Effects of timing and duration of low voltage electrical stimulation of light and heavy carcasses on meat quality of South African feedlot cattle

By

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LIST OF ABBREVIATIONS

a*	meat redness
ADP	adenosine di-phosphate
AMPC	Australian Meat Processors Corporation
AMSA	American Meat Science Association
ANOVA	analysis of variance
ATP	adenosine tri-phosphate
b*	meat yellowness
^{0}C	degree celcius
C*	chroma
Ca^{2+}	calcium ion
CL	cooking loss
cm	centimeter
CP	creatine phosphate
CPK	creatine-phospho-kinase
CW	carcass weight
DL	drip loss
DCB	dark cutting beef
DFD	dark firm dry meat
dpm	days post-mortem
ES	electrical stimulation
EU	European Union
ES t	electrical stimulation time
ES d	electrical stimulation duration
FAO	Food and Agricultural Organisation
g	gram
GLM	general linear model
GP	glycolytic potential
G-6-P	glucose-6-phosphate
H^+	hydrogen ion
H*	hue angle
h pm	hour post-mortem
HTLP	High temperature low pH
HVES	high voltage electrical stimulation
Hz	Hertz
IMP	Ionosinemonophosphate
kg	Kilogram
KJ	Kilo joule
L*	meat lightness
LL	*Longissimus dorsi et lumborum*
LVES	low voltage electrical stimulation
MANOVA	multiple analysis of variance
MFL	myofibril fragment length
MbO_2	oxymyoglobin
Mg^{2+}	magnesium ion
MLA	Meat and Livestock Australia
MSA	Meat Standard Australia
ms	milli-second
MRA	Metmyoglobin reductase activity

MVES	medium voltage electrical stimulation
N	Newton
NES	non-electrically stimulated
P	phosphorus
PCA	perchloric acid
pm	post-mortem
PSE	pale soft exudative meat
pH_u	ultimate pH (pH 24 hour post-mortem)
SA	South Africa
SD	Standard deviation
SF	shear force
Sf	subcutaneous fat
SL	sarcomere length
SMEQ	Sheep meat eating quality
TCA	tri-caboxylic-acid
USA	United States of America
V	voltage
WHC	water holding capacity
µmol	micro-molar

DEDICATION

To my loving wife, Nancy and children, Shola and Victoria Agbeniga

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DECLARATION

I declare that this thesis for the degree PhD (Agric) Meat Science at the University of Pretoria is my original work and has not been submitted by me for a degree at any other university.

Babatunde Agbeniga
February 2019.

ABSTRACT

The study was designed to evaluate the effects of timing and duration of low voltage electrical stimulation (LVES) of light and heavy beef carcasses on meat and carcass quality of South African commercial feedlot cattle. It was inspired by the variations in meat quality that were emanating from the various electrical stimulation protocols by meat processors and the negative impact of this quality variation on the profitability of the meat industry. It was also inspired by the increasing live and carcass weight of cattle owing to better nutritional interventions and the use of feed additives and the impact on post-slaughter processing in relation to meat quality. It is believed that more meat could be produced from fewer heavier cattle compared with larger number of smaller or lighter cattle. However, the quality of meat, for example, shear force (SF), water holding capacity (WHC), drip loss (DL) and colour, from these heavier animals needs to be evaluated in order to ascertain the advantages and/or disadvantages, compared with lighter carcasses. To better understand the effects of treatments on meat quality, proteolytic enzyme activity, muscle energy metabolites and histological parameters were evaluated. Carcasses were subjected to LVES (110 V) applied early (7 min) or late (45 min) post-mortem (pm), for shorter (30 s) or longer (60 s) duration on heavy or lighter carcasses. Meat quality evaluations were done to ascertain the effects of the treatments (i.e. carcass weight, ES duration and time of ES application) and their interactions at 3 and 14 days pm (dpm). This was done to determine the values of the various quality parameters at the abattoir/butchery (3 dpm) and display period (14 dpm). Analyses revealed that early application of electrical stimulation (ES), especially on the heavier carcasses produced the highest rigor temperature (> 35 °C), which produced the lowest ($P < 0.05$) meat SF at 3 and 14 dpm. However, early ES application was disadvantageous for meat WHC and DL, as higher ($P < 0.05$) DL and lower WHC were recorded, especially in the heavier carcasses. The interaction of shorter ES and heavier carcass weight favoured ($P < 0.05$) meat SF at 3 days pm. Longer ES did not favour WHC at 3 and 14 dpm. Heavier carcasses exhibited lower ($P < 0.05$) WHC and higher DL at 3 dpm, but at 14 days of ageing, there were no significant differences between the two weight categories. In terms of meat lightness (L*), early ES and longer ES produced the lightest ($P < 0.05$) meat at 2 and 14 dpm regardless of carcass weight. The interaction of early ES and longer ES also produced the highest ($P < 0.05$) L* at 2 and 14 days pm. Early ES produced the highest ($P < 0.05$) chroma (C*), at 2 dpm. The interaction of early ES and heavier carcasses also produced a significantly higher ($P < 0.05$) C* at 2 days pm. On the other hand, the interaction of late application and longer duration of ES produced higher ($P < 0.05$) C* at the display period (14 dpm). Regarding hue angle (H*), early ES produced higher ($P < 0.05$) H* at 2

dpm, but at 14 days pm, late ES produced higher ($P < 0.05$) H*. Heavier carcasses exhibited higher but not significant H* at 2 dpm, but at the display period, heavier carcasses exhibited lower ($P < 0.05$) H*. Furthermore, the interaction of early ES and longer duration of ES brought about higher ($P < 0.05$) H* at 2 and 14 dpm. Overall, early LVES on heavier carcasses favoured SF and C* especially at the butchery period but as the meat aged, the advantages diminished. Drip loss and WHC were also marginally disadvantaged by early ES, but these could be minimized by shorter ES. This shows that quality heavier carcasses, which favour slaughter house pricing, could be produced and processed alongside smaller carcasses with better managed LVES. The use of LVES has the potential to reduce variability in meat quality while reducing occupational risk to meat workers.

