) Malaysian Protocol (AQIS, 2006).

P (penetrative): Penetrative stunning using a captive bolt pistol humane killer (Cash 8000 Model Stunner, 0.22 calibre, 4.5 grain cartridge) followed by exsanguination using transverse incision of the neck.

All carcasses were delivered to the slaughter floor immediately following exsanguination, and processed using the operator's normal dressing procedures, which did not include any electrical inputs.

Muscle sampling and storage

All quality measurements were assessed in both the *longissimus* (LL) and *semitendinosus* (ST) muscles, which were removed at 45 min post-mortem from the carcasses. As the study was carried out at a commercial abattoir located some way from the laboratory, it was not possible to retain the carcasses with the muscles *in situ* for the required ageing period. The muscles were not controlled for shortening, which is likely to have an effect on shear force. If the muscles had remained in the carcass, the skeletal structure would have reduced the shortening effect. However, all muscles were treated in the same manner, so comparison between groups within this study is still possible.

Immediately after removal, each muscle sample (1,000 g±100 g) was cut into four equal portions. Each portion was assigned to one of four different ageing periods: i) 0 d (no ageing), ii) 1 d, iii) 7 d and iv) 14 d post-mortem, care being taken to ensure that muscle quadrants were evenly represented within each ageing period and treatment group combination. The d 0 samples further cut to sub samples for color (approximately 30 g, 10 mm thick, 30 mm×50 mm), TBARS (approximately 20 g) and water holding capacity (approximately 1 g) determination. The samples for color and TBARS were placed into zip-lock plastic bags (40 mm×70 mm) and the samples for water holding capacity were placed into 1.5 mm micro tubes (1.5 ml PP, Sarstedt, Aktiengeselischaft & Co, D-50588 Nümbrecht, Germany). All samples were temporarily kept on ice until transported to CSIRO Food and Nutritional Sciences Laboratory, Cannon Hill, Queensland. On arrival at the laboratory, all muscle samples were vacuum-packaged and the samples for 1, 7 and 14 d post-mortem ageing stored in a chiller (4°C), labeled according to their ageing period. Samples from d 0 post-mortem were transferred to a -80°C freezer (Ultra-low Temperature Freezer, Forma Scientific, Model: 8425, USA) immediately after packaging. At each ageing period, the appropriate packs were removed from the chiller, and the muscle samples for each different ageing period were further cut to sub samples as described above. All sub-samples were transferred to -80°C frozen storage until further analysis was carried out.

pH determination

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 at least 1cm below the surfaces. Data were recorded once the readings had stabilized. Day 0 pH was measured at the abattoir prior to muscle portioning. The instrument was calibrated prior to and immediately after each session using pH 4 and pH 9 standards as per the manufacturer's instructions.

Water holding capacity

Water holding capacity was determined according to the methods of Kristensen and Purslow (2001) and Bouton et al. (1971). Briefly, samples were removed from the -80°C freezer thawed overnight at 4°C. Small pieces of muscle (approximately 0.28 to 0.30 g) were sliced along the grain of the muscle fibres, approximately 3 mm thick and 15 mm long, weighed (M1) and placed in mobicols (LIFM1002, Quantum Scientific, Murrarie, QLD) containing 90 μm filters. The samples were centrifuged (Bench Centrifuge, Model: Eppendorf Minispin Plus, USA) at 4°C at 26 G for 1 h and reweighed (M2). The samples were dried in an oven at 105°C for 24 h and weight was recorded again (M3). Based on the weights, centrifugation loss (expressed juice), total water content and water holding capacity were calculated according to the following formulae:

Centrifugation loss (Expressed Juice EJ) = M1-M2

Total water content (TWC) = M1-M3

Water holding capacity (WHC) = 1-(%EJ/%TWC)

Cooking loss determination

Samples were removed from the -80°C freezer and thawed overnight at 4°C. The thawed meat samples were then cut (125±5 g), weighed and recorded as W1 (raw meat weight). The samples were then placed in plastic bags and cooked at 70°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 a chiller at 4°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 by using the following equation:

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

Warner-Bratzler shear force analysis

Samples for texture analysis were taken from the previous cook loss samples. Assessment of meat texture was made using the Warner-Bratzler shear force measurement on samples cooked at 70°C for 60 min, 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

assessed and the samples cut according to the protocols outlined by Bouton and Harris (1972) and Bouton et al. (1971). Six subsamples of a rectangular cross-section of 15 mm wide by 6.7 mm deep (1 cm^2 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 peak force as an indication of tenderness.

