

# **Influence of carcass weight on meat quality of commercial feedlot steers with similar feedlot, slaughter and post-mortem management**

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Short title: Influence of carcass weight on carcass and meat quality

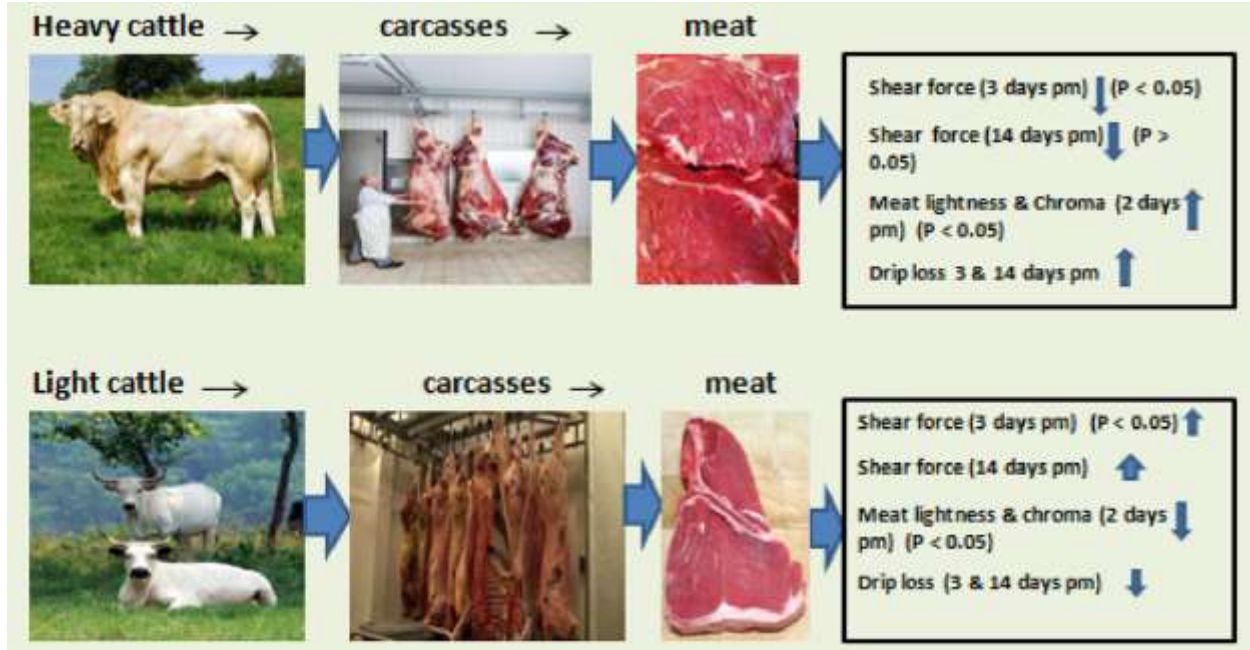
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## **Highlights**

- Heavier carcasses from feedlot cattle produced meat with significantly higher tenderness early post mortem.
- Heavier carcasses from feedlot cattle showed a more favorable meat color early post-mortem.
- Meat from heavy carcasses from feedlot cattle produced marginally higher drip loss than meat from lighter carcasses.
- Heavy carcasses can be conveniently processed alongside lighter carcasses using low voltage electrical stimulation.
- The production of heavier carcasses from feedlot cattle should be encouraged.

## Graphical abstract



## Abstract

The effects of beef carcass weight on muscle pH and temperature profile and selected meat quality attributes were evaluated. Twenty-six carcasses from light ( $\leq 260$  kg,  $n = 15$ ) and heavy ( $\geq 290$  kg,  $n = 11$ ) feedlot steers were randomly allocated and stimulated with low voltage electrical stimulation (LVES) for 30 s at 7 min post-mortem (pm). Quality evaluations were carried out on samples from the *Longissimus et lumborum* (LL) muscle from the left side of each carcass. Heavier carcasses showed faster pH decline and slower ( $P < 0.05$ ) temperature decline at 45 min, 3, 6, 12 and 24 h pm. Heavier carcasses passed through the heat shortening window (i.e. at pH 6 temperature was  $> 35^{\circ}\text{C}$ ) but there was no sign of sarcomere shortening in any carcass. Significantly lower ( $P < 0.05$ ) shear force values were recorded in the heavier carcasses at 3 days pm but at 14 days pm, heavier carcasses had numerically lower but not significantly different shear force. Heavier carcasses produced numerically higher but not significant ( $P > 0.05$ ) drip loss at 3 and 14 days pm as well as higher  $L^*$  (meat lightness) ( $P < 0.05$ ) and  $C^*$  (chroma) ( $P < 0.05$ ) values early (2 days) pm. However, at 14 days pm, there were no significant differences between the light and heavy carcasses in terms of  $L^*$  and  $C^*$ . No significant difference was observed between

heavy and light carcasses in terms of H\* at 2 and 14 days pm. The study showed that heavier carcasses which favor slaughter house pricing can be produced and processed alongside lighter carcasses without significant detrimental effects on meat quality by using low voltage electrical stimulation (LVES).

