Timing and duration of low voltage electrical stimulation on selected meat quality characteristics of light and heavy cattle carcasses

E. C. Webb [A B] and B. Agbeniga [A]

[A] Department of Animal and Wildlife Sciences, University of Pretoria, Private Bag X20, Hatfield, 0028, Pretoria, South Africa.

[B] Corresponding author. Email: edward.webb@up.ac.za

Abstract

Context: The present study investigated the effects of several electrical-stimulation parameters with conventional chilling of heavy- and light-grade carcasses from commercial feedlot cattle on selected meat-quality attributes.

Aims: The aim was to determine the combination of electrical-stimulation parameters that produced the most desirable results in terms of meat quality, which will serve as a guide to processors seeking to enact best processes in the meat industry.

Methods: Low-voltage electrical stimulation (110 V peak, 17 pulses/s, 5-ms pulse width) was applied either early post-mortem (PM) at 7 min or late PM at 45 min, for either 30 or 60 s on steer carcasses (n = 98) divided into two weight categories (light (≤260 kg) and heavy (≥290 kg) grades). The *Longissimus lumborum* muscle was evaluated for sarcomere length, myofibril fragment length (MFL), calpain-1, calpastatin, shear force and drip loss (3 and 14 days PM).

Key results: There were no significant differences in sarcomere length and no sarcomere shortening was observed. There were minor inconsistencies where early stimulation coincided with marginally longer MFL at 3 and 14 days PM, while late stimulation produced the shortest MFL at 14 days PM. Higher decline in calpain-1 concentration (mean 36.2%) was recorded in the early stimulated carcasses compared with the late stimulated carcasses (mean 29.7%) from 1 to 24 h PM, while calpastatin concentration decreased at a similar rate (mean 24%). Early stimulation resulted in lower shear force ($P < 0.05$) at 3 days PM, especially in the heavier carcasses, indicating that higher initial temperature did accelerate tenderisation. At 14 days PM, there were no significant differences in shear force as regards stimulation time or carcass weight. Higher drip loss was however recorded in the early stimulated carcasses.

Conclusions: Early application of low-voltage electrical stimulation produced faster tenderisation early PM, due to higher rigor temperature, but, after aging for 14 days, all meat was acceptably tender with a lower variability, regardless of the stimulation time and carcass weight. Higher drip loss was associated with higher tenderness, which is normal and not a defect.

Implications: The use of low-voltage electrical stimulation should be encouraged for its ability to reduce variability in meat quality due to carcass-weight differences, especially in the current feedlot systems.

Additional keywords: drip loss, meat aging, meat tenderness, proteolytic enzymes.

Introduction

According to the report of Savell (2012), beef carcass weights have been on a steady increase in recent times, which is attributed to factors such as improved nutrition and feeding methods and the use of feed additives to improve feed-conversion efficiency and carcass yield. Savell (2012) reported an increase of 1–1.5 kg/year in beef carcass weight in the USA, while Strydom *et al*. (2009) reported an increase of ~14 kg in carcass weight of steers fed with zilpaterol hydrochloride (Zilmax) in South Africa (SA). In Australia, average carcass weight increased from 180 kg/head in 1983–1984 to 287 kg/head in 2011 (MLA 2013). Likewise, according to Pesonen *et al.* (2012), average carcass weight of slaughtered bulls in Finland has increased from 275 kg (1996) to 335 kg (2008) and this heavy carcass weight is said to favour slaughterhouse pricing. Currently, there is not enough information and conclusive data on carcass traits and meat-quality parameters of such heavy beef carcasses, especially from intact carcasses under commercial feedlot conditions (Kim *et al.* 2014; Warner *et al.* 2014). A recent trend in SA is that feedlot managers feed their cattle to heavier weights for a longer period due to increasing grain prices, so as to recover the expenses on the higher-priced grains. This increase in carcass weight raises concerns in terms of carcass and meat quality because these heavy carcasses are being processed using the same electrical-stimulation (ES) protocols and chilling facilities that have been designed over the years to accommodate lighter carcasses. In a recent study by Warner *et al*. (2014) across seven beef-processing plants in Australia, it was discovered that ~75% of the carcasses surveyed had the incidence of high rigor temperature (temperature above 35°C, at pH 6; Thompson 2002). The high rigor temperature was attributed to grain feeding, which resulted in heavier carcasses. Other factors that were identified as possible contributing factors to the high rigor temperature were a high amount of electrical inputs and higher plasma insulin concentrations at slaughter. The relationship between carcass weight and meat quality has produced various reports that focus on a variety of experimental conditions. Authors, such as Shorthose and Harris (1990), have reported that heavier carcasses have less tender *longissimus* muscle compared with lighter carcasses, while Sañudo *et al*. (2004) reported more tender meat from heavier carcasses than from lighter carcasses, depending on animal breeds and types. Electrical stimulation has become a standard practice in most of the commercial abattoirs in SA, but questions are still being raised about the 'ideal' application of this technology in terms of voltage, timing and duration of application. According to Thompson (2002), MSA abattoir audits showed that heavy carcasses require less ES than do lighter carcasses, to achieve similar glycolytic rate. A major contributing factor to the increased carcass weight in SA is the use of β adrenergic agonists such as zilpaterol hydrochloride, which increases carcass yield, and is becoming more popular among feedlot operators in SA, but comes with a challenge of reduced beef tenderness due to increased calpastatin activity (Koohmaraie *et al*. 1991*b*). Researchers, such as Hope-Jones *et al*. (2010), have concluded that ES in combination with post-mortem aging could improve, but not fully overcome, the adverse effects of β-adrenergic agonist feeding on beef loin tenderness.

According to Miller *et al*. (1995), consumers have become more aware of the high variability and inconsistency in beef quality and these variations pose a serious economic challenge to the meat industry in terms of consumer acceptance. A proven intervention in alleviating the problems of variability in meat quality is the use of ES (Hopkins and Toohey 2006). Meat quality is a function of post-mortem glycolysis, which is influenced by ES in muscles (O'Halloran *et al*. 1997; Ferguson and Gerrard 2014). Electrical stimulation enhances faster glycolysis, thereby reducing the risk of cold-shortening during chilling, as well as accelerating proteolysis, being mediated by time–pH–temperature interaction, which affects

enzyme stability and activity (Hwang *et al.* 2003). Improved tenderness has been reported with the use of high- and medium-voltage ES (Gariépy *et al*. 1992; Polidori *et al.* 1996), but due to the high cost of installation and safety concerns for its operators, low-voltage electrical stimulation (LVES) is now used more frequently in many countries to replace high-voltage units. LVES is regarded as the safest and has been proven to be equally effective in improving meat quality (Aalhus *et al*. 1994; Polidori *et al*. 1999), but must be applied early post-mortem to be effective (Chrystall *et al*. 1980). There is a need to improve on existing technology and the use of ES in SA, simply because there is no uniform protocol, especially at the commercial level, which is a major source of variation in carcass and meat quality. Furthermore, there is no recommendation on pH–temperature window, nor a robust beef-classification or -grading scheme such as the Meat Standard Australia (MSA 2007–2008), in SA (Polkinghorne and Thompson 2010); rather, most processors tend to follow the SA *Meat Safety Act* of 2000 and the EU parliament regulation of 2004, which states that carcasses destined for the local or export market must attain a minimum of 7°C at the time of dispatch from the abattoir.