Lipid oxidation

Samples were removed from the -80°C freezer and thawed overnight at 4°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 min in a water bath and cooled in ice prior to determination. Samples were then homogenised in trichloroacetic acid solution and filtered. TBARS were calculated from a standard curve of malondialdehyde (MDA), freshly prepared by acidification of 1,1,3,3-tetraethoxypropane (TEP), and calculated as mg MDA per kg sample.

Instrumental color measurement

Samples were removed from the -80°C freezer and subjected to overnight thawing at 4°C. They were removed from the packaging and allowed to bloom in air for 20 min prior to color measurement. Meat color was measured using a MINOLTA CR300 (Minolta Camera Co. Ltd, Osaka, Japan) colorimeter under light source D65, with an aperture size of 8 mm, a closed cone and 10° Standard observer. The Minolta Chroma meter was calibrated against a white tile (L* = 97.06, a* = 0.41 and b* = 1.72). CIELAB L* (lightness), a* (redness) and b* (yellowness) were measured in triplicate on each sample at d 0, 1, 7 and 14 post-mortem.

Statistical analysis

Statistical analyses were performed using the mixed model procedures of SAS version 9.1.3 (SAS Institute, 2005). The following univariate mixed model was employed: $Y_{ij} = \mu+A_i+B_j+\varepsilon_{ij}$, where Y_{ij} is the response of the parameter of interest, μ is the overall mean, A_i describe the effects of different stunning methods (US, HPNP, LPNP, P) and B_j describe the random factors in the design (source of animals, lairage duration and slaughter sequence), while ε_{ij} represent the residual error. pH was included as covariate when comparing the effects of stunning methods and ageing period on meat quality parameters. All statistical analysis was conducted at 95% confidence level.

RESULTS AND DISCUSSION

Key meat quality results are presented in Table 1. In this experiment, pH values at 0, 1, 7 and 14 d post-mortem in both the LL and ST were not affected by the stunning treatments. Irrespective of the stunning methods, significant declines in muscle pH were only noticed at d 1 of post-mortem ageing and these were consistently observed in both muscles. However, the pH did not exhibit any further

Table 1. Effects of stunning on water holding capacity, TBARS and tenderness at different day(s) of ageing (Mean±SEM)

	Day 0		Day 1		Day 7		Day 14	
	LL	ST	LL	ST	LL	ST	LL	ST
Water holding capacity								
HPNP	0.90±0.01	0.92±0.01	0.85±0.02	0.87±0.01^{c}	0.89±0.01^{b}	0.90±0.01^{b}	0.94±0.04	0.94±0.03
LPNP	0.90±0.02	0.92±0.01	0.89±0.01	0.91±0.01^{ab}	0.96±0.01^{a}	0.93±0.01^{a}	0.89±0.03	0.95±0.01
P	0.90±0.01	0.91±0.02	0.89±0.01	0.95±0.02^{a}	0.88±0.01^{b}	0.90±0.01^{b}	0.91±0.01	0.90±0.01
US	0.90±0.02	0.90±0.01	0.88±0.01	0.90±0.01^{bc}	0.90±0.01^{b}	0.89±0.01^{b}	0.87±0.03	0.91±0.01
Tenderness (Peak force; kg)								
HPNP	12.15±1.37	14.49±0.99^{a}	11.88±1.00	13.08±1.43	9.58±0.70	13.07±0.78^{a}	8.22±1.02	12.73±1.03^{a}
LPNP	10.59±0.95	10.99±0.88^{bc}	14.20±1.50	9.83±0.71	9.19±1.37	12.54±1.17^{ab}	7.30±0.81	8.47±0.72^{b}
P	9.75±0.88	12.43±0.92^{ab}	11.80±0.84	10.72±1.17	9.91±0.97	9.47±0.92^{c}	7.11±0.75	8.62±1.07^{b}
US	12.22±1.10	9.51±0.87^{c}	12.99±0.97	10.37±0.82	9.96±0.72	10.19±0.84^{bc}	8.69±0.91	9.85±0.55^{b}
TBARS (mgMDA/kg meat)								
HPNP	0.28±0.02^{a}	0.28±0.03^{a}	0.25±0.02^{a}	0.26±0.02^{a}	0.29±0.02^{b}	0.41±0.02^{a}	0.38±0.04^{ab}	0.52±0.02^{a}
LPNP	0.19±0.02^{b}	0.14±0.02^{b}	0.28±0.03^{a}	0.09±0.02^{b}	0.42±0.02^{a}	0.18±0.02^{c}	0.44±0.03^{a}	0.21±0.02^{c}
P	0.24±0.05^{ab}	0.17±0.02^{b}	0.31±0.04^{a}	0.22±0.02^{a}	0.43±0.04^{a}	0.30±0.05^{b}	0.30±0.04^{b}	0.20±0.02^{c}
US	0.06±0.01^{c}	0.17±0.03^{b}	0.11±0.01^{b}	0.21±0.02^{a}	0.20±0.02^{c}	0.27±0.02^{b}	0.31±0.02^{b}	0.28±0.02^{b}