SUMMARY OF RESULTS

One hundred and forty-four (140) cattle were sampled. They were mostly steers in "A" age class of composite breeds of mostly Bonsmara type, representative of typical South African feedlot cattle. Low voltage electrical stimulation (LVES) (110 V) was applied early (7 min) or late (45 min) post-mortem (pm) for a shorter (30 s) or longer (60 s) duration on light (≤ 260 kg) or heavy (≥ 290 kg) carcasses from cattle treated with zilpaterol hydrochloride (Zilmax). Non-electrically stimulated (NES) carcasses represented the controls. The combination of these treatments, including controls, made up the 10 treatment groups that were studied. Carcass and meat quality attributes that were evaluated were, pH and temperature decline (45 min pm, 3, 6, 12 and 24 h pm), subcutaneous fat (Sf) and sarcomere length (SL) measurement (3 dpm), meat shear force (SF), cooking loss (CL), drip loss (DL), water holding capacity (WHC), myofibril fragment length (MFL) (3 and 14 dpm), colour attributes (L* (lightness), a* (redness), b* (yellowness), C* (chroma) and H* (hue angle)) at 2 and 14 dpm, on *Longissimus dorsi et lumborum* (LL) samples, extracted from 11th to 13th ribs. To better ascertain the effects of electrical stimulation (ES) on tenderness, muscle proteolytic enzymes activities (calpain-1, calpain-2 and calpastatin) were determined on samples taken on LL at 1 and 24 h pm. To better ascertain the effects of ES on glycolytic metabolism in relation to other quality attributes, muscle energy metabolites (glycogen, glucose, lactate, glucose-6-phosphate (G-6-P), creatine phosphate (CP) and adenosine tri-phosphate (ATP)) were evaluated on LL samples taken at 1, 4 and 24 h pm.

Analysies revealed significantly ($P < 0.05$) higher subcutaneous fat in the heavier carcasses compared to lighter carcasses. Most of the early stimulated carcasses passed through the heat shortening (currently referred to as rigor shortening) window (pH 6 or less at temperature > 35 °C), but sarcomere length was not affected in any of the treatment groups. There was no sign of cold shortening in any of the treatment groups. Heavy, early stimulated carcasses had the highest rigor (pH at 6) temperature (> 35 °C). In terms of SF, early ES produced significantly ($P < 0.05$) lower shear force at 3 dpm compared with late ES and NES, while heavier carcasses exhibited lower but not significantly different SF at 3 and 14 dpm, compared with lighter carcasses. The effect of ES duration was not significant on SF at 3 and 14 dpm, but the interaction of heavy carcass weight and shorter duration (30 s) of ES produced significantly ($P < 0.05$) lower shear force at 3 dpm. At 14 dpm, early ES (especially in the heavier carcasses) produced the lowest ($P = 0.06$) but not significantly different SF. On the other hand, the lower SF in the early ES carcasses was not reflected in the myofibril fragment length (MFL), in that, the late stimulated carcasses had the lowest MFL's at 3 and 14 dpm. However, there were low variations among treatments at 3 and 14

day pm and numerical differences were low among treatments in terms of MFL. There were no significant differences among most of the carcass groups, but early ES for 60 s, produced the longest ($P < 0.05$) MFL at 14 dpm. This implies that, late ES produced a higher MFL degradation tempo (mean 32%), while early ES produced a lower degradation tempo (mean 24%).

Analysies revealed no significant differences in cooking loss among treatment groups in terms of ES time, ES duration and carcass weight at 3 and 14 dpm. There were low variations among the groups at 3 and 14 dpm. Heavy carcasses produced significantly ($P < 0.05$) higher DL at 3 dpm but at 14 dpm, there were no significant differences between the two carcass weights, even though the heavier carcasses produced numerically higher DL. Early ES produced significantly ($P < 0.05$) higher DL at 3 and 14 dpm. ES duration did not affect drip loss significantly but longer duration (60 s) produced marginally higher DL at 3 and 14 dpm. The interaction of heavy carcass weight and early ES also produced significantly ($P < 0.05$) higher DL at 14 dpm and a high tendency to be significant ($P = 0.077$) at 3 dpm. Regarding WHC, there were mixed reactions among treatment groups and there were low but significant variations among treatment groups at 3 and 14 dpm. Heavier carcasses had marginally lower but significant ($P < 0.05$) water holding capacity (WHC), compared with the lighter carcasses at 3 dpm. Early ES produced significantly ($P < 0.05$) lower WHC at 3 and 14 dpm. Longer ES duration (60 s) produced marginally lower but significant ($P < 0.05$) WHC at 3 dpm, with a high tendency to be significant ($P = 0.052$) at 14 dpm, compared with shorter duration (30 s). The interaction of heavier carcass weight and early ES had a high tendency to produce significantly ($P = 0.077$) lower WHC at 3 dpm. Likewise, the interaction of early ES and longer ES duration, showed a high tendency to produce significantly ($P = 0.061$) lower WHC at 3 dpm.