Excessive drip loss and low water-holding capacity (WHC), which is known to reduce the commercial yield of meat, is also a major detrimental factor caused by high rigor temperature, especially in heavy feedlot carcasses (Jacob and Hopkins 2014; Warner *et al.* 2014). Over-stimulation from ES (Hildrum *et al*. 1999), causing faster pH decline at higher carcass temperature, which could lead to protein denaturation, is also a major factor to be considered when it comes to meat WHC. That is why it is paramount to have a proper balance between pH decline and carcass temperature, so as to produce better-quality carcasses and meat, and that was part of the reason why LVES was considered for this trial. Another important factor that could cause a major significant variation in shear force and drip loss, especially early post-mortem, is the ultimate pH, which is also influenced by ES (Offer 1991). According to Thompson (2002), for 'ideal pH decline' to be realised in beef, pH should be greater than 6.0 when temperature is above 35°C and pH should be below 6.0 before temperature falls below 12°C. Time and duration of application of ES are also very critical in achieving an optimum pH decline at the appropriate carcass temperature (Chrystall *et al*. 1980; Hwang and Thompson 2001; Strydom and Frylinck 2014), and that is why a combination of treatments, which included early or late ES and shorter or longer ES, were included in the present trials.

In the wake of the ever-increasing carcass weight, and in light of earlier evidences that heavier carcasses require less ES (Thompson 2002; Hopkins *et al.* 2007*a*; Warner *et al.* 2014), there is a need to re-evaluate the effects of ES on carcass and meat quality, especially using LVES. Therefore, the objective of the present study was to evaluate the influence of LVES applied early or late post-mortem (PM), for shorter or longer duration, on meat tenderness and drip loss of light and heavy bovine carcasses from feedlot cattle.

Materials and methods

Source of animals and pre-slaughter processes

The trial was conducted at a high throughput abattoir located in the Free-state province of South Africa. The animals, which comprised mixed breeds and non-descript cross breeds,

were first reared and weaned on a rangeland at ~7–8 months of age, before their transfer to the feedlot where they were fed with a standard commercial feedlot ration, which provided them with ~10.5 MJ metabolisable energy (ME) kg^{-1} DM, available *ad libitum*, for ~3–4 months before slaughter. All animals were supplemented with zilpaterol hydrochloride, which was included in the feed (0.15 mg/kg liveweight) at 1 month before slaughter, followed by a 3-day withdrawal period pre-slaughter. Ninety-eight feedlot cattle in the 'A' age group (with no permanent incisors), representative of a typical South African feedlot-type cattle, were sampled. The fatness score was mainly 2 and 3 (lean to medium) according to the South African Beef Carcass Classification System (Meat Classification Regulation No. 863 in Government Gazzette, September 2006). The average subcutaneous fat on the 12/13th rib of the light carcasses (≤260 kg) was 3.59 mm, while that of the heavy carcasses (≥290 kg) was 6.50 mm. At each slaughter time, the cattle were transported at night by truck for 2 h (100 km) to the abattoir, with ~6-h lairage period, with free access to water but without feed. An average of 24 animals was slaughtered per time (one day in a week), using the same number of animals from each treatment group (Table 1), over a 4-week period in a conventional and humane way according to standard commercial procedure (Agbeniga and Webb 2012). The abattoir is registered with the Red Meat Abattoir Association (RMAA) and International Meat Quality Assurance Services (IMQAS), and ISO certified (FSSC ISO 22 000, 14 001, 9001 and OHAS 18 001).

Table 1. Experimental design for treatment groups

Item	Corn grain	Sunflower pelleted meal	Diet
Dry matter (%)	91.0	92.0	91.2
Organic matter (%)	97.5	92.9	97.0
Crude protein (%)	8.7	33.5	12.4
Neutral detergent fibre (%)	6.3	35.9	10.7
Ether extract (%	4.4	1	4.5
Starch (%)	73.0	–	61.6
In vitro digestibility (%)	82.0	65.0	79.4
Metabolisable energy (MJ/kg)	12.33	10.49	12.03

ES, electrical stimulation; CW, carcass weight; ES d, ES duration; Es t, time of ES application; 30 s, 30 s ES; 60 s, 60 s ES; L, light carcass grade (≤260 kg); H, heavy carcass grade (≥290 kg); A, late stimulation (after evisceration, 45 min post-mortem); B, early stimulation (before evisceration, 7 min post-mortem); *n*, number of samples. Location of stimulation is cut neck region; muscle sampled is *M. longissimus et lumborum* (LL)

Slaughter and post-slaughter processes

After sticking, each carcass was suspended by a shackle on one of the hind legs while it bled out. Early stimulation (before evisceration, B) was performed at 7 min PM, during bleeding, while late stimulation (after evisceration, A) was performed at 45 min PM (after dressing). The settings of the stimulator (Jarvis Product Corporation, South Africa, Pty Ltd) were as follows: voltage = 110 V, frequency = 17 Hz, pulse = 5 m/s. Electrical stimulation was performed by hooking the sharp positive end of the electrode on the cut neck region of each carcass. After evisceration and ES, carcasses were moved to a chiller room of 0–2°C temperature, with air speed of ~1.5 m/s^2 at relative humidity of 95%, at ~1 h PM.