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**Keywords:** heavy carcasses, light carcasses, electrical stimulation, meat shear force, drip loss, meat color

## 1. Introduction

There has been a steady increase of bovine carcass weight in most parts of the world in recent time (Savell, 2012; Pesonen et al., 2012). This can be attributed to a number of factors including better management, better nutrition, genetic improvement, use of steroidal growth enhancers and beta agonist such as zilpaterol hydrochloride (Zilmax), which was approved for use in South Africa (Registered by Intervet S.A. (Pty) Limited; Reg. No 91/06580/07) in 1997 (Delmore et al., 2010; Strydom et al., 2009). According to Savell (2012), carcass weight increases by about 1 to 1.5 kg yearly in the USA. Delmore *et al.* (2010) also reported about 10 and 15 kg increase in live and carcass weight respectively in Zilmax fed cattle. In South Africa, Strydom *et al.* (2009) reported about 14 kg increases in carcass weight when steers were fed Zilmax. Beta-agonists (e.g Zilmax) acts as repartitioning agents by redirecting nutrients which are used for growth towards increased rate of muscle protein synthesis leading to larger muscles (hypertrophy) and away from fat deposition (reduced lipogenesis). The repartitioning effects occur through beta-adrenergic receptors on skeletal muscles and adipose membranes which generate signals that control metabolic activities in the cells. Consequently, there is reduced feed intake at greater or equal rate of body weight gain (improved feed conversion efficiency) (Koochmaraie et al., 1991b; Strydom et al., 2009). The use of feed supplements like Zilmax also has a disadvantage of decreased meat tenderness due to increased calpastatin activity (Koochmaraie *et al.*, 1991b). Calpastatin, which is associated with the calpain proteolytic enzyme family, is known to be an endogenous calpain-specific inhibitor which reduces calpain activity during proteolysis (Wendt, Thompson and Goll, 2004). Beta-agonists like Zilmax have been linked with elevated calpastatin activity and mRNA expression which are known to increase meat

toughness (Dunshea et al., 2005). A recent trend in South Africa is that cattle are fed to a higher weight for a longer period at the feedlot in order to compensate for the increase in price of grains. The money spent on the higher priced grain is recovered through the increased live weight, since the animals are sold by weight (Spies, 2016; Agri Africa, 2016- [www.farmingportal.co.za](http://www.farmingportal.co.za)). However, this increase in live and carcass weight raises concerns in terms of carcass and meat quality because the heavier carcasses have to be processed using the same facilities that were designed over the years to accommodate smaller carcasses. It has been reported that heavier carcasses require less electrical stimulation than lighter carcasses to achieve similar glycolytic potential (Thompson, 2002). This was one of the reasons for using LVES in this study. The study of Hopkins et al. (2007) showed faster pH decline in most of the heavier carcasses that were not electrically stimulated and most carcasses did not comply with the MSA (Meat Standard Australia) temperature/pH window. Furthermore, heavier carcasses may have a higher risk of high temperature rigor and entering the heat shortening window (pH 6 at loin temperature above 35°C- Thompson, 2002) due to insulation from higher subcutaneous fat thickness and faster glycolytic rate (Warner et al., 2014). This could reduce tenderness and ageing potential due to early exhaustion of proteolytic enzyme activity at higher carcass temperature and low muscle pH (Simmons et al., 1996). A recent study by Warner et al. (2014), which was the first to investigate the phenomenon of high temperature rigor across a beef industry, discovered an unacceptably high occurrence (72%) of high rigor temperature in beef carcasses across Australia. This calls for an urgent investigation of carcass and meat quality especially in the heavier carcasses that are currently produced from feedlot cattle in South Africa. The recent review of Kim et al. (2014) also pointed out that knowledge gaps still exists in the understanding of the underlying mechanisms by which proteolytic enzymes and other metabolic processes are influenced by high temperature rigor (which is prevalent in heavier carcasses), especially in intact beef carcasses under commercial conditions.

A major intervention in alleviating the problems of variability in meat quality is the use of electrical stimulation (ES) (Hopkins and Toohey, 2006) which has been adopted by commercial meat processors in many countries including South Africa. ES was originally developed in New Zealand in the

late 1970's to manage meat toughening by reducing cold-shortening in large amount of lamb carcasses that were rapidly frozen after slaughter for export purposes (Simmons et al., 2008). It was later discovered that the ES improves meat quality in other species (e.g. bovine, swine and poultry). ES depletes energy reserves in muscle at a faster rate through the process of glycolysis thereby reducing the risk of cold or sarcomere shortening (which causes reduced tenderness), while carcasses are chilled. According to Ferguson and Gerrard (2014), the glycolytic rate and energy metabolism play a crucial role in transforming living muscle into high quality meat. Glycolytic rate is a function of the rate of pH decline, cooling rate (which is affected by subcutaneous fat), residual muscle glycogen content and plasma insulin level at slaughter (Warner et al., 2014). Warner et al. (2014) indicated that heavier carcasses displayed higher rigor temperature (Temp at pH6). This was associated with fat depth, carcass weight and blood plasma insulin which affects pH and temperature decline rate and hence, glycolysis. According to these authors, the higher the carcass weight, the higher the temperature at pH6. These authors also acknowledged that there is little information on factors that impact high temperature rigor in beef carcasses. A secondary effect of ES is the acceleration of proteolysis, through activation of  $Ca^{2+}$  which in turn activates calpain enzyme activity, a process which is mediated by time-pH-temperature interaction (Hwang et al., 2003). The study of Savell, Smith, Carpenter & Parish (1979) on heavy carcasses showed that ES (400V) produced higher ratings for tenderness, flavor, color and overall palatability compared to non-ES sides. However, these qualities were not compared to the meat quality of lighter carcasses in that study. Care should be taken in choosing the type of stimulation systems to be used as this could lead to over or under- stimulation which can result in quality defects such as reduced tenderness, color defects and excessive drip loss (Hildrum *et al.*, 1999). Low voltage ES, which was used in this study, is now frequently used in many countries because it is regarded as safer and cheaper to procure and install, compared to high and medium voltage equipment. It has also been proven to be as effective as high or medium voltage electrical stimulation in enhancing carcass and meat quality but according to Chrystall, Devine and Davey (1980), it must be applied early pm in order to be effective. Currently in SA, most commercial abattoirs use either medium or high voltage ES systems and as indicated earlier, LVES

remains the safest and the cheapest to use. Furthermore, it is difficult to have two different stimulation systems based on carcass weights at any abattoir and that is why LVES was chosen as it has been known to be generally effective for beef carcasses (Bouton et al., 1978).