For colour attributes, analysis revealed that, early ES produced significantly ($P < 0.05$) higher L* at 2 and 14 dpm. Longer ES duration (60 s) produced significantly ($P < 0.05$) higher L* at 2 and 14 dpm, compared with shorter duration (30 s). Regarding carcass weight, there was no significant difference between the heavy and lighter carcasses in L* value. However, heavier carcasses had a high tendency to produce significantly ($P = 0.059$) higher L* at 14 dpm. The interaction of early ES and longer duration of ES also produced significantly ($P < 0.05$) higher L* at 2 and 14 dpm. With regard to a* value, analysis revealed low but significant variations among treatments at 2 and 14 dpm. The early ES carcasses had the highest but not significant a* values at 2 dpm. In carcass weight, there were no significantly different a* among treatments at 2 dpm. At 14 dpm, there were mixed reactions and no significant differences among most of the treatment groups in terms of ES time and duration, and carcass weight. All treatment groups except the heavy early ES carcasses,

had marginal increases in a* values from 2 to 14 dpm. With regard to b* value, at 2 dpm, early ES carcasses especially the heavy ones had the highest ($P < 0.05$) b* values compared with the late ES carcasses and controls. At 14 dpm, there were mixed reactions and no significant differences among treatment groups in terms of ES time and duration and carcass weight. Also, all treatment groups had marginal increases in b* value except the early ES heavy carcasses (which decreased) from 2 to 14 dpm.

Early ES produced higher chroma (C*) value especially in the heavier carcasses ($P < 0.05$), compared with the late ES carcasses and controls, at 2 dpm. Electrical stimulation duration showed no significant influence at 2 dpm. At 14 dpm, there were lower variations and numerical differences among treatment groups. All carcasses had marginal increase in C*, from 2 to 14 dpm, except the early stimulated heavy carcasses, which had marginal decreases. The interaction of heavy carcass weight and early stimulation produced significantly higher C* at 2 dpm. Likewise, the interaction of late ES and longer duration (60 s) produced significantly ($P < 0.05$) higher C* at 14 dpm. With regard to H*, analysis revealed low but significant variations among treatments at 2 and 14 dpm. At 14 dpm, analysis revealed significant lower ($P < 0.05$) H* in the heavier carcasses compared with lighter carcasses. Also, early ES produced marginally higher but significant ($P < 0.05$) H*, but at 14 dpm, early ES produced marginally lower but significant ($P < 0.05$) H* compared with late ES carcasses and controls. The interaction of early ES and longer ES duration (60 s), produced significantly ($P < 0.05$) higher H* at 2 and 14 dpm.

In terms of the energy metabolites and glycolytic metabolism, analyses revealed moderate to high significant variations in most of the energy metabolites especially at 1 and 4 h pm. Early ES brought about significantly ($P < 0.05$) higher muscle lactate and glucose content at 1 and 4 h pm. Of the early ES carcasses, longer stimulation (60 s) produced the highest muscle lactate and glucose content at 1 and 4 h pm. Carcass weight did not produce any noticeable influence on muscle lactate. At 24 h pm, there were no significant differences among treatment groups. Regarding glycogen, there were mixed reactions from the treatment groups at 1 h pm, but at 4 h pm, the early ES carcasses displayed significantly ($P < 0.05$) lower muscle glycogen compared with the late ES carcasses and controls. At 24 h pm, there were no significant numerical differences among most of the treatment groups but the early ES carcasses still displayed numerically lower muscle glycogen. The influence of carcass weight and ES duration was not noticeable on muscle glycogen of the early ES carcasses. However, carcasses stimulated late for longer duration (60 s) had the highest muscle glycogen at 24 h pm. For creatine phosphate (CP), analyses revealed lower but not significantly different CP in the early ES carcasses, especially the

heavier ones at 1 and 4 h pm. At 24 h pm, there were no significant differences among treatments. All meat samples decreased in CP content from 1 to 24 h pm. A similar pattern of reaction to CP was observed for ATP (adenosine triphosphate) content at 1, 4 and 24 h pm. Early ES carcasses especially the heavier ones had lower ($P < 0.05$) muscle ATP content at 1 and 4 h pm. At 24 h pm, there were no significant differences among treatments in muscle ATP content. With regards to glucose-6-phosphate (G-6-P), analyses revealed a general increase in muscle G-6-P from 1 to 24 h pm. At 1 h pm, there were mixed reactions among treatments but at 4 h pm, early ES produced numerically higher muscle G-6-P content, but not significantly different from the late ES carcasses and the controls. Similar reaction patterns were observed at 24 h pm from the treatment groups but no significant numerical differences were observed. In terms of glycolytic potential (GP), most treatment groups had increases in GP from 1 to 24 h pm except the heavy, late ES carcasses, stimulated for shorter duration (30 s). The group had the highest initial glycogen content of all treatment groups. There were mixed reactions from the treatment groups in terms of carcass weight and ES time. However, longer ES (60 s), produced significantly ($P < 0.05$) higher GP at 1 and 4 h pm, irrespective of time of ES and carcass weight. The interaction of heavy carcasses and early ES brought about significantly higher ($P < 0.05$) GP at 24 h pm. Also, the interaction of early ES and longer duration of ES brought about significantly higher ($P < 0.05$) GP at 1 and 4 h pm.

In terms of proteolytic enzymes, there were low variations among the treatment groups and there were no significant differences among most of the ES groups. The controls, however, were numerically and significantly lower in enzyme concentration than the ES groups, especially the early ES groups. At 24 h pm, there were no significant differences among most of the treatment groups. However, early ES groups (mean 36.2%) showed higher calpain-1 decline from 1 to 24 h pm, compared with late ES groups (mean 29.7%), and controls (mean 12.3%). Calpain-2 showed mixed reactions at 1 h pm and there were no significant differences among most of the treatment groups. However, the early ES treatment groups had numerically lower calpain-2 concentrations, but differences were not significant. A similar scenario to 1 h pm was observed at 24 h pm for calpain-2. In terms of change in calpian-2, there were mixed results. Some treatment groups had an increase in calpain-2 activity, some decreased and some remained unchanged at 24 h pm. With regards to calpastatin activity, analysis revealed very low and insignificant variation among treatment groups. Numerical differences were low among treatments and there were no significant differences among most of the groups at 1 and 24 h pm. Early and late ES groups had similar calpastatin decline (mean 24.6 and 24.8% respectively), while the controls had lower decline (mean 15.8%) from 1 to 24 h pm.