The categorisation of carcasses at SA abattoirs in terms of carcass weight is as follows: light (200–225 kg), medium (226–275 kg) and heavy (over 275 kg) carcasses. Prior to slaughter at the abattoir, the liveweight of each animal was recorded, so as to estimate and to group the

carcasses according to their weights. For the light carcasses, the minimum weight was 200 kg, while, for the heavy carcasses, the maximum carcass weight was 350 kg. Carcasses were further grouped on the basis of the time of stimulation (early or late stimulation) and according to the duration of stimulation (30 or 60 s). The different combinations of these treatments, i.e. carcass weight, ES time and ES duration, made up the eight treatment groups that were compared in terms of meat-quality attributes (Table 1). This was performed to determine the combination of treatments (carcass weight, ES time and ES duration) that gives the best or poorest results, regarding meat-quality attributes. *Longissimus dorsi et lumborum* (LL) samples from each treatment group were evaluated for sarcomere length (SL) at 3 days PM, MFL, shear force (SF) and % drip loss at 3 and 14 days PM, while calpain-1 and -2 and calpastatin were evaluated on samples at 1 and 24 h PM. In addition to the comparisons among various treatment groups in terms of meat quality (Tables 2–4), the means of the main effects (individual treatments), namely, carcass weight, ES time and ES duration, and their interactions, on all carcasses were also evaluated and compared (pair-wise) as shown in Table 3. The significance of the main effects on SF and drip loss of all meat samples, irrespective of treatment groups, were also evaluated (Table 4). In other words, Table 3 shows the mean of each treatment effect (main effects) on the meat-quality attributes (SF and drip loss) of all samples, while Table 4 shows the significance of the means of the main effects and their interactions on meat SF and drip loss of all samples at 3 and 14 days PM. These analyses were performed to ascertain the effects of treatment combinations, and to determine and compare the effects of each treatment on meat-quality attributes.

Table 2. Mean ± s.e. of calpain-1 and calpastatin from feedlot *M. longissimus et lumborum* (LL) samples at 1 and 24 h post-mortem (PM)

Behaviour	Definition
Eating	Head in or above feed bunk
Ruminating	Chewing regurgitated boluses of food out of food bunk standing or lying
Resting	Includes lying or standing inactive
Drinking water	Head in or above paddle waterer
Licking mineral salts	Head in or above salt feeders

ES, electrical stimulation; A, late stimulation; B, early stimulation; L, light carcass grade; H, heavy carcass grade; 30, 30-s stimulation; 60, 60-s stimulation. Means in the same column followed by the same letter are not significantly different (at $P = 0.05$)

Table 3. The effect of carcass grade, time of stimulation application and stimulation duration on shear force (SF) and percentage drip loss (% DL) of meat samples for aging at 3 and 14 days post-mortem

Parameter	ADLIB	REST	s.e.m.	*P*-value
Productive traits				
Initial bodyweight (kg)	318.20	318.60	9.99	0.97
Final bodyweight (kg)	388.16	388.99	11.94	0.95
ADG (kg)	1.04	1.05	0.06	0.91
Dry-matter intake (kg)	7.91	7.21	0.09	<0.01
Dry-matter intake (% liveweight)	2.24	2.04	0.06	0.03
Feed conversion (kg feed/kg liveweight)	7.61	6.89	0.29	0.12
UFT initial (mm)	5.69	5.17	0.46	0.30
UFT final (mm)	9.69	8.66	0.52	0.20
UFT rate (mm/day)	0.05	0.05	0.01	0.17
ULMA initial (cm^2)	52.46	48.86	1.10	0.03
ULMA final (cm^2)	68.30	61.76	1.63	<0.01
ULMA rate (cm^2/day)	0.23	0.19	0.02	0.11
Carcass traits				
HCW (kg)	210.65	205.20	6.05	0.53
Carcass yield (%)	57.70	56.07	4.47	0.02
FT (mm)	6.7	6.6	0.63	0.11
REA (cm^2)	57.01	55.46	1.42	<0.01

Values are means ± s.d. Experimental categories are as follows: light (L) and heavy (H) carcass grades with early stimulation, before evisceration (B) and after evisceration (A), with stimulation duration of either 30 s or 60 s. Means in each category in the same column followed by different letters are significantly different (at $P = 0.05$)

Table 4. Significance of carcass weight, electrical stimulation (ES) time and ES duration (main effects) and their interactions on meat shear force (SF) and % drip loss (% DL) at 3 and 14 days post-mortem (PM)

Behaviour	ADLIB	REST	s.e.m.	*P*-value
Eating				
(min/day)	170	176	1.50	<0.01
(sessions/day)	11.3	10.0	0.34	0.02
(min/session)	15	17.6	0.55	0.06
Ruminating				
(min/day)	231	228	1.73	0.07
(sessions/day)	14.4	13.7	0.36	0.20
(min/session)	16.1	16.7	0.40	0.33
Resting				
(min/day)	1017	1006	3.36	0.02
(sessions/day)	20.6	19.5	0.38	0.06
(min/session)	49.4	51.6	1.25	0.23
Drinking water				
(min/day)	16.0	21.0	0.45	<0.01
(sessions/day)	2.9	3.4	0.19	0.11
(min/session)	5.6	6.1	0.18	0.03
Licking mineral salts				
(min/day)	6.2	9.6	1.41	0.09
(sessions/day)	1.1	1.6	0.45	0.25
(min/session)	5.7	6.0	0.75	0.84

CW, carcass weight; ES t, ES time of application; ES d, ES duration; $P = 0.05$

Table 1 shows the experimental design for the treatment groups.

Materials and methods

Carcass pH and temperature were recorded using a portable pH meter fitted with a temperature probe (RS 232, 2003 model, Oakton Instruments, Vernon Hills, IL, USA) at 45 min, 3, 6, 12 and 24 h PM. The pH glass electrode and the temperature probe were inserted into an incision (~2 cm deep) on the LL, at the 12th to13th ribs. About 500 g of LL sample was dissected from the left side of each carcass between the 11th and 13th ribs after the 24-h pH and temperature readings. The samples were packed in vacuum bags and kept at 4°C until analysis at 3 days PM. For the measurement of sarcomere length at 3 days PM, ~2 g of the sample was taken from the dissected samples and homogenised according to Hergarty and Naude (1970). Sarcomere length was measured by means of a video imaging analyser, using an Olympus B340 microscope (Olympus, Tokyo, Japan) at a magnification of ×31 000 at 3 days PM. The system was equipped with a CC12 video camera (Olympus). The processing and the quantification of measurements were undertaken with AnalySIS Life Sciences software package (Soft Imaging System Gmbh, Munster, Germany). Five sarcomeres were measured, and the readings were divided by 5, so as to obtain the average length of each sarcomere (µm) due to the small size of each sarcomere (Frylinck *et al*. 2013).