Currently, no elaborate study has been undertaken to compare the merits and/or demerits of producing heavier beef carcasses compared to lighter carcasses in terms of carcass and meat quality. Most of the research conducted to date has been done on muscles of other species, smaller ruminants, animals from extensive systems or different production systems where high or medium voltage and other electrical inputs were used (Ellies-Oury et al., 2017; Kim et al., 2014; Warner et al; 2014; Sanudo et al., 2004). It has been reported that heavier cattle had less tender meat (Shorthose & Harris, 1990) while other researchers reported contrarily that heavier cattle had more tender meat (Sanudo et al., 2004). The latter authors acknowledged that most of the researches carried out have been on cattle from extensive systems and that there is lack of information on intensively fattened cattle. Warner et al. (2014) recommended that further research needs to be carried out on cattle fed on grain for over 70 days to ascertain the impact of weight on high rigor temperature. It is known that slaughter house pricing favors heavy beef carcasses (Pesonen et al., 2012) in many countries including South Africa. In addition, there is an increase in demand for good quality animal protein in the developing countries where hunger and malnutrition is prevalent (Webb, 2013). In view of the contrasting reports surrounding carcass weight and beef quality and because of the earlier evidence that heavier carcasses exhibited a faster pH decline and glycolytic rate and did not comply with the MSA temperature/pH window compared to smaller carcasses (Hopkins et al., 2007; Warner et al., 2014), it is important to evaluate the effects of carcass weight on carcass and meat quality attributes. Therefore the objective of this study was to evaluate the influence of carcass weight on selected quality attributes of beef from light and heavy commercial feedlot cattle, with similar feeding regime, slaughter and post-slaughter management. .

## 2. Materials and methods

### 2.1. Experimental animals and abattoir

Heavy ( $\geq 290$  kg;  $n = 11$ , mainly Bonsmara, Drakensberger and their crosses) and light ( $\leq 260$  kg;  $n = 15$ , mainly Bonsmara, Drakenberger and their crosses) carcasses from twenty-six feedlot cattle of mixed breeds (e.g. breeds or crosses of Brahman, Bonsmara, Tuli, Drakensberger and Nguni) representative of typical South African feedlot steers (*ca* 12 months old) were randomly selected at a high throughput commercial abattoir. Fatness scores (subcutaneous fat thickness- SF) for the lighter carcasses were class 2 (1 to 3 mm) and a few 3 (3 to 5 mm) with a mean value of  $3.59 \pm 1.08$  mm (lean to medium). Heavy carcasses fall under class 4 (5 to 7 mm) (fat) with a mean subcutaneous fat thickness of  $6.5 \pm 2.05$  mm on 12/13<sup>th</sup> rib, according to the South African Beef Carcass Classification System (Meat Classification Regulation No 863 in Government Gazzette, September 2006). The animals were first reared on a rangeland and weaned at about 7 to 8 months prior to their transfer to the feedlot where they were fed with commercial ration which provided about 10.5 MJ ME Kg<sup>-1</sup> DM, available *ad libitum* for 3 to 4 months prior to slaughter. Zilmax was included in their feed (0.015 mg/kg live weight) one month before slaughter followed by a three day withdrawal period according to industry procedure (Hope Jones et al., 2010; Strydom et al, 2009). Animals were transported by truck to the abattoir at night for about 2 h with about 6 h lairage time, with access to water but without feed. All animals were slaughtered the same day in a conventional way i.e. stunning was done with a pneumatic captive bolt gun in a humane slaughter box. After the stun, each animal was shackled on one hind leg and moved to the bleeding rail where the carcasses were bled, according to standard commercial procedures (Agbeniga and Webb, 2012). The abattoir is registered with the Red Meat Abattoir Association (RMAA) and International Meat Quality Assurance Services (IMQAS) and ISO (22000, 14001, 9001 and OHAS 18001) certified.

### 2.2. Slaughter and Post-slaughter processes

Each animal was stuck with a sharp knife while suspended with a shackle on one hind leg for the carcass to bleed. Seven minutes after sticking, each carcass was electrically stimulated with a low voltage

stimulator while the carcass was still bleeding. The settings of the portable stimulator (Jarvis Product Incorporated, R.S.A, PTY LTD) was: voltage = 110V, frequency = 17 Hz, pulse = 5 m/s and AC current = 1A. The positive electrode of the electrical stimulator was hooked on the cut neck region of each carcass for 30 s during stimulation. Bleeding was followed by carcass evisceration, splitting and grading. Carcasses were then moved at 1 h pm to a chiller room of  $\pm 2^{\circ}\text{C}$  and air speed of about  $1.5 \text{ m/s}^2$  after evisceration and carcass splitting. The first pH and temperature (45 min pm) reading was done at the grading area on the hot carcasses before being moved to the chiller room. In the chiller room, subsequent carcass pH and temperature measurements were done using the same portable pH meter fitted with temperature probe (Oakton Instrument, RS 232, 2003 model) at 3, 6, 12 and 24 h pm. The pH and temperature probes were inserted into an incision (about 2 cm deep) on the LL, at the 12<sup>th</sup> to 13<sup>th</sup> ribs about 5 cm from the medial line. After the 24 h pH and temperature measurements, about 500 g of LL sample was dissected from the left side of each carcass between the 11<sup>th</sup> and 13<sup>th</sup> ribs. Samples were packed in vacuum bags and transported to the research station at about  $4^{\circ}\text{C}$ , for about 5 h. On arrival at the research station, the samples were stored at  $4^{\circ}\text{C}$ . At 48 h pm, the vacuum bags were opened and samples for the 3 and 14 day quality analyses were dissected and kept in a chiller at  $4^{\circ}\text{C}$ . Samples (*ca* 30 g) for 3 day determination of drip loss were also dissected and prepared for storage (24 h) and evaluated for drip loss at 3 days pm (see section 2.4). The first color measurement was done at 48 h pm because the color measuring system could not be moved to the other laboratory where cooking for 3 day shear force measurement and the determination of other attributes would take place.