CHAPTER 1

INTRODUCTION

In recent times, there has been a substantial increase in the live and carcass weight of cattle, especially feedlot cattle that are slaughtered in South Africa and many other parts of the world. Carcass weights have increased on average of 1 to 2 kg each year in recent time in USA (Savell, 2012). This is according to the findings of the National Beef Quality Audit (Lorenzen et al., 1993; Moore et al., 2012). In Australia, average slaughter weight increased from 180 kg in 1983/84 to 232 kg in 2003/2004 (Department of Agriculture, Fisheries and Forestry, 2005) and to 287 kg in 2011 (Meat and Livestock Australia (MLA)). In Finland, the average carcass weight of slaughtered bulls increased from 275 kg (1996) to 335 kg (2008) over twelve years (Pesonen et al., 2012). Currently in Finland, bulls are slaughtered at an average carcass weight of 400 kg (Pesonen et al., 2012). The increase in carcass weight in South Africa is due to a number of factors, including higher grain prices, because of the current drought and economic downturn. This compels feedlot farmers to feed their cattle for longer, to attain higher live weight, and to cover the cost on the higher priced grains. Other factors include better management practices such as genetic improvement, improved nutrition and the use of feed additives and growth regulating molecules including hormonal implants and beta-agonists. According to Ellies-Oury et al. (2017), the link between carcass weight and meat quality has produced various results depending on experimental conditions such as production systems, breed and animal type.

One such beta-agonist that is approved and commonly used in South Africa is zilpaterol hydrochloride (Zilmax®). Delmore et al. (2010) reported about 10 to15 kg increase in live and carcass weights, respectively, when cattle were fed with Zilmax, while Strydom et al. (2009) reported about 14 kg increase in carcass weight. Heavier carcasses favour slaughter house pricing in many countries, including South Africa (Pesonen et al., 2012).

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Also, the production of heavier carcasses through better nutritional interventions, and the use of feed additives and improved genetic selection, has the potential to produce more beef with fewer cattle which implies lower waste, and less use of land, water and energy.

However, little research has been done to compare meat qualities from heavy and lighter carcasses. On the other hand, the increase in carcass weight presents challenges to the processors, in that, these heavier carcasses have to be processed with the same systems and facilities that were designed for lighter carcasses, decades ago. There are also reports that these heavier carcasses require less ES to achieve a similar glycolytic rate than lighter carcasses (Thompson 2002). According to Hopkins et al. (2007b) and Warner et al. (2014a), most of the heavier carcasses in their experiments exhibited faster pH decline and did not meet the MSA (Meat Standard Australia) pH/temperature window, compared with lighter carcasses. The phenomenon of faster pH decline at high carcass temperature (i.e. pH6 at temperature above 35 °C –Thomson, 2002), especially in heavier carcasses, has recently become the subject of interest and investigation. In a study across the beef industry in Australia, 72% of the carcasses exhibited high rigor temperature which occurred mostly in the heavier carcasses from feedlot (Warner et al., 2014). This was attributed to higher blood plasma insulin resistance, increased electrical inputs and increased number of days on feed. DiGiacomo et al. (2014) indicated that high energy feed such as in feedlot, results in heavier cattle and increased fat level which subsequently reduces carcass cooling. Warner et al. (2014a) reported that insulin resistance could lead to decreased heat tolerance and, as a result, increased carcass temperature at slaughter.

An important intervention in reducing the problems of variability in meat quality is the use of electrical stimulation (ES) (Hopkins & Toohey, 2006), which has now been adopted by most commercial slaughter house in many countries including South Africa. Electrical stimulation was adopted initially to accelerate post-mortem (pm) glycolysis, so that rapid cooling can take place, without the risk of cold shortening (Davey & Chrystall, 1980). It was later discovered that ES played a huge role in meat tenderization and colour enhancement and has since been adopted as a method of meat tenderization and colour enhancement in beef, lamb, and goat carcasses (Geeesink, van Laak, Banier, & Smulders, 1994; Savell, Smith, Dutson, Carpenter, & Sutter, 1977; McKeith et al., 1981). Electrical stimulation has been adopted as a processing procedure by commercial meat processors in South Africa. Low voltage electrical stimulation (LVES) has been described by a number of authors as more practical, more attractive, cheaper and easy to install. It

therefore attracts less stringent rules in terms of safety and occupational health harzards from government regulating agencies compared with high voltage equipment (Fabianson & Reutersward, 1985; Hawrysh, Shand, Wolfe, & Price, 1987). LVES has also been reported to achieve similar muscle glycolytic response and meat quality traits to high voltage electrical stimulation (HVES) (Aalhus, Jones, Lutz, Best, & Robertson, 1994; Taylor & Marshall, 1980). According to Bouton et al. (1978), LVES (110 V) lowered muscle pH effectively and improved meat tenderness. This was one of the reasons that 110 V electrical stimulator was used in this trial. However, some authors reported negative results on the effectiveness of LVES on meat quality (Unruh, Kastner, Kropf, Dikeman, & Hunt, 1986) while some some reported little or no effects (Rodbotten, Lea, & Hildrum, 2001), others reported deleterious effects (Hector et al., 1992; Simmons et al., 2008). Timing and duration of application of LVES are important factors to be considered in relation to meat quality. Chrystall et al. (1980) suggested that for LVES to be effective, it must be applied within a short time of slaughter. On the contrary, Hwang and Thompson (2001b) reported that early stimulation (3 min pm) is detrimental to meat tenderness compared with late application (40 min pm) irrespective of the type of stimulation (LVES or HVES). The authors emphasized that timing and type of stimulation would affect pH decline, enzyme activity and hence shear force of meat. The duration of stimulation is equally important. Roeber et al. (2000) reported that duration of ES and voltage intensity affected beef colour and tenderness. Gursansky, O'Halloran, Egan and Devine (2010) stated that HVES delivered satisfactory results in all circumstances but low voltage could be equally advantageous as long as the duration was not too short (> 40 s as opposed to 10 s). Strydom and Frylinck (2014) also concluded that shorter duration of stimulation (15 s, compared with 45 or 90 s) is more beneficial to water holding capacity and tenderness, where pre-slaughter stress is minimized.