^{ab} Means within a section column with no common superscripts differ at $p<0.05$.

HPNP = High power non-penetrative percussive stunning prior to slaughter. LPNP = Low power non-penetrative percussive stunning prior to slaughter. P = Penetrative percussive stunning prior to slaughter. US = Penetrative percussive stunning after slaughter.

decline at d 7 and 14 post-mortem in both muscles.

The present results are in agreement with the findings of Petersen and Blackmore (1982), Vergara and Gallego (2000) and Velarde et al. (2003) who also reported no differences in muscle pH between non-stunned and electrically stunned lambs. A previous study in cattle reported a significantly higher muscle pH at 15 min post-mortem in the animals subjected to percussive stunning (non-penetrative) than the animals subjected to either electrical stunning or no stunning (Ö nenc and Kaya, 2004). These authors also observed no difference in pH at 24 h post-mortem. In their study, the use of percussive stunning resulted in a significantly faster rate of pH decline compared to electrical stunning or no stunning. In another related study in lambs, Paulick et al. (1989) documented significant differences in muscle pH at 45 min post-mortem with stunning method. However, pH at 15 min post-mortem (the nearest to at death muscle pH) as well as other subsequent time points over the first 24 h post-mortem were not monitored in this study, therefore no conclusions can be reached regarding pH decline. Nevertheless, pH values after 24 h were considered to be in the normal range for beef.

At d 0, pH values in the LL of cattle that were subjected to HPNP, LPNP and US were found to be higher than those in the ST, although it would be expected that the ultimate pH of ST would be higher than the LL. The differences between the results observed could be explained by differences in metabolic and contractile characteristics between the two types of skeletal muscles (Melody et al., 2004). Day 0 pH was measured at 45 min post-slaughter, during the development of rigor, and long before the ultimate pH was reached. It is likely that the rate of pH decline in ST and LL differ, leading to the apparently unusual observations on d 0. The calculated magnitudes of decline in pH over the first 24 h in LL (0.68/h in HPNP, 0.53/h in LPNP and 0.55/h in US) were numerically greater than the ST (0.33/h in HPNP, 0.33/h in LPNP and 0.33/h in US).

The effects of stunning on WHC were dependent on the days of post-mortem ageing. In LL, the effects of stunning on WHC were only present in the 7 d aged samples. Similar to the US group (control), the HPNP and P stunning methods resulted in significantly lower WHC than the LPNP group. Among the stunning groups, differences in WHC over the 14 d of ageing were only seen in the LPNP samples, whereby higher WHC ($p<0.05$) was found in the 7 d aged samples. In the ST, significant differences in WHC among the stunning treatments were observed at d 1 and 7 of ageing. At d 1 post-mortem, the WHC of the P and LPNP groups was significantly higher than in HPNP and US groups. In the 7 d aged samples, LPNP resulted in higher WHC ($p<0.05$) compared to HPNP, P and US treatments. In general, significantly higher WHC was found in both LL and ST following the LPNP stunning method. Although no differences were found between the US, HPNP and P groups, HPNP resulted in a significantly lower WHC than the LPNP group. Ö nenc and Kaya (2004) reported higher WHC in meat samples of the un-stunned than the percussively and electrically stunned cattle, and in lamb, a higher drip loss was found in stunned compared to un-stunned animals (Linares et al., 2007).