### *2.3. Meat color determination*

Meat color attributes were measured at 2 and 14 days pm with a Minolta meter (Model CR200, Osaka, Japan) fitted to a computer system. Fresh cut steaks of about 1.5 cm thick from each excised LL samples were allowed to bloom for 1 h at  $4^{\circ}\text{C}$  before taking the readings (Frylinck et al., 2013). Color attributes were recorded as lightness,  $L^*$  value (lightness),  $a^*$  value (redness) and  $b^*$  value (yellowness) (CIE, 1986). The physiological attribute of chroma ( $C^*$ ), which is the intensity of red color (saturation



index) was calculated as:  $(a^{*2} + b^{*2})^{1/2}$  (MacDougall, 1977) and hue angle ( $H^*$ ), which indicates discoloration was calculated as  $\tan^{-1} (b^*/a^*)$  (Young et al. 1999). The values of the color attributes were recorded with spectral components excluded (SCE) and D65 lamp was used for analyses.

#### 2.4. Drip loss determination

Percentage drip loss was determined by cutting out cubes of meat (about 30 g, devoid of fat) from the excised LL samples (stored at 2 to 4°C) at 48 h pm. 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 achieved by drilling two holes through the lid and passing the wire through the sample and the holes in order to suspend the meat sample without touching the wall of the container. In doing so, the meat sample was able to release its drip directly to the floor of the container. The bottle containing the samples were then stored for 24 h at 2 to 4°C. After storage, each sample was taken out of the container, gently blotted dry and weighed (Frylinck et al., 2013; Honikel, 1998). Duplicate samples were also prepared and stored in bottles for drip loss measurement at 14 days pm, at 2 to 4°C. Drip loss was expressed as the percentage of the initial weight:

$$\% \text{ Drip loss} = \frac{\text{weight loss after drip}}{\text{Initial sample weight}} \times \frac{100}{1}$$

#### 2.5. Proteolytic enzyme determination

Calpain-1 and 2 and calpastatin activity were determined from about 5 g of the LL muscle samples taken 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 increase in absorbance at 366 nm of 1 h at 25°C while one unit of calpastatin activity was defined as the amount that inhibits one unit of calpain activity. Data was expressed as unit/g of muscle activity (Frylinck et al., 2015). Protein concentration of the samples was determined by the Biuret method of Cornall et al. (1949).

## 2.6. Sarcomere length determination

Sarcomere length (SL) was measurement at 3 days pm, using about 2 g from the each dissected LL sample which was homogenized according to Hergarty and Naude (1970). Sarcomere length was measured with the aid of a Video Imaging Analyzer (VIA), by means of an Olympus B340 microscope at a magnification of 31000 at 3 days pm. The VIA system was equipped with a CC12 video camera (Olympus, Japan). Quantification and processing were done with AnalySIS Life Science software package (Soft Imaging System GmbH, Munster, Germany). The mean of 5 sarcomeres represented the sarcomere length for each sample (Frylinck *et al.*, 2013).

## 2.7. Myofibril fragment length determination

Myofibrillar fragment length (MFL- $\mu\text{m}$ ) (a function of myofibrillar fragment index) as a predictor of enzymatic degradation and tenderness was measured at 3 and 14 days pm using about 2 g of meat sample from each excised LL muscle. Myofibrils were extracted according to Culler *et al.* (1978) and as modified by Heinz and Bruggermann (1994). MFL measurement was done with an Olympus BX41 system microscope at a magnification of 400X (VIA- Soft Imaging System, Olympus, Japan). One hundred myofibrillar fragments per sample were examined and measured. The mean values represented the MFL for each sample (Frylinck *et al.*, 2015).

## 2.8. Shear force determination

Shear force was determined by boiling about 200 g of samples from each dissected LL muscles in a transparent plastic bag placed in a water bath to a core (internal) temperature of 75°C for about 1 h (Honikel, 1998). The internal temperature was monitored by poking a temperature probe into the core center of the boiling meat sample. Samples were then cooled on ice slurry and stored for the next 12 h at 4°C. A hollow metal probe of 1.27 cm and 8 cm long was used to take out 6 round cores, parallel to the fiber direction (AMSA, 1995; Honikel, 1998). Each core was sheared perpendicular to the fiber direction, using Warner Bratzler shear device mounted on Universal Instron apparatus (Model 4301, Instron Ltd,

Buckinghamshire, England) at a cross speed of 200 mm/min. The mean value of 6 readings was used as the shear force value (N) for each sample at 3 and 14 days pm.