Myofibril fragment length (MFL, µm) was measured at 3 and 14 days PM, by using ~2 g of the LL muscle that was excised and stored at 4°C. Myofibrils were extracted according to Culler *et al*. (1978) and as modified by Heinz and Bruggemann (1994). Hundreds of samples were examined and measured with an Olympus BX41 system microscope at a magnification of ×400 (video imaging analyser, Soft Imaging System, Olympus).

Calpain-1, calpain-2 and calpastatin were extracted from 5 g of the LL samples at 1 and 24 h PM, as described by Dransfield (1996). Enzymes were separated by a two-step gradient ion-exchange chromatography, as described by Geesink and Koohmaraie (1999). One unit of calpain activity was defined as an increase in absorbance at 366 nm of 1.0/h at 25°C, while one unit of calpastatin activity was defined as the amount that inhibits one unit of calpain activity. Data were expressed as units/g of muscle activity (Frylinck *et al*. 2015). Protein concentration of the samples was determined by the Buiret method of Cornall *et al*. (1949).

Percentage drip loss was determined by cutting out cubes of meat (~30 g, devoid of fat) from the excised LL samples at 48 h PM and the weight of each sample was recorded. Each sample was suspended with a thin wire from the lid of a sealed, transparent plastic bottle. This was done by drilling two holes through the lid and passing the wire through the sample and through the holes to suspend the meat sample without touching the walls of the container. By so doing, the meat was allowed to release the drip directly to the floor of the container. The bottles were stored for 24 h at 0–2°C. After storage, each sample was taken from the container, gently blotted dry and weighed (Honikel 1998; Frylinck *et al*. 2013). Drip loss was expressed as a percentage of the initial sample weight:

$$\% \text{ drip loss} = \text{weight loss after drip} / \text{initial sample weight} \times 100$$

Shear-force was determined by cooking blocks of meat (~200 g; 2.54 × 15.24 cm) in transparent plastic bags placed in a water bath, to an internal temperature of 75°C, for ~1 h (Honikel 1998). Samples were cooled down on ice slurry and stored overnight in a chiller at 4°C. A hollow metal probe (1.27 cm in diameter and 8 cm in length) was used to take out six round cores, parallel to the fibre direction (AMSA 1995; Honikel 1998). Each core was sheared perpendicular to the fibre direction, through the centre, by using Warner Bratzler

shear device mounted on Universal Instron apparatus (Model 4301, Instron Ltd, Buckinghamshire, UK; cross speed = 200 mm/min). The mean value of six readings was used as the shear-force value (N) for each sample at 3 and 14 days PM.

General linear model (GLM, SAS 9.3; SAS Institute Inc. Cary, NC, USA) was used to compare the variation among the mean values of each quality attribute (SL, MFL, SF, % drip loss, calpain-1 and calpastatin) among the treatment groups. Pair-wise comparison was used to compare and rank (in order of magnitude) the means of the treatment groups (in terms of each quality attribute), to determine significant differences among treatment groups. For MFL, SF and drip-loss analyses, ANOVA was used to determine the variance contrast at 3 and 14 days of measurement. The significance of the main effects or individual treatments (carcass weight, ES time and ES duration) and their interactions were also determined on all carcasses, in relation to SF and drip loss, irrespective of treatment groups, at 3 and 14 days PM, using GLM. Means were separated and compared using Fisher's protected least significant difference (l.s.d.) at a 5% level of probability (Snedecor and Cochran 1980). Correlation analyses were performed using the CORR procedure to determine the relationships among the measured carcass and meat-quality attributes. Days/week of slaughter was included in the ANOVA as both covariate and as a random term, to determine whether there was any day-of-slaughter effect on meat- and carcass-quality attributes. Carcasses that went through high rigor temperature (Temp@pH6 above 35°C) were identified using the following equation from Warner *et al*. (2014):

$$\text{Temp@pH6} = \text{TempA} = (\text{pH}_\text{A} - 6) \times (\text{Temp}_\text{A} - \text{Temp}_\text{B})/ (\text{pH}_\text{A} - \text{pH}_\text{B})$$

where Temp_A and pH_A stand for the first temperature and pH measurement above pH 6, while Temp_B and pH_B represent the first measurement taken below pH 6. High rigor-temperature carcass was defined as Temperature@pH6 of >35°C (Thompson 2002).

Results

Analysis revealed no significant differences ($P > 0.05$) in meat-quality parameters (SF and drip loss) due to day-of-slaughter differences.

Figure 1 shows the pH and temperature plots, which indicated the trend of pH decline at a specific carcass temperature, for treatment groups from 1 to 24 h PM. It also gives an indication of carcass groups that went through high rigor temperature (pH 6 of >35°C). The measurement time points as shown in Fig. 1 are 45 min, 3, 6, 12 and 24 h PM.

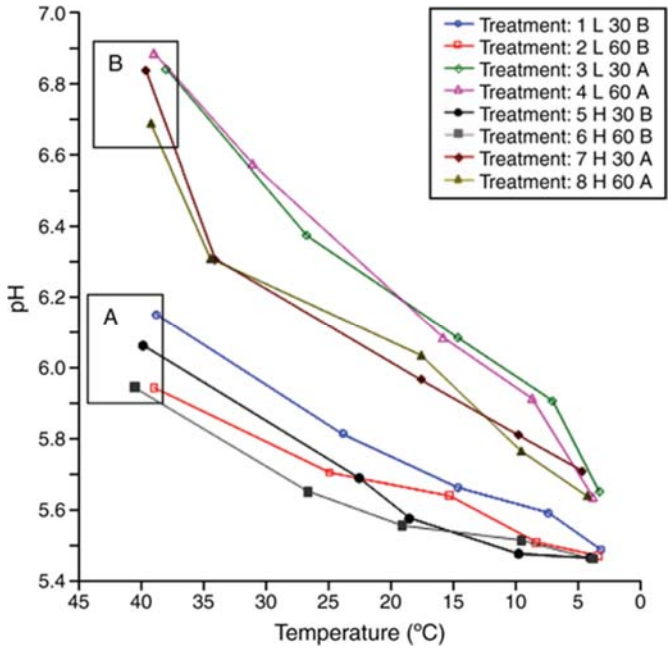

Fig. 1. Plot of pH and temperature (°C) decline for treatment groups during the first 24 h post-mortem (PM). A, late-stimulated carcasses; B, early stimulated carcasses; Temperature, carcass temperature; L, light carcass grade; H, heavy carcass grade; 30, 30-s stimulation; 60, 60-s stimulation. Time-points for measurements are 45 min, and 3, 6, 12 and 24 h PM.