Although the observed differences in pH were not significant, they could be attributable to earlier onset of rigor development (Vergara and Gallego, 2000) and myofibrillar proteolysis in the muscles from stunned animals (Schafer et al., 2002; Melody et al., 2004). Moreover, it has also been suggested that degradation of the cytoskeletal proteins would increase WHC of meat during ageing (Kristensen and Purslow, 2001). However, pH declines and protein analysis were not included in the current study, so no firm conclusions can be drawn from the results.

Neither stunning method nor ageing period had a significant effect on cooking loss in both muscles and this is in agreement with previous findings in lambs (Vergara et al., 2005), pigs (Channon et al., 2002), broiler chickens (Mohan Raj et al., 1990) and turkeys (Northcutt et al., 1998). Recently, however, higher cooking losses have been reported in meat from electrically stunned and percussively stunned cattle compared with those without stunning (Ö nenc and Kaya, 2004).

In LL, significant effects of stunning on meat toughness expressed as peak force were noted at d 1 and 7 post-mortem ageing time points. At d 1, the highest peak force value was shown by the LPNP group with no difference ($p>0.05$) seen between the HPNP, P and US groups. In the 7 d aged samples, the values presented by the HPNP and LPNP groups were higher ($p<0.05$) than P and US groups. In the case of ST muscle, the effect of stunning treatments on peak force values was only present at d 14 with significant differences observed only between the HPNP and P groups. Similarly, the highest peak force values were indicated by the HPNP stunning group. The results indicate that the effects of stunning method on the shear force values of the LL and ST muscles were influenced by the days of ageing. In general, cattle subjected to the HPNP and LPNP stunning methods produced tougher meat than those assigned to the P and US treatments.

Previous reports on the effects of stunning on meat tenderness are rather inconsistent. A study in cattle demonstrated lower shear force values in longissimus muscle at 24 h post-mortem in the percussive stunning groups compared with the electrical stunning and non-stunned groups (Ö nenc and Kaya, 2004). This was

supported by Vergara et al. (2005) who also reported more tender meat from stunned compared to unstunned lambs. However, an earlier study by Vergara and Gallego (2000) reported a tendency for the meat from non-stunned lambs to be more tender than that from electrically stunned lambs. Meanwhile, Linares et al. (2007) documented no significant differences in toughness of lamb longissimus at d 3 and 7 post-mortem between different stunning methods (electrical stunning, gas stunning and without stunning). Although not significant, Northcutt et al. (1998) reported higher shear force values in non-stunned turkey breast muscle than those assigned to electrical and CO_2 stunning. It is interesting to note that the significant effect of stunning methods on toughness of ST muscle appeared later (d 14 post-mortem) compared to those shown by the LL muscle (d 1 and 7 post-mortem). This could be explained by differences in metabolic and contractile properties between the muscles.

It is well accepted that ST muscle is mainly involved in locomotion whilst the LL is more postural in function. At Day 0 post-mortem, the pH values in the ST were also found to be lower than the LL. In this experiment, although the rate of glycogen depletion was not measured, we speculate that the HPNP stunning method could have resulted in a more sudden and rapid rigor development in the affected muscle which in turn, may have caused more rapid biochemical change affecting pH decline and protein degradation, although the observed differences are not large. 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). In the current study temporal changes in pH over the first 24 h post-mortem were not monitored, and removal of the muscles from the hot carcass is likely to have led to shortening. Despite these omissions, because all muscles in the current study were handled in a similar fashion, the observed differences between treatments warrants further investigation.