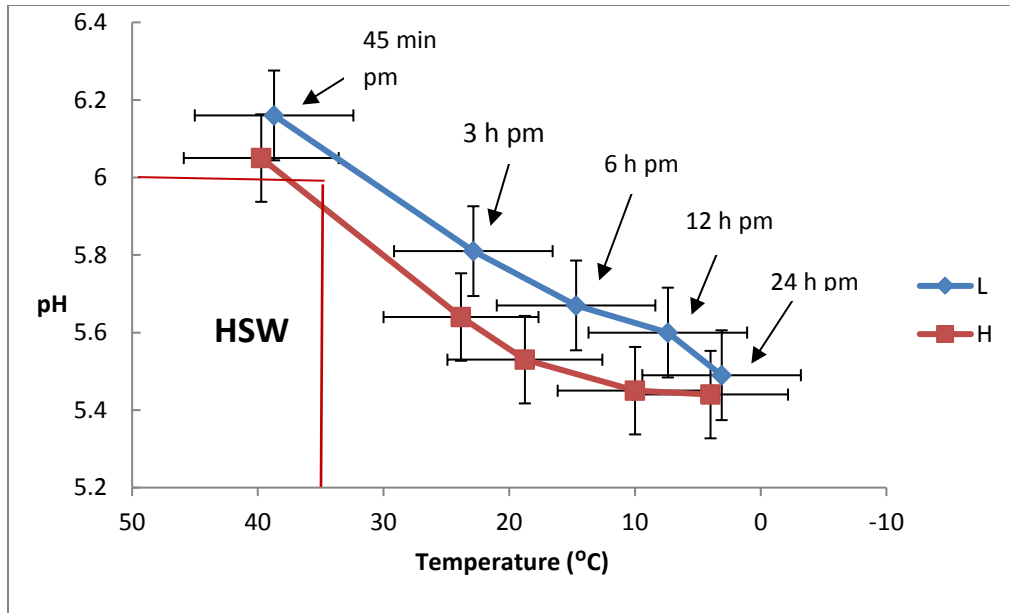
### *2.9. Statistical analysis*

ANOVA was done using the General linear model procedure (GLM) (SAS 9.3 TS1M1, 2011; SAS Institute, Cary NC) to determine the variation (R-square) between the mean values of treatments (light and heavy weights- *factors*) in relation to meat quality attributes (SF, % DL, SL, MFL, proteolytic enzymes and meat color- *response variables*) at the different times and days of measurements. Pair-wise comparison was used to determine the means and significant differences between means of treatment groups (carcass weight) in relation to quality attributes. Test of within subject effects (light and heavy carcasses) was also done using GLM to determine significant differences between carcass weights in terms of all quality attributes (subcutaneous fat, shear force, drip loss, color, sarcomere length, myofibre length and enzymes). Means were separated using Fisher's protected least significant difference (LSD) at 95% level of probability (Snedecor & Cochran, 1980).

## **3. Results**

### *3.1. pH and temperature profile*

Figure 1 shows the pH and temperature decline for small and heavy carcasses at 45 min, 3, 6, 12 and 24 h pm.



**Fig 1.** pH and temperature decline for small and heavy carcasses showing heat shortening window (HWS). L, light carcasses (lean to medium); H, heavy carcasses (fat)

As shown in Fig 1, heavier carcasses had a faster pH decline compared to the lighter carcasses at all times. Heavier carcasses also exhibited a slower ( $P < 0.05$ ) temperature decline at all times of measurement compared to the lighter carcasses. The heavier carcasses passed through the heat shortening window (pH 6 @ temperature  $> 35^{\circ}\text{C}$ ) and the heavier carcasses were over  $4^{\circ}\text{C}$  higher than the lighter carcasses at rigor temperature (Temp at pH6). At all times of measurement in the pH profile, heavier carcasses had lower pH values but at 3, 6 and 12 h pm, heavier carcasses had significantly ( $P < 0.05$ ) lower pH compared to the lighter carcasses.

### 3.2. Mean values of color attributes

The mean values of the color attributes (at 2 and 14 days pm) are presented in Table 1.

**Table 1**

Mean values and standard error of means (SEM) of light and heavy carcasses for meat lightness (L\*), chroma (C\*), and hue value (H\*) at 2 and 14 days pm

	CW n	L* 2d pm	L* 14d pm	C* d2 pm	C* d14 pm	H* d2 pm	H* d14 pm
<b>L</b>	15	33.10 ± 0.62 <sup>a</sup>	34.97 ± 0.76	16.61 ± 0.67 <sup>a</sup>	17.71 ± 0.54	49.53 ± 0.84	41.42 ± 0.88
<b>H</b>	11	35.07 ± 0.73 <sup>b</sup>	36.81 ± 0.88	18.85 ± 0.79 <sup>b</sup>	17.23 ± 0.63	49.25 ± 0.98	39.52 ± 1.03

CW, carcass weight; L, lighter carcasses (lean to medium); H, heavier carcasses (fat); d2, day 2 pm; d14, day 14 pm; n, number of samples

<sup>a, b</sup> Means in the same column with different superscript letter differ ( $P < 0.05$ ).

As shown in Table 2, at 2 days pm, heavier carcasses had higher L\* ( $P < 0.05$ ) than the lighter carcasses, however, at 14 days pm, the difference was not significant ( $P > 0.05$ ). For Chroma, heavier carcasses had significantly higher C\* ( $P < 0.05$ ) at 2 days pm but at 14 days pm, the differences were not significant ( $P > 0.05$ ). There was no significant ( $P > 0.05$ ) difference between the lighter and heavier carcasses in terms of H\* at both times of measurement but the lighter carcasses showed numerically higher values at 14 days pm. Lightness increased in all carcasses from 2 to 14 days pm while H\* values decreased in all carcasses from 2 to 14 days pm. It is noteworthy to see an increase in C\* values for the lighter carcasses (from 2 to 14 days pm) while C\* decreased in the heavier carcasses at 14 days pm.

### 3.3. Mean values and percentage change in calpain-1 and calpastatin

To further investigate the effects of treatments on shear force, calpain and calpastatin activity were evaluated from the meat samples (LT) from the lighter and heavier carcasses. Percentage decline of each proteolytic enzyme was determined from 1 to 24 h pm. The results are presented in Table 2.