The effects of ES on meat quality attributes have been researched for decades but despite this intensive research, a lot of unanswered questions remain, and, the fundamental mechanisms and generally acceptable commercial application remain obscure. There needs to be an understanding of the mechanisms of stimulation under varying conditions for optimizing the effects of ES on meat quality (Morton & Newbold, 1982). According to Troy (2006), the meat industry has gradually transformed from being consumer driven to being commodity driven. Consumers are now more aware of the quality of products and demand consistent eating quality, acceptable appearance, and safe and healthy meat products (Miller et al., 1995). There is a need to create a balance in the effective application of ES to optimize meat quality. Currently, in South Africa and other parts of the world, there is no uniform protocol regarding the use of ES. This is a major

cause of variability and lack of consistency in terms of quality, which differs from one processor to the next. Therefore, for South Africa to remain competitive in the meat industry, variability in meat quality must be reduced. Electrical stimulation stands out as a dependable technology to achieve this. According to Adeyemi and Sazili (2014) and other reports, the major factors in the application of ES to optimize the conversion of muscle to meat are timing and duration of application and the type of ES. In light of the increasing live and carcass weights, which influence processing conditions in relation to meat quality, ES technology should be re-evaluated, especially on the recently produced heavier carcasses to achieve desirable qualities from South African carcasses and meat. In addition, most of the reports on the use of electrical stimulation in relation to rigor temperature and meat quality are old. Most of the recent reviews on this topic are also devoid of hard data and are subject to personal opinions. This study presents current data on the use of LVES, which is regarded as the safest, in terms of occupational hazards, in relation to carcass weight and meat quality of intact muscles, from carcasses of recently produced commercial feedlot cattle.

The objective of this project was to evaluate the effects of low voltage electrical stimulation (110 V) applied early (7 min) or late (45 min) post-mortem for a shorter (30 s) or longer (60 s) duration on carcass and meat quality of light or heavy carcasses from cattle treated with zilpaterol hydrochloride. Non-electrically-stimulated (NES) carcasses represented the control groups.

CHAPTER 2

LITERATURE REVIEW

2.1 Biochemical and structural changes in carcass and meat during the first 24 hour post-mortem

The biochemical processes and structural changes that occur in muscles within the first 24 h post-mortem (pm), play major roles in meat quality and are influenced by the chilling conditions and other pm treatments that carcasses are subjected to, after slaughter (Lawrie, 1998; Savell et al., 2005). Post-mortem energy metabolism or glycolysis in muscle plays an important role in determining the final quality of meat (Ferguson & Gerrard, 2014). At the time of death of the animal, the various tissues carry on with their metabolism under local control. Although the muscles are not actively contracting at this time, energy (ATP) is being used to maintain the temperature and the organizational well-being of its cells against spontaneous tendency to breakdown. Essentially, ATP is hydrolysed ($ATP + H_2O \leftrightarrow ADP + P_i + H^+$) to provide the energy to regulate and drive muscular contraction (Ferguson & Gerrard, 2014). The ATP-ase of the sarcoplasm is one of the enzymes that are involved in the release of ATP (Bendall, 1973). The first change caused by bleeding is the elimination of the blood-borne oxygen supply to the muscles and consequent fall of oxidation potential, which marks the beginning of anaerobic metabolism. As a result, the cytochrome enzyme system can no longer operate, and the re-synthesis of ATP becomes impossible. The immediate response in restoring or maintaining ATP levels in muscle is the supply of a phosphate group from the store of creatine phosphate (CP) to ADP via an enzyme called creatine phosphokinase ($CP + ADP + H^+ \leftrightarrow$ creatine + ATP) (Ferguson & Gerrard, 2014). Hydrogen ion is consumed in the process, as shown in the equation. The continuing operation of sarcoplasmic ATP-ase depletes the ATP level, simultaneously producing inorganic phosphate which hastens the breakdown of glycogen to lactic acid, part of which causes a decrease in pH (Swatland, 1984; Lawrie, 1998). Other processes are discussed in detail below.

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2.1.1 pH decline

The pH in muscles, normally decrease from around 7.2 at slaughter to approximately 5.4 to 5.8 (ultimate pH) (Smulders, Toledra, Flores & Prieto, 1992; England, Matarneh, Scheffler & Gerrard, 2017). The decline in pH is linked to the accumulation of lactic acid resulting from the process of glycolysis. However, the review of Robergs et al. (2004) describes this as a popular misconception. They indicated that the major source of H^+ is from ATP hydrolysis (see reaction in the paragraph 2.1 above). The attainment of ultimate pH coincides with the end of glycolysis, which is attributed to lack of substrate (glycogen) or the inactivation of glycolytic enzymes because of the acidic condition caused by the accumulation of lactic acid (Lawrie, 1998). Beef usually completes its pH decline within 18-40 hours pm (Smulders et al., 1992). Hwang and Thompson (2001b) reported that the rate of pH decline, in-situ, had the largest effect on eating quality. Morton, Bickerstaffe, Kent, Drandfield and Keeley (1999) reported that pH 3 h pm correlated strongly with shear force. In their work, muscles with high pH 3 h pm were slow to tenderize. This is in accord with the work of Marsh, Ringkob, Russell, Swartz, & Pagel (1987) and Kastner et al. (1993), who identified that the rate of pH decline is associated with the rate of tenderization. Morton et al. (1999) also showed that carcasses with the most rapid pH decline showed the highest loss of calpain 1/2 activity over 24 h period pm. This could reduce tenderness and ageing potential owing to early exhaustion of the proteolytic enzymes (especially calpain) at higher carcass temperature (Simmons et al., 1996). Zamora et al. (1996) and Whipple et al. (1990) reported that pH decline was 3 times faster in ES carcasses than that observed in non-stimulated carcasses. As pH declines, it nears the iso-electric point. At this point, all the negatively and positively charged amino-acid side chains are equal, which causes maximum attraction between the two. This attraction holds the filaments closely together and does not allow water to come in, thereby greatly reducing the water holding capacity (WHC) of the meat (Smulders et al., 1992). pH decline occurs because of the need for muscles to regenerate ATP, which is accomplished mainly post-mortem through glycolysis and the build-up of lactic acid as a result. When temperature is quickly lowered post-mortem, it reduces the velocity of these chemicals and biochemical reactions, thereby reducing the rate of pH decline (Lawrie, 1998). On the other hand, slower chilling brings about a more rapid drop in pH (Marsh et al., 1987; Jacob and Hopkins, 2014). Electrical stimulation brings about a rapid drop in pH and apart from ES, pH is not as actively managed in beef processing as it is in pork processing. The time-course of pH decline in beef varies but ultimate pH is usually achieved within 24 h pm (Simmons et al., 2008). The quality issue most associated with pH in beef is dark cutting beef (DCB) which causes about 1 to 3% loss. It occurs when the ultimate pH is ≥ 6.0 as compared with 5.6 for normal beef owing to