Figure 2 shows the distribution of the mean SF and MFL for treatment groups at 3 and 14 days PM and the corresponding percentage change in calpain-1 between 1 and 24 h PM.

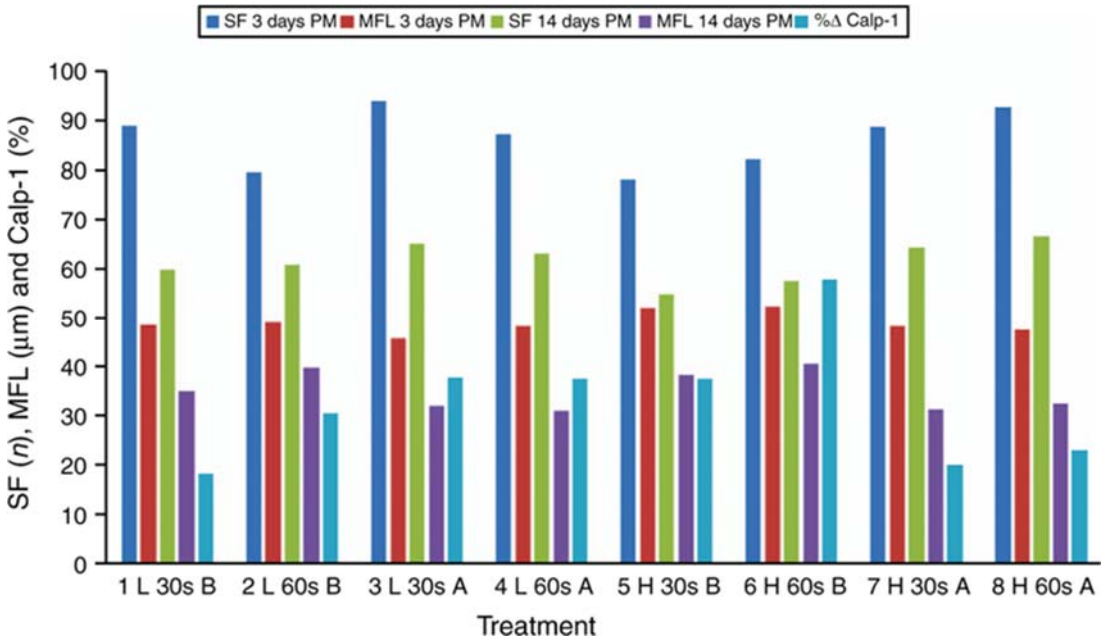

Fig. 2. Distribution of mean shear force (SF) and myofibril fragment length (MFL) at 3 and 14 days post-mortem (PM) and percentage change in calpain-1 (Calp-1) between 1 and 24 h PM for treatment groups. L, light carcass grade; H, heavy carcass grade; 30s, 30-s stimulation, 60s; 60-s stimulation; B, early stimulation; A, late stimulation.

Sarcomere lengths (SLs) for all the treatment groups were >1.9 μm and there were no significant differences among the treatment groups. This indicates lack of cold-shortening in all carcasses, according to the threshold groupings of Starkey *et al*. (2016). The early stimulated carcasses, except for Treatment group 1 (at 3 days PM), had the lowest SF at 3 and 14 days PM. In addition, at 14 days PM, the early stimulated heavier carcasses (Treatment groups 5 and 6) had numerically lowest SFs of all treatment groups. There were weak correlations between SL and most of the pH readings, as follows: SL vs $pH_{3\ h}$ (r = –0.23), $pH_{6\ h}$ (r = –0.28) and $pH_{12\ h}$ (r = –0.24). SF values and pH readings showed moderate to strong correlations, as follows: SF 3 days PM vs $pH_{45\ min}$ (r = 0.30), $pH_{3\ h}$ (r = 0.43), $pH_{6\ h}$ (r = 0.54), $pH_{12\ h}$ (r = 0.45) and $pH_{24\ h}$ (r = 0.40). SF 14 days PM vs $pH_{45\ min}$ (r = 0.24), $pH_{3\ h}$ (r = 0.40), $pH_{6\ h}$ (r = 0.48), $pH_{12\ h}$ (r = 0.36) and $pH_{24\ h}$ (r = 0.15). Initial carcass temperatures also correlated moderately with SF at 3 and 14 days PM, as follows: SF 3 days pm vs $T_{45\ min}$ (r = –0.33), SF 14 days PM vs $T_{45\ min}$ (r = –0.40).

Regarding MFL, at 3 days PM, there was no significant difference among the treatment groups, except between Treatment group 3 and Treatment groups 5 and 6. At 14 days PM, early stimulated carcasses (Treatment groups 1, 2, 5 and 6) surprisingly had numerically longest MFL, and of these groups, Treatment groups 2 and 6 (60 s stimulated) displayed the longest (P < 0.05) MFL. Overall, late-stimulated carcasses (Treatment groups 3, 4, 7 and 8) had the numerically shortest MFL. SF at 14 days PM showed a moderate positive correlation with MFL PM(r = 0.30), while MFL at 14 days PM showed weak negative correlations with $pH_{45\ min}$ (r = –0.29), $pH_{3\ h}$ (r = –0.23) and $pH_{24\ h}$ (r = –0.23).

As shown in Fig. 2, the early stimulated carcasses had a slightly higher combined average shear-force decline (29%) than did the late-stimulated groups (28%) from 3 to 14 days PM and the lowest SF at 3 and 14 days PM. The early stimulated heavy carcasses (Treatment groups 5 and 6) displayed the numerically lowest SF at 14 days PM, followed by the early stimulated lighter carcasses (Treatment groups 1 and 2) and the late-stimulated carcasses respectively. However, the late-stimulated carcasses (Treatment groups 3, 4, 7 and 8) showed higher aging tempo (MFL degradation; mean 31.9%) than did the early stimulated carcasses (mean 24.9%), which suggests that late stimulation favours MFL degradation compared with early stimulation (Fig. 2). However, this was not advantageous to meat tenderness of the late-stimulated carcasses.

Table 2 shows the mean calpain-1 and calpastatin (1 and 24 h PM) and their percentage decline for treatment groups.