**Table 2**

Calpain and calpastatin activities in muscles of light and heavy carcasses

Enzyme	CW	n	Time 1 (1 h pm) ± SD	Time 2 (24 h pm) ± SD	%Δ (decline)
<b>Calpain-1</b>	<b>L</b>	11	1.6 ± 0.23	1.4 ± 0.27	12.7
	<b>H</b>	15	1.7 ± 0.22	1.0 ± 0.44	37.6
<b>Calpastatin</b>	<b>L</b>	15	2.8 ± 0.22	1.8 ± 0.45	33.6
	<b>H</b>	11	2.4 ± 0.38	2.0 ± 0.20	13.9

CW, carcass weight; L, light carcasses (lean to medium); H, heavier carcasses (fat); n, number of samples; SD, standard deviation

Calpain-2 was excluded from Table 2 because there were no numerical differences between the lighter and heavier carcasses. Heavier carcasses showed a higher ( $P < 0.05$ ) decline in calpain-1 (37.6%) compared to lighter carcasses (12.7%) at 24 h pm. On the contrary, heavier carcasses showed a much lower (13.9%) decline ( $P < 0.05$ ) in calpastatin compared to lighter carcasses (33.6%) at 24 h pm.

### 3.4. Mean values for SL, SF, MFL and %DL of meat

**Table 3**

Mean values and standard error of means (SEM) of light and heavy carcasses for meat SL, SF, MFL and %DL at 3 and 14 days pm

CW	SL	SF	SF	MFL	MFL	%DL	%DL
(kg)	3d pm	3d pm	14d pm	3d pm	14d pm	3d pm	14d pm
	(μm)	(N)	(N)	(μm)	(μm)		
<b>L</b>	1.94±0.019	90.23 ± 2.86 <sup>a</sup>	59.48 ± 2.11	48.95 ± 2.37	35.05 ± 2.67	2.90 ± 0.44	4.23 ± 0.59
	(n=15)						
<b>H</b>	2.00±0.022	77.10 ± 3.35 <sup>b</sup>	56.14 ± 2.46	52.39 ± 2.77	38.74 ± 3.12	3.76 ± 0.51	5.41 ± 0.69
	(n=11)						

CW, carcass weight; L, light carcasses (lean to medium); H, heavy carcasses (fat); SL, sarcomere length; SF, shear force; MFL, myofibril fragment length; DL, drip loss; n, number of samples. <sup>a,b</sup> Means in the same column with different superscript letter differ ( $P < 0.05$ ).

As shown in Table 1, SL's of samples from the light and heavy carcasses are numerically close and there was no significant difference between the light and the heavier carcasses. At 3 days pm, the heavier carcasses had significantly lower ( $P = 0.006$ ) shear force compared to the lighter carcasses. At 14 days pm, the heavier carcasses were not significantly ( $P > 0.05$ ) different in terms of shear force, compared to the lighter carcasses. The lighter carcasses exhibited a higher (34%) decline in SF compared to the heavier carcasses (27%) at 14 days pm. Samples from the heavier carcasses also showed longer MFL at 3 and 14 days pm compared to the lighter carcasses but the differences were not statistically significant ( $P > 0.05$ ). A similar scenario played out for drip loss at 3 and 14 days where the heavier carcasses had higher drip losses but differences were not statistically significant ( $P > 0.05$ ). Marginal increase in drip loss was also observed in all carcasses from 3 to 14 days pm.

#### **4. Discussion**

As indicated in the introduction, the relationship or link between carcass weight and meat quality has produced various results based on different experimental conditions (Ellies-Oury et al., 2017). Some authors have reported some advantages while others have reported negatively on the influence of heavier carcasses on meat quality. However, there has not been comprehensive research to address the specific influences of carcass weight on carcass and meat quality attributes, especially from feedlot cattle which produces a significant percentage of beef that are consumed in most of the developed and developing countries including South Africa. A number of researchers have reported on the effects of faster pH decline, faster glycolysis and high temperature rigor, especially from heavier cattle (Warner et al., 2014; Jacob and Hopkins, 2014). Recent studies have shown that there is still a knowledge gap and there are few reports on high rigor temperature/PSE-like conditions in beef carcasses relative to heavy carcass weights (Kim et al., 2014). As highlighted in the introduction, a recent study by Warner et al. (2014) at seven processing plants in Australia revealed that 72% of beef carcasses exhibited high rigor temperature, which calls for a concern and further investigation. One of the recommendations of Warner et al. (2014) is to find new ways of identifying high temperature rigor carcasses in grain fed cattle. The focus of this

research however was not to dwell so much on the general effects of high rigor temperature on beef but rather to investigate the specific influences of carcass weight (which affects rigor temperature, proteolytic activity, and glycolytic rate) on selected meat quality attributes of heavier and lighter carcasses from typical South African feedlot cattle. The study was designed to highlight the advantages and possible disadvantages (in terms of meat quality) of producing these heavier carcasses which favors slaughter house pricing. The use of ES has generated some controversies on its effects on carcass cooling and rigor temperature according to the review of Jacob & Hopkins (2014). ES has been reported not to affect the rate of carcass cooling by many authors (Li et al., 2006; Jeremiah et al., 1985). Rosenvold et al. (2008) suggested that ES may in fact protect against high temperature rigor by accelerating rigor and further stated that the attachment of myosin heads to actin protects myosin from denaturation. Kim et al. (2012) also found that ES may protect myosin, but not calpain-1 against denaturation by advancing rigor onset. However, the effect of ES is not the primary focus of this study as this has been covered by other authors (Hwang et al., 2003) and moreover, the same amount of stimulation was applied to all carcasses in this study.