lack of sufficient lactic acid build up as a result of limited glycogen store in living animal (Savell, 2012).

2.1.2 Rigor mortis

'Rigor' is a term that is applied to individual muscle fibres when they become ATP depleted (from pH 6) while 'rigor mortis' refers to the muscle stiffness that occurs as a result of the rigor (Hwang et al., 2003). According to Devine and Graafhuis (1995), tenderization begins at the point of rigor mortis. Rigor mortis does not take place in all muscles simultaneously with an accompanying fall in pH (Jeacocke, 1984). Jeacocke (1984) showed that there was a contracture as the final ATP disappeared (rigor mortis) and individual fibres had their own time course depending on their initial glycogen level. The stiffness that occurs in the fibres at full rigor is significant when the muscle reaches a pH of approximately 6.0. This shows that rigor is a cumulative process and as individual fibres enter rigor sequentially, protection from cold shortening occurs gradually (Jeacocke, 1977). Jeacocke (1977) suggested that the minimum rate of pH decline occurs at *ca* 13 °C which was attributed to ATP-ase activity. The author indicated that the rate of lactate production was proportional to the rate of ATP hydrolysis. This theory is also supported by studies which reveal there is tension throughout the rigor process but the tension fades away as proteolysis kicks in at which time more fibres age and tenderise (Devine et al., 1999).

Development of rigor mortis in muscles starts when the stores of ATP are no longer in sufficient quantities to break the bonds between actin and myosin filaments. This is when permanent cross-bridges called actomyosin are formed between the actin and myosin filaments. A study by Honikel, Roncales and Hamm (1983) showed that rigor shortening starts in the neck muscle of beef carcass at pH 6.3 - 6.0 and at about 2 µmol ATP/g of muscle. Rigor begins in normal meat at pH of between 5.7-5.8 (Hannula & Puolanne, 2004). During the early phase of rigor which is the delay phase, the muscle is extensible owing to the availability of ATP to bind with Mg^{2+}, which assists in disconnecting the actin/myosin cross-bridges which allows the muscles to relax. During this delay phase creatine phosphate is depleted and as a result, the phosphorylation of ADP to ATP is inhibited. This marks the onset phase of rigor. At this phase, little ATP is available to breakdown the actin/myosin bond, and muscles can no longer relax and therefore become inextensible, which leads to the completion phase of rigor mortis. High chilling temperatures cause a more rapid rigor development while low chilling temperature will cause a slower rigor development (Savell, 2012). Fibres that enters rigor early, attain faster tenderization (Graafhuis,

Lovatt & Devine, 1992). The rate at which pH and temperature declines during rigor development are probably the most important factors, that affect meat quality in terms of tenderness, color and WHC (Mancini & Hunt, 2005; Savell et al., 2005). A recent review by Kim et al. (2014) showed that high rigor temperature (i.e. temperature above 35 °C when pH is 6) is prevalent in heavy carcasses from feedlot. This is attributed to factors such as carcass fatness, blood plsma insulin and days on feed (Warner et al., 2014). This causes protein denaturation, reduces ageing potential, lowers water holding capacity and causes low color stability (Kim et al., 2014). According to Kim et al. (2014), there is a knowledge gap in the understanding of high rigor temperature in beef. Figure 2.1 shows muscle contraction and relaxation according to the sliding filament theory.

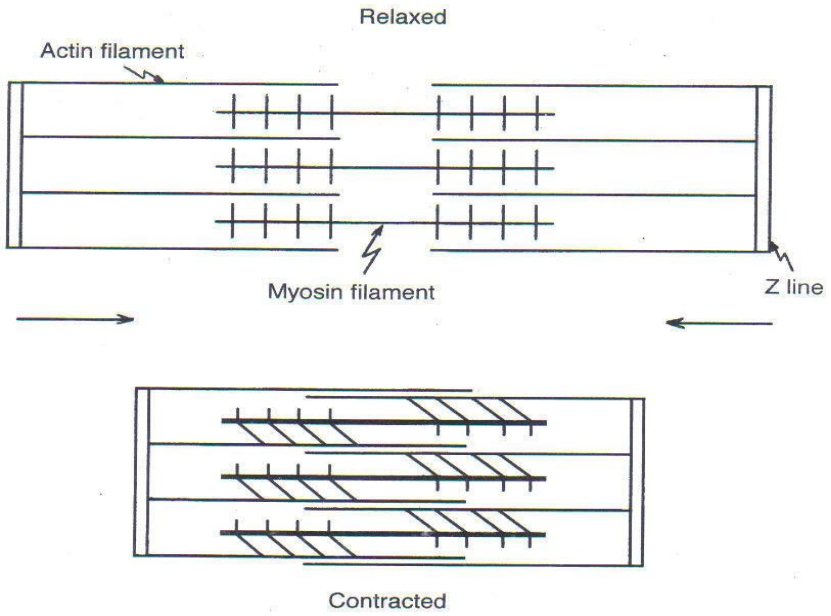


Figure 2.1 Muscle contraction and relaxation according to the sliding filament theory-sarcomere shortening
(Gregory, 1998)

Figure 2.2 depicts the fibrous and micro-structure of meat. It shows the thick (myosin) and thin (actin) filaments that reflects the end of rigor mortis.