As shown in Table 2, there was a general decrease in calpain-1 from 1 to 24 h PM in all treatment groups but early ES (mean-36.15%; 1, 2, 5 and 6) produced a higher decline in calpain-1 than did late ES (mean 29.7%; Treatment groups 3, 4, 7 and 8). Treatment group 6 (early stimulation) had the highest calpain-1 decline (P < 0.05; 58.1%) and the third-lowest SF at 3 days PM, while, at 14 days PM, it had the second-lowest SF. The lowest decline in calpain-1 at 24 h PM was recorded in Treatment group 1 (early ES; 18.35%), which produced the third-lowest SF at 14 days PM. The result suggests that LVES had an influence on calpain-1 autolysis and decline. Carcasses stimulated earlier, except for Treatment group 1, had an overall faster and higher calpain-1 decline than di carcasses stimulated late, as shown in the level of extractable calpain-1 at 24 h PM. Treatment group 1, which was stimulated early, had an unusually low calpain-1 decline (18.35%) at 24 h PM, compared with the other early stimulated groups, as well as the highest amount of calpastatin (2.77 units/g) at 3 days PM, as shown in Table 2, which might have inhibited the activity and extractability of

calpain-1 at 24 h PM. The late-stimulated heavy carcasses (Treatment groups 7 and 8) also had distinctly lower % decline of calpain-1, which was reflected in the relatively high shear-force values, compared with the early stimulated counterparts, especially at 14 days PM.

Calpastatin had a general decrease from 1 to 24 h PM in all treatment groups, but there was no significant difference in decline between the early stimulated (mean 24.6%) and the late-stimulated (mean 24.8%) carcasses. At 24 h PM, there were no significant differences among all the treatment groups. Calpain-2 did not show significant differences at 1 and 24 h PM among the treatment groups and that is why it was excluded from the data.

Figure 3 shows the distribution of the mean values of percentage drip loss for all treatment groups at 3 and 14 days PM.

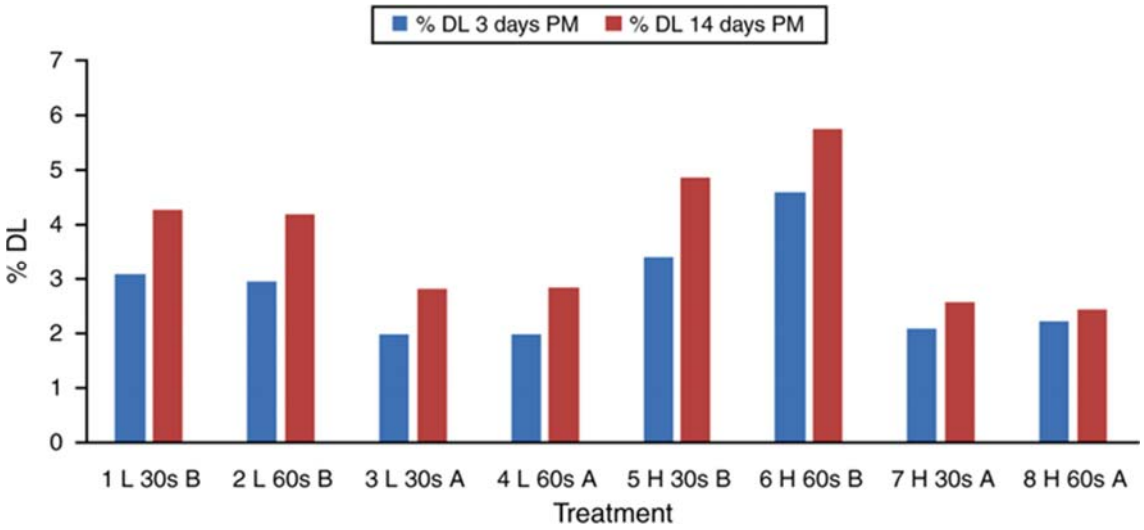

Fig. 3. Distribution of mean percentage drip loss (DL) of treatment groups at 3 and 14 days post-mortem (PM). L, light carcass grade; H, heavy carcass grade; 30s, 30-s stimulation; 60s; 60-s stimulation; B, early stimulation; A, late stimulation.

There was a clear pattern of reaction at 3 and 14 days PM, regarding drip loss. Early stimulated carcasses (Treatment groups 1, 2, 5 and 6) had but significantly higher drip losses ($P < 0.05$) than did the late-stimulated carcasses (Treatment groups 3, 4, 7 and 8). In terms of the duration of stimulation, there were mixed reactions from the treatment groups. Heavy carcasses (Treatment groups 5 and 6) from the early stimulated groups had the overall highest drip loss at 3 and 14 days PM. This is consistent with the faster rate of tenderisation of the early stimulated carcasses, especially the heavy ones, as shown in Fig. 2. The relationship between SF and drip loss are shown in Fig. 4, and this shows a linear relation between the two parameters.

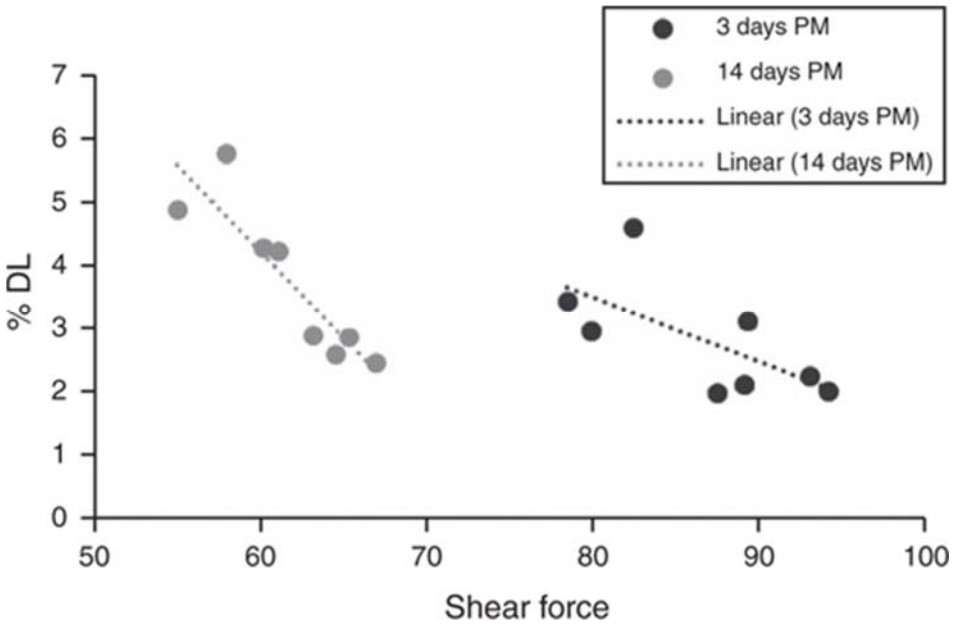

Fig. 4. Relationship between shear force and percentage drip loss (DL) of meat at 3 and 14 days post-mortem (PM).