As shown in Figure 1, heavier carcasses passed through the heat shortening window ( $\text{pH} > 6$  when temperature is  $> 35^{\circ}\text{C}$ ) according to Thompson (2002). However, there was no indication of heat or cold shortening in the SL of both carcass weights. Some authors have reported that, the longer the SL, the more tender the meat (Devine et al, 1999; Marsh and Leet, 1966). While some authors denied the existence of shortening /toughening of meat (Smith et al., 1979b; Seideman et al., 1987), others reported that SL does not play appreciable role in meat tenderness (Uyterhaegan et al., 1992). Furthermore, Buts et al. (1990) concluded that tenderness is highly dependent on sarcomere shortening in slow glycolysing muscle but completely independent in muscles with more rapid pH decline, which was the case in this study. According to Marsh and Leet (1966), SL of 1.7 – 2.0  $\mu\text{m}$  is regarded as moderately tender meat and going by this, none of the SL investigated in this study could be regarded as out of range. A credible reason or cause is the time and condition at which muscle enters rigor mortis. According to the report of Goll *et al.* (1997), contraction is impeded by low muscle pH, hence, the proportion of myosin cross-



bridges in the strong-binding state will be less if ATP levels decrease slowly. In that way, muscle pH is less than 6.3 before intracellular calcium concentrations reach a level high enough to initiate contraction. According to the authors, such conditions would decrease the proportion of myosin cross-bridges in the strong binding state and therefore accounts for the lack of relationship between SL and tenderness in muscles that attain a pH of 6.3 or less within 3 h pm (Smulders et al., 1990). In the present study, all carcasses attained pH of less than 6 before 3 h pm and the reason given above is strongly attributed to why there was no difference in SL and no effects of SL on tenderness was seen because all carcasses were stimulated early, causing a faster pH decline in all.

Regarding MFL, there was no significant difference between the heavy and light carcasses as shown in Table 1. According to Hopkins and Taylor (2002), the final tenderness of meat depends on the degree of alteration of the muscle structure and associated proteins. Also, according to Goll et al. (1995), pm tenderization is a complex process that is affected by a large number of factors like the rate of pH decline, temperature decline, pm muscle cell osmolarity, myofibrillar/cytoskeletal protein degradation and inherent animal genetic make-up among others. Myofibrillar proteins constitute 50 to 55% of total protein content of muscle tissue but according to Devine et al. (1999), MFL alone does not give a complete indication of the extent of tenderization and proteolytic ageing. In this study, significantly higher MFL degradation was expected in the heavier carcasses considering the lower shear force as a result of faster pH decline at higher carcass temperature and the subsequent higher calpain activation and less calpastatin activation in the heavier carcasses (Koochmaraie et al., 1987; Robson et al., 1991). This suggests that other tenderizing mechanisms may have been involved in the lower meat shear force of the heavier carcasses. According to Koochmaraie and Geesink (2006), the factors that basically determine meat tenderness are the amount and solubility of connective tissue, sarcomere shortening during rigor development and pm proteolysis of myofibrillar and other associated proteins. Eliminating the effects of sarcomere shortening (for which there was no significant difference between the heavy and light carcasses) and solubility of connective tissue which was not measured in this study; the extent of proteolytic activity which is known to be affected by pm energy metabolism remains the causal factor to be considered. Judging from the low

pH and higher temperature profile and their effects on tenderness in the heavier carcasses in this study, it is obvious that the proteolytic enzymes (Calpain-1) were more activated in the heavier carcasses. Calpain-1 enzyme, which are widely known to contribute to meat tenderness (Koochmaraie & Geesink, 2006) were found to be activated more in the heavier carcasses (37.6% decline) compared to the lighter carcasses (12.7% decline) from 1 to 24 h pm. The review of Kim et al. (2014) indicated that increased tenderness of unaged meat (1-2 day pm) that has gone through high temperature rigor (temperature above 35°C at pH 6 according to Thompson, 2002 and Warner et al., 2014) could be explained by the early activation of the calpain enzyme and subsequent reduced ageing as observed in the heavier carcasses. This could explain why the heavier carcasses had significantly lower shear force at 3 days pm but at 14 day pm, there was less tenderization (reduced ageing), compared to the lighter carcasses. Similarly, Hwang et al. (2004) reported improved tenderness of high rigor muscle (beef LD) early (1 day) pm. These authors indicated that early activation of calpain by elevated free Ca<sup>++</sup> combined with high pre-rigor temperature resulted in faster myofibrillar protein degradation. However, this resulted in early exhaustion of proteolytic enzyme due to autolysis, causing decreased proteolysis and reduced tenderization with extended ageing. Also, calpastatin, which is known to inhibit the calpain enzymes (Goll et al., 2003) was less activated in the heavier carcasses (13.9% decline) compared to the lighter carcasses (33.6% decline) from 1 to 24 h pm (Table 2). This corroborates the reason why the heavier carcasses had significantly ( $P < 0.05$ ) lower shear force values at 3 days pm. Another credible reason according to Li et al. (2011) is that more of the lysosomal enzymes might have been released for access to the myofibrillar proteins to enhance tenderness in the heavier carcasses (Hopkins and Taylor, 2002). This is because lower pH and higher carcass temperature, as recorded in the heavier carcasses, can enhance the disruption of the lysosomal membrane (O'Halloran et al., 1997), thereby releasing the lysosomal enzymes [especially cysteine (cathepsins B, H, L and X)] as muscles enter rigor as a result of ATP depletion. The shear force values in the present study were slightly high at 2 and 14 days pm, going by the threshold of Shorthose et al. (1986). This could be associated with the effects of Zilmax which was fed as a supplement to all cattle used in this study as well as reduced ageing potential due to the higher temperature at rigor especially in the heavier carcasses. As

reported by Dunshea et al. (2005), beta agonists reduced muscle protein degradation due to elevated calpastatin activity and this was observed in this study. Furthermore, Hope-Jone et al. (2010) reported that ES could only reduce the toughening effect of Zilmax but not completely get rid of it. As indicated in the review of Kim et al. (2014), there are various compounding factors that could influence tenderization when investigating the effects of accelerated pH decline at higher temperature. Such factors include animal background (breed, age, sex, castration status and feeding regime), pre-slaughter factors (e.g. stress and transportation), environmental factors and variation in muscle fiber types and location, which are difficult to incorporate in a single study.