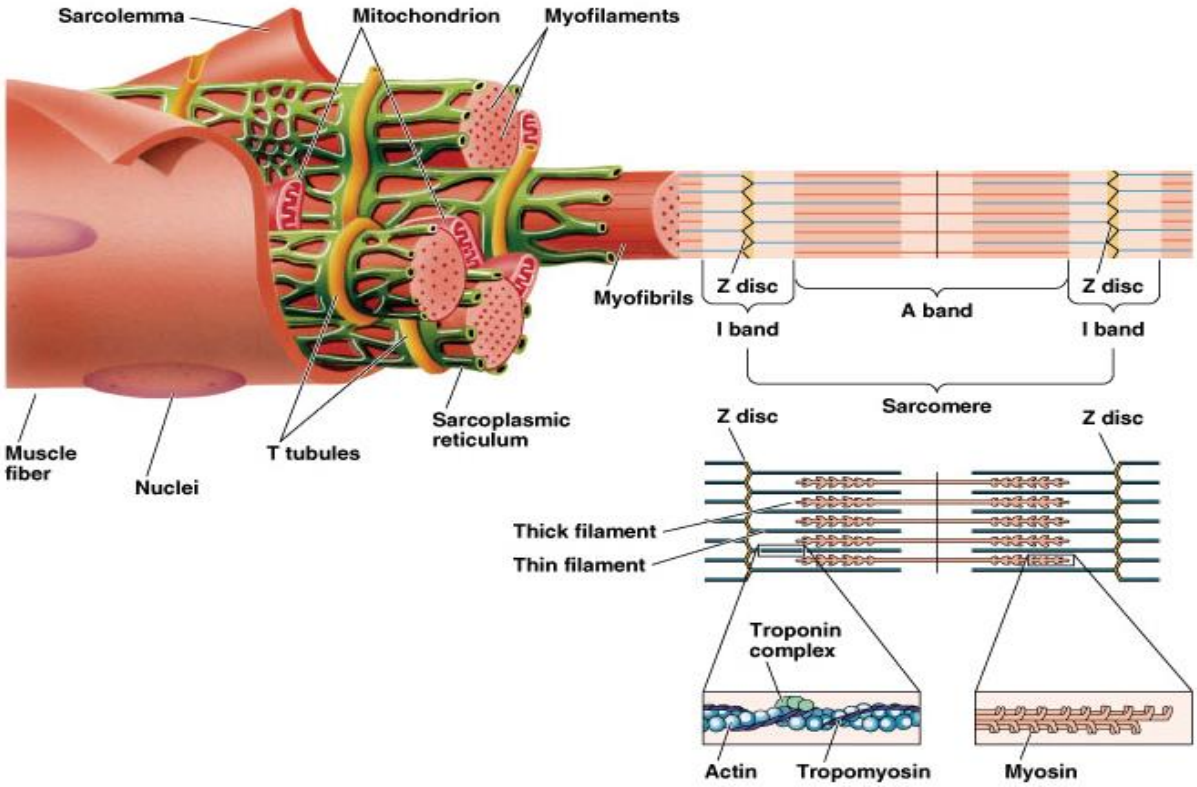


Figure 2.2 Fibre and micro-structure of meat

(Gunenc, 2007)

2.1.3 Cold and heat shortening or toughening

Cold shortening has been known and studied for over 50 years (Locker, 1985). Cold shortening could be defined as a rapid decline in muscle temperature to or less than 14-19 °C before the onset phase of rigor mortis (Locker & Hagyard, 1963). Cold shortening can begin at around pH 7.0 at full ATP concentration (4µmol ATP/g muscle) in the muscle, while it is completed at 0.5µmol ATP/g muscle or at about 10% of the initial concentration. Locker (1960) found that the degree of muscular contraction was related to tenderness and that exposure to specific cold temperatures early post-mortem before rigor could result in a phenomenon called cold shortening (sarcomere shortening). This discovery turned the attention of the meat science community to the evaluation of myofibrillar components of meat as primary contributor to meat tenderness (Locker, 1985). Bendall (1973) also found that muscles at less than 10 °C are susceptible to cold shortening until a pH of 6.2 is reached. Cold shortening occurs when the pH is greater than 6.0 while ATP is still available for muscle contraction and muscle temperature has dropped to less than 10 °C (Pearson & Young, 1989). The work of Thompson (2002) and Hopkins et al. (2005) reveal that when the carcass reaches pH 6.0 and enters rigor, the temperature can be used to predict meat quality. Cold

contracture can be noticed at temperature as high as 25 °C but its severity increases as temperature drops (Davey & Gilbert, 1975; Honikel, Roncales & Hamm, 1983).