The effects of stunning method on lipid oxidation as indicated by the values of TBARS (mg malondialdehyde/kg meat) were observed in LL and ST samples aged for 1, 7 and 14 d post-mortem. Differences in TBARS ($p<0.05$) were noted among the stunning methods in the pre-rigor (d 0) samples. In LL muscle, on d 0, the highest and lowest TBARS values were presented by the HPNP and US group, respectively. However, no differences ($p>0.05$) in TBARS values were seen between HPNP and P and between LPNP and P groups. At d 1 and 7, the TBARS values in US samples remained significantly lower than the other groups. In the 14 d aged LL samples, although not significantly different ($p>0.05$) to HPNP, the LPNP group showed significantly higher TBARS values than the P and US samples.

In the ST, the highest TBARS values were consistently found in the HPNP group at all ageing times, while LPNP resulted in lower TBARS values at all ageing times. In general, compared to the other stunning methods employed in this study, HPNP significantly accelerated muscle lipid oxidation as indicated by the TBARS values.

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 pork (Buckley et al., 1995). In pigs, stress and handling of animals before and during slaughter influences the degree of lipid oxidation in meat (Juncher et al., 2003). In the present study, the use of HPNP stunning resulted in a higher level of TBARS ($p<0.05$) which indicates greater lipid oxidation in both muscles. The results suggest that the HPNP stunning employed in this study could have resulted in greater physiological change in HPNP animals compared to those subjected to the US, LPNP and P treatments. A recent study in lambs reported significant differences in lipid oxidation between different gas stunning methods and electrical stunning at 7 d post-mortem (Bórnez et al., 2009). However, in the current study, the significant effects of stunning on the level of TBARS were detected in both muscles as early as at d 0 post-mortem and continued to be present at d 1, 7 and 14 of post-mortem ageing. There are three critical phases of lipid oxidation in meat and meat products, of which the second phase of oxidative damage occurs immediately pre-slaughter and during the early post-slaughter period: which are greatly influenced by pre-slaughter handling including stunning and other related stressors. The rate and extent of lipid oxidation also depend on the degree of tissue damage in the live animal (Morrissey et al., 1998). In agreement with previous findings in beef (Insausti et al., 2001), lambs (Berruga et al., 2005; Linares et al., 2007; Bórnez et al., 2009), pork (Martinez et al., 2005) and ostrich (Seydim et al., 2006), lipid oxidation as indicated by TBARS level increased with storage time in all treatment groups and in both muscles .

Color results are presented in Table 2. The lightness (L*) values shown by LL were not affected ($p>0.05$) by stunning method, whereas significant differences in the L* values were seen in the ST from different stunning groups, particularly at 0, 1, and 7 d post-mortem. At d 0, significant differences in L* of the ST were seen between HPNP and US, and between LPNP and US. At d 1, significant differences in L* were only noticed between LPNP and P. As at d 7 post-mortem, the L* values of P and US were

Table 2. Effects of stunning on colour coordinate values (*L**, *a**, *b**) at different day(s) of ageing (Mean±SEM)