In terms of drip loss, the heavier carcasses had higher drip losses at 3 and 14 days pm, but the differences were not significant (Table 1). A slight increase in drip loss was generally expected for all carcasses in this study. This is because electrical stimulation is known to reduce meat water holding capacity. A recent study by Hopkins et al. (2014) showed increased drip loss in beef strip loins that were electrically stimulated and had rapid pH decline at high muscle temperature. A plausible explanation for the increased drip loss in the heavier carcasses is that the heavier carcasses went through faster pH decline at lower carcass temperature compared to lighter carcasses as shown as shown in Fig 1. According to Puolane and Halonem (2010), faster pH decline decreases net charges on the surface of the myofilaments and the lactate formed due to faster glycolysis acts as an anionic chaotrope which impairs the interaction between water and proteins thereby reducing water holding capacity. The quicker formation of actomyosin at rigor due to faster pH decline also reduces the intermyofibrillar space, leading to drip loss (Offer and Cousins, 1992). According to Offer (1991), the denaturation of myosin (caused by high temperature rigor) causes shortening of the myosin head and a reduction in myofibrillar filament spacing thereby inducing water to be expelled from muscle cells into the extra-cellular space. Another plausible reason for the higher drip loss in the heavier carcasses is that heavier carcasses have higher myofibrillar protein density compared to smaller carcasses (Bertram et al., 2002). Increase in protein density reduces intra-myofibrillar myowater content and increases inter-myofibrillar water content as muscles increase in weight and size (Bertram et al., 2007). This inter-myofibrillar water is then released as drip as protein

denatures at lower pH and higher temperature (Rosenvold et al., 2008) as seen in the heavier carcasses. Drip loss has also been linked with reduced proteolysis resulting from limited autolysis of calpain-1, which induces shrinkage of muscle cells thereby creating channels for drip out of muscle bundles (Huff-Lonergan and Lonergan, 2007). On the contrary, more proteolytic activity and autolysis was observed in the heavier carcasses (Table 2), which was accompanied with more drip.

Regarding color attributes, heavier carcasses recorded higher ( $P < 0.05$ ) meat lightness ( $L^*$ ) and chroma values at 2 days pm but at 14 days pm, there were no significant differences between the light and the heavy carcasses. Heavier carcasses also displayed numerically lower  $H^*$  (indication of discoloration; Kim et al., 2014) at 2 and 14 days pm.

Electrical stimulation is known to affect meat color but the extent of color change depends on pH-temperature relationship and the type of muscle observed (Ledward, 1985). Faster glycolysis enhances meat  $L^*$  value according to Moeller et al. (2003). These authors found a positive correlation ( $r = 0.33$ ) of  $L^*$  with loin glycolytic potential and this could explain why there was higher  $L^*$  in the heavier carcasses which persisted till 14 days pm. According to the review of Kim et al. (2014) faster denaturation of sarcoplasmic protein (due to high temperature rigor) increases the amount of light reflected from meat surface. This is coupled with reduction in mitochondrial activity and reduced oxygen consumption rate, making the meat lighter and redder at the early post mortem stage. This is supported by other researchers who reported that carcasses that went through faster glycolysis accumulates less metmyoglobin (Renerre, 1984) and displayed more appealing light bright red color (Eikelenboom et al., 1985) especially at the early post-mortem period. This effect is attributed to enzymes systems which are responsible for oxygen consumption (Ledward et al., 1986) and as ageing progresses, oxygen consumption decreases. The increase in  $L^*$  value from 2 to 14 days in all carcasses was mainly due to accelerated denaturation of mainly sarcoplasmic proteins (particularly myoglobin) and myofibrillar lattice shrinkage as reported by Buts et al. (1986). At a rapid pH decline/higher temperature, the redox stability of myoglobin (which is the primary pigment responsible for meat color) is adversely impacted by autoxidation (Kim and Hunt, 2011). Another plausible reason why the heavier carcasses exhibited higher  $L^*$  and  $C^*$  ( $P < 0.05$ ) early

pm is the higher subcutaneous fat thickness. According to Bruce et al. (2004), subcutaneous fat correlated strongly with  $L^*$  ( $r = 0.63$ ) and  $a^*$  ( $r = 0.60$ ) at 1 day pm. As shown in the result of this study, the subcutaneous fat thickness of the heavier carcasses was almost double that of the lighter carcasses. Going by the statements above, it is obvious that the color attributes of the heavy carcasses in this study were slightly enhanced at the early post-mortem period. This is due to the faster pH decline at significantly ( $P < 0.05$ ) higher carcass temperature due to the higher subcutaneous fat thickness which brought about higher rigor temperature compared to the light carcasses. As ageing progressed, oxygen consumption decreased in all carcasses but the heavier carcasses had numerically lower  $H^*$ . It is noteworthy to observe a slight increase in  $C^*$  value in the lighter carcasses from 2 to 14 days pm. At 14 days pm, the heavier carcasses lost the initial advantage of higher  $C^*$  values and there was no longer significant difference between the heavy and lighter carcasses. According to Sammel et al. (2002a) metmyoglobin reductase activity of meat decreased and metmyoglobin activity increased (Ledward, 1985) causing the meat to be less stable in color as the display period increased. .

## 5. Conclusions

It is concluded that heavier carcasses from the current South African feedlot systems favor meat quality in terms of shear force but this was associated with moderately higher drip loss which may marginally reduce carcass yield. Initial meat color was also better in heavier carcasses but this advantage diminished in all carcasses as ageing progressed. It is recommended that heavier carcasses be produced and that more research is conducted on ways to mitigate the negative effects on quality attributes such as drip loss and beef color after longer ageing periods. The production of heavier carcasses should be encouraged because heavier carcasses favor slaughter house pricing in many countries including South Africa. Heavy carcasses can also be produced alongside lighter carcasses using low voltage electrical stimulation without significant detrimental effect on meat quality. Lastly, the production of heavier carcasses either through better nutritional interventions or the use of feed additives such as Zilmax has the

potential to produce more beef with fewer cattle which implies less waste, less use of land, water and energy.

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