The mean values of the effects of carcass weight, ES time and ES duration (main effects) on shear-force values and % drip loss are presented in Table 3.

Table 4 shows the significance of the effects of carcass weight, ES time and ES duration (main effects) and their interactions on meat SF and drip loss at 3 and 14 days PM.

Overall, there was no significant difference among carcass weights in terms of meat SF, but heavier carcasses had a slightly lower SF, as shown in Table 3, while early stimulated carcasses had a significantly higher drip loss than did the late-stimulated carcasses at 3 and 14 days PM. Overall, heavier carcasses produced a significantly ($P < 0.05$) higher drip loss than did lighter carcasses at 3 days PM (Tables 3, 4). Early stimulation produced a significantly ($P < 0.05$) lower SF at 3 days PM (Tables 3, 4). At 14 days PM, early stimulated carcasses produced a marginally, although not significantly, lower SF than did the late-stimulated carcasses. Early ES produced a significantly ($P < 0.05$) higher drip loss at 3 and 14 days PM (Tables 3, 4) than did the late ES, while the early stimulated heavy carcasses produced the highest drip loss at 3 and 14 days PM. There was no significant influence of ES duration on meat SF, but 60 s ES produced a marginally lower SF (at 3 days PM) and a marginally higher drip loss at 3 and 14 days PM than did a shorter duration of ES (30 s; Table 3). The interaction of carcass weight and duration of ES also produced a significant influence on meat SF at 3 days PM (Table 4). Heavier carcasses stimulated for 30 s produced a significantly ($P < 0.05$) lower SF than did smaller carcasses stimulated for 30 s or heavier carcasses stimulated for 60 s (Table 4).

Discussion

Because the SL was >1.90 μm in all carcass groups, meat samples in all groups could be regarded as tender (Starkey *et al.* 2016; Hopkins *et al*. 2007*b*). However, late stimulation produced a higher SF, as shown in Fig. 2, with an average SF of ~64 N, compared with that in the early stimulated carcass groups of ~57 N, after aging, and could be regarded as slightly tough (Huffman *et al.* 1996). Currently, there is no consensus on protocols and a threshold for acceptable tenderness; however, most surveys have indicated that SF in the range of 30–60 N is acceptable by consumers, ranging from extremely tender to slightly tough meat (Huffman *et al.* 1996; Miller *et al.* 2001). Early stimulation produced a faster pH decline, which brought about a significantly lower SF at 3 days PM and, although it produced a lower SF at 14 days PM, there was no significant difference. The heavier carcasses also produced a numerically lower SF at 3 and 14 days PM (Tables 3, 4). Most of the early stimulated carcasses (Treatment groups 4, 7 and 8) passed through the rigor-shortening window (pH < 6 at t > 35°C) (Fig. 1), but tenderness was not affected. This result partially agrees with the report of Smulders *et al*. (1990), who indicated that tenderness is less dependent on SL in fast-glycolysing muscles and in muscles with rapid pH decline. According to Smulders *et al*. (1990), when muscle pH decreases to <6.3 before intracellular calcium concentration reaches a level high enough to initiate contraction, such conditions would reduce the proportion of myosin cross-bridges in the strong binding state. This accounts for the lack of relationship between SL and tenderness in muscle that attains a pH of 6.3 or less within 3 h PM. The lower SF in the early stimulated heavy carcasses (Treatment groups 5 and 6, Fig. 2) implies that heavy carcasses treated with an early application of LVES have the tendency of having a lower SF, even at 14 days of aging, despite the rapid decline in pH at higher carcass temperatures early PM. It also suggests that aging potential is affected to a lower extent using LVES. This agrees with the report of Thompson (2002) and Warner *et al*. (2014), which showed that heavier carcasses require less ES to achieve a similar glycolytic rate than do lighter carcasses. This indicates a positive synergy among carcass weight, shorter duration and early application of LVES, in reducing variation in meat tenderness from heavy and lighter feedlot cattle.

In terms of MFL degradation and tenderness, the late-stimulated carcasses (mean 32%) had a higher mean degradation at 14 days PM than did the early stimulated carcasses (mean 24.7%). This indicated a higher aging tempo in the late-stimulated carcasses, with a higher pH or a less rapid pH decline at all time-points, and this agrees with the report of Contreras-Castillo *et al.* (2016). This could be attributed to a smaller decline in calpain-1 in the late-stimulated carcasses, which made more calpains available during aging. The result indicated that, despite the reduction in aging tempo in the early stimulated carcasses (Fig. 2), lower SF was achieved at 3 and 14 days PM, using LVES. However, Devine *et al*. (1999) cautioned that MFL alone may not be a complete indicator of the extent of tenderisation during proteolytic aging. Li *et al*. (2011) reported that LVES applied after dressing did not affect sarcoplasmic or myofibrillar protein in the LL. However, they showed that the concentration of sarcoplasmic protein increased slightly with time, which could be attributed to the release of some encapsulated proteins such as lysosomal enzymes and proteolytic fragments from myofibrillar proteins, which could increase aging tempo and, hence, tenderness. This could be related to the unexpectedly higher aging tempo in the late-stimulated carcasses.

It is well documented that the rate of pH and temperature decline PM in carcasses influence the binding of calpain to calpastatin, and the action of calpains on myofibrillar proteins to initiate tenderisation (Dransfeld 1993; Morton *et al.* 1999). Morton *et al*. (1999) reported that

pH had no direct influence on tenderness, but muscles with a high pH at 1 h PM had significantly slower declines in calpain-1 and calpastatin concentrations. These authors found a strong correlation of pH at 3 h PM with SF, using high-voltage electrical stimulation (900V) and they indicated that carcasses with the most rapid pH decline, at higher temperatures, had the highest loss of calpain-1 activity at 24 h PM. Although in their study, they had a much higher decline of ~80% in both calpain and calpastatin activity at 24 h PM, this was probably due to the high-voltage electrical stimulation that was used. In the present study, higher calpain-1 decline (mean 36.2%) was recorded in the early stimulated carcasses, compared with 29.7% in the late-stimulated carcasses at 24 h PM. Authors such as Huff-Lonergan *et al*. (1996) have suggested that the degradation of structural proteins such as titin, nebulin, desmin and troponin-T, which went through similar proteolysis as did myofibrils incubated with calpain-1, could potentially contribute to meat tenderness. The mixed concentration of the proteolytic enzymes in the present study showed that inherent variabilities in meat should be taken into account when it comes to meat quality. However, despite the mixed results in calpain-1 activity, the lower mean SF in the early stimulated carcasses at 3 and 14 days PM still corresponds with the higher percentage of calpain-1 decline than in the late-stimulated carcasses. This implies that LVES, compared to high voltage electrical stimulation, has the capacity to produce meat of acceptable tenderness with a relatively lower calpain decline and autolysis due to rapid glycolysis. The early stimulated carcasses had more tender meat ($P < 0.05$) at 3 days PM and a lower SF at 14 days PM. It is possible that Ca^{2+} was activated to a lesser extent with LVES, but the produced meat was still of acceptable tenderness. This suggests that more calpains would be available for extended aging in both the early and late-stimulated carcasses. Calpain-2 showed negligible variations at 1 and 24 h PM and it was speculated that Ca^{2+} activated calpain-2 to a very low extent (Dransfeld 1994; Morton *et al.* 1999), and it was concluded that calpain-2 played very little role in proteolysis due to lack of autolysis (Veiseth *et al.* 2001, 2004).