	Day 0		Day 1		Day 7		Day 14	
	LL	ST	LL	ST	LL	ST	LL	ST
*L** (lightness)								
HPNP	33.4±0.47	36.29±0.72 ^{bc}	34.7±0.68	38.40±0.93 ^{ab}	35.1±0.36	37.08±0.67^{b}	37.1±0.53	40.01±0.88
LPNP	34.4±0.53	35.84±0.53^{c}	35.5±0.79	37.13±0.69 ^{b}	35.7±0.82	37.15±0.91 ^{b}	36.6±1.12	39.40±0.86
P	33.8±0.70	38.14±0.73^{ab}	34.4±0.93	41.05±0.84^{a}	36.0±0.54	40.19±0.72^{a}	36.9±0.98	40.66±0.98
US	34.6±0.84	38.76±0.70^{a}	35.7±0.97	39.44±1.05 ^{ab}	36.7±0.80	40.27±1.15 ^{a}	37.0±0.84	41.59±1.03
*a** (redness)								
HPNP	14.7±0.39	14.99±0.40	14.8±0.57	14.08±0.41	16.7±0.52	15.85±0.54	18.1±0.73	15.95±1.43
LPNP	14.9±0.40	14.82±0.41	16.3±0.65	15.31±0.30	18.3±0.47	16.34±0.67	18.7±0.66	17.66±0.51
P	14.0±0.20	14.55±0.63	14.8±0.44	14.68±0.63	17.5±0.66	16.26±0.70	18.9±0.24	18.05±0.39
US	14.2±0.58	14.94±0.40	15.1±0.25	13.81±0.47	16.8±0.26	15.43±0.26	17.2±0.50	16.17±0.83
*b** (yellowness)								
HPNP	-1.62±0.48^{b}	-0.11±0.46	0.13±0.57^{b}	0.94±0.51^{b}	0.93±0.79^{b}	1.26±0.73	0.08±0.47^{b}	0.46±0.24^{c}
LPNP	0.57±0.45^{a}	-0.12±0.53	2.56±0.53^{a}	1.79±0.21^{ab}	3.61±0.41^{a}	2.2±0.71	2.67±0.63^{a}	1.92±0.42^{a}
P	-1.51±0.40^{b}	-0.07±0.47	0.25±0.63^{b}	2.53±0.60^{a}	1.51±0.69^{b}	2.80±0.70	0.59±0.39^{b}	1.74±0.42^{ab}
US	-0.73±0.59^{ab}	-0.99±0.64	0.32±0.51^{b}	0.92±0.39^{b}	1.22±0.77^{b}	2.10±0.59	-0.72±0.40^{b}	0.66±0.41^{bc}

^{ab} Means within a column with no common superscripts differ at p<0.05.

HPNP = High power non-penetrative percussive stunning prior to slaughter. LPNP = Low power non-penetrative percussive stunning prior to slaughter. P = Penetrative percussive stunning prior to slaughter. US = Penetrative percussive stunning after slaughter.

significantly higher than those from HPNP and LPNP. In general, the brightest meat color was observed in the US and P stunning treatments. The redness (a*) values of LL and ST did not differ (p>0.05) between the stunning treatments and these were consistently seen over the 14 d of post-mortem ageing. However, a* values in LL and ST muscles were significantly affected by the days of ageing. In general, there were significant increases in a* values as ageing progressed in both muscles.

The presence of interactions between stunning methods and days of ageing indicate that the effect of stunning on the values of yellowness (b*) in LL and ST depended on the ageing period. In LL, the effect of stunning method on b* values was significant at d 0, 1, 7 and 14 of ageing. Interestingly, LPNP stunning resulted in significantly higher b* values in LL than the other stunning methods at d 1, 7 and 14 of ageing. Unlike the LL, the influence of days of ageing on the significant effects of stunning on b* values in ST were only noticed at d 1 and 14. At d 1, significant differences in yellowness were seen between HPNP and P, and between P and US. Compared with the HPNP and US treatments, the highest yellowness values were indicated by P. At d 14, b* values differed significantly between HPNP and LPNP, HPNP and P, and between LPNP and US. In general, the highest yellowness values in the 14 d aged ST were found in the LPNP stunning group.

In contrast to our findings, previous studies found no differences in color values among different stunning methods in lambs (Vergara and Gallego, 2000; Velarde et al., 2003; Vergara et al., 2005; Vergara et al., 2009) and cattle (Ö nenc and Kaya, 2004). In the current study, except for the b* (yellowness) values, the color values of the LL muscle were not affected by the stunning method. However, in the case of ST muscle, L* (Lightness) and b* values were significantly different with stunning methods. Brighter meat color (as indicated by higher L* values) were exhibited by the P and US samples at 0, 1 and 7 d post-mortem compared with those in the HPNP and LPNP groups . These results disagree with previous findings by Linares et al. (2007) who showed that meat from un-stunned lambs was darker (lower L* value) than that of stunned.

IMPLICATIONS

The effect of stunning methods on meat quality of two different muscles has been determined in this study. In general, stunning treatments, particularly HPNP, did affect beef quality in the LL and ST muscles, although the differences observed were not large and may not be detectable by consumers. The meat quality achieved using P, LPNP and US treatments under the constraints of this study was comparable, and no treatment stood out as considerably better than another.

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