When carcass temperature reduces too quickly before the onset of rigor, muscles can be affected by cold-induced shortening or toughening (Tornberg, 1996). Hannula and Puolanne (2004) found that temperature and pH relationship at the time of rigor onset were the decisive factors in the degree of cold shortening. When the muscle temperature is reduced to 0 °C-15 °C before the onset phase of rigor mortis, the sarcoplasmic reticulum ceases to function properly, and is unable to bind calcium, which leaves a lot of calcium in the sarcoplasm. At this point, a lot of ATP is left in the muscle. This makes the muscle contract at a maximum level, causing the filaments to slide over one another, while eliminating the I-band of the sarcomere. At internal temperatures of 1 °C-2 °C, the sarcoplasmic reticulum becomes least functional (Aberle et al., 2001). Herring, Cassens and Briskey (1965) showed the direct relationship of sarcomere length to fibre diameter and toughness. They postulated that the more contracted the sarcomere, the larger the fibre diameter becomes as a result of the filaments sliding over one another. They also noted that after cooking, meats with larger fibres were tougher. Bendall (1973) also found that muscle types differ in their potential to shorten. Red fibres are more susceptible than white fibres because the white fibres have higher amounts of glycogen and experience a more severe drop in pH earlier in the rigor process. Hannula and Puolanne (2004) found that the combination of time, temperature and pH varies within muscles and within species, which shows that not all muscles are affected by cold shortening to the same extent.

Other forms of shortening conditions include thaw and hot /rigor shortening. Thaw rigor develops when pre-rigor muscles are frozen and later thawed (Aberle et al., 2001). Contraction is produced when the frozen muscle is thawed and this produces a sudden release of calcium ion into the sarcoplasm, causing a physical shortening of 60-80% with the release of large quantities of meat juices and severe toughening. On the other hand, hot rigor occurs when muscles are maintained at high temperature up to 40^0C causing a rapid depletion of ATP which in turn creates severe shortening and the onset of early rigor (Aberle et al., 2001). Thompson (2002) observed that heat-induced shortening occurred when the combined effect of high temperature and low pH in the muscle exhausted proteolytic activity owing to accelerated protein denaturation. According to this author, the window set to avoid this phenomenon is when pH is greater than 6 when muscle temperature is lower than 35 °C. Also, Devine et al. (1999) reported that meat entering rigor at 15^0C produced more tender meat but at rigor temperature of 35-38^0C, there was increased toughness and reduced tenderization when meat was aged. Reduced ageing potential could be

caused by exhaustion of proteolytic enzyme activity or elevated protein denaturation, which limits proteolysis in meat from high rigor temperature (Kim et al., 2014). Figure 2.3 presents a plot of pH and temperature decline of beef carcasses from various production systems, showing the cold and hot shortening windows.

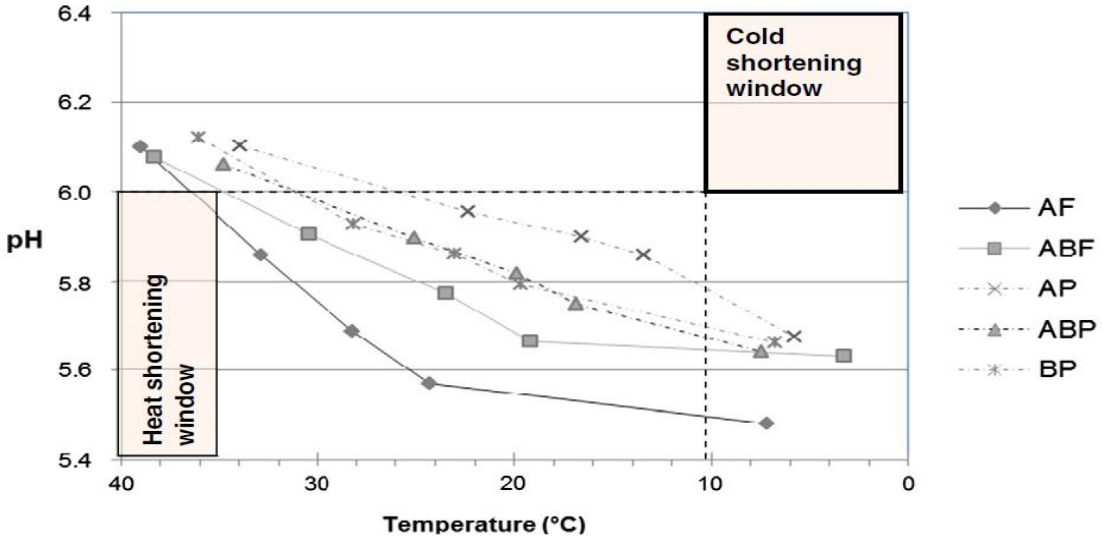


Figure 2.3 Cold shortening and heat/rigor shortening windows

(Frylinck et al., 2013; Thompson, 2002)

2.2 Trends and effects of carcass weight increase on carcass and meat quality

Over the past few years, the weights of live cattle and carcasses have increased substantially. This presents challenges for abattoir processors, retailers and foodservice (Savell, 2012; Pesonen et al., 2012). As shown in Figure 2.5, beef carcass weights have increased over time by an average of 1 to 1.5 kg each year (Lorenzen et al., 1993; Boleman et al, 1998; Mckenna et al., 2002; Garcia et al., 2008). Also, from Figure 2.6, steers carcass weights are about 60 pounds (27.2 kg) heavier than heifer carcasses and this presents more challenges during chilling. Ribeye areas have also increased with an almost one square inch (6.45 cm^2) over 20 years. These challenges beef processors in terms of uniform chilling and, hence, grading (Savell, 2012). Apart from improved nutritional interventions and improved management practices, a major factor in the carcass weight increase could be attributed to the adoption of growth enhancement technologies which includes implants and beta-adrenegic agonists such as zilpaterol hydrochloride (Zilmax) (Savell, 2012). Delmore et al. (2010) showed that live weights were increased by about 10 kg and carcass weights increased by about 17 kg. However, these heavier carcasses still have to be chilled and processed using systems that were originally designed for lighter carcasses decades ago.

Figure 2.4 and 2.5 shows the carcass weight profile over time in the USA.