It is well documented that zilpaterol hydrochloride (which was administered to animals in the present study) and other β-agonists reduce meat-tenderisation process (Dunshea *et al*. 2005; Hope-Jones *et al*. 2010), mainly due to increased calpastatin activity, which is known to reduce PM aging (Koohmaraie and Shackelford 1991; Geesink *et al*. 1993). Hope-Jones *et al*. (2010) concluded that ES reduced the negative effect of zilpaterol hydrochloride on tenderness, but could not completely eradicate its negative effect on aging. In the present study, the average decline in calpastatin for the early and late-stimulated carcasses was 24%, which suggests similar levels of binding and inhibition of calpastatin in both groups at 24 h PM. This indicates some complexities in the role of calpastatin. Nevertheless, it is likely that the initial high rigor temperature in the early stimulated carcasses played a significant role in calpain autolysis. This is in accord with the findings of Thomson *et al*. (2008) and Warner *et al*. (2014) where high temperature–low pH, especially in heavier feedlot carcasses, resulted in reduced aging. According to Warner *et al*. (2014), the higher rigor temperature in the heavier carcasses was associated with fat depth, carcass weight, increased electrical inputs and blood plasma insulin, which influenced the pH–temperature decline and glycolysis. Researchers such as Sañudo *et al*. (2004) have also reported that heavier cattle have more tender meat, while other researchers such as Shorthose and Harris (1990) have reported contrarily. The review of Kim *et al*. (2014) showed that knowledge gap still exists in understanding the underlying mechanisms that affect proteolytic enzymes and other metabolic processes as influenced by high rigor temperature, especially in intact carcasses from feedlot cattle raised under commercial conditions. Despite the differences in pH decline and chilling rate between the heavy and lighter carcasses, the results obtained in the present study show that heavier

carcasses could be conveniently processed alongside smaller carcasses, using LVES without detrimental effects on tenderness.

Regarding drip loss, early stimulated carcasses had the highest drip loss ($P < 0.05$) early post-mortem, and this could be attributed mainly to the rapid pH decline at a high initial carcass temperature, which led to more protein denaturation and cytoskeletal degradation that comes with tenderisation (den Hertog-Meischke *et al*. 1997; McGlone *et al*. 2005; Kim *et al*. 2014). However, some researchers have shown that high pre-rigor temperature may not necessarily affect WHC (Rosenvold *et al.* 2008; Devine *et al.* 2014), concluding that drip loss was mainly caused by continuous post-mortem cytoskeletal degradation that accompanies tenderisation as meat ages. However, most of the experiments took place under different controlled conditions, such as wrapping and the use of water bath, and these researchers (Devine *et al*. 2014) admitted that the results could be different for intact muscles undergoing normal chilling procedures with variable airflow and carcass sizes. Moreover, most of the early stimulated carcasses in the present study attained pH of 6 at ~38–40°C (Fig. 1), which was quite high; hence, the effect of pre-rigor temperature cannot be ruled out, as this can influence myosin denaturation (Offer 1991). It has also been shown that SF is related to drip loss, especially at the early PM period and especially when ES is administered, but the drip that is released during aging is not dependent on the initial high temperature and low pH, but, rather, the rate of proteolytic activities and tenderisation that follows (Devine *et al.* 2014). In other words, SF has a linear relationship with drip loss, and this has been demonstrated in Fig. 4. During aging, more drip is lost mainly due to cytoskeletal protein degradation as proteolysis advances (McGlone *et al*. 2005; Rosenvold *et al.* 2008). Furthermore, the ultimate pH in the early stimulated carcasses was slightly lower (~5.48) than that in the late-stimulated carcasses (~5.68), as shown in Fig. 1, and this might have also contributed to the higher drip loss in the early ES carcasses (Offer 1991). Additionally, the quicker formation of actomyosin at rigor, as a result of a faster pH decline, due to ES, reduces the inter-filamental space, leading to drip loss (Offer and Cousins 1992). Heavier carcasses had a significantly higher drip loss at 3 days PM and this could be attributed to the higher protein density. According to Bertram *et al*. (2007), heavier carcasses are known to have higher myofibrillar protein density, which reduces intra-myofibrillar water content, thereby increasing inter-myofibrillar water content. On application of ES, this inter-myofibrillar water could easily be flushed out, as a result of lactate accumulation due to faster glycolysis and faster pH decline at a higher carcass temperature as myosin heads denature, thereby reducing the ability to bind water (Offer 1991). Overall, the drip loss in all carcasses was within the acceptable range (<5.0%, van Laack *et al*. (1994)), except in the early stimulated heavy carcasses that were stimulated for a longer duration (60 s; Table 3). This shows that longer or more intense stimulation is not good for early stimulated heavy feedlot carcasses, and this agrees with the recent findings of Warner *et al*. (2014).

Conclusions

The results of the study suggest that current LVES procedure, i.e. with either early or late stimulation, or for short or long duration, is equally effective in producing acceptably tender meat for heavy or light carcasses from current feedlot systems. However, early stimulation resulted in marginally earlier tenderness and this is likely to be due to the higher temperatures at rigor mortis, which resulted in rapid tenderisation. This ensured tender meat at the abattoir or butchery on display at 3 days PM. However, tenderisation was equally effective for all

situations at 14 days PM. There was a corresponding reduction in proteolytic enzymes and increased myofibrillar degradation consistent with such tenderisation. Higher drip loss was associated with rapid tenderisation, which is normal, and not a defect.

Conflicts of interest

The authors declare no conflict of interest.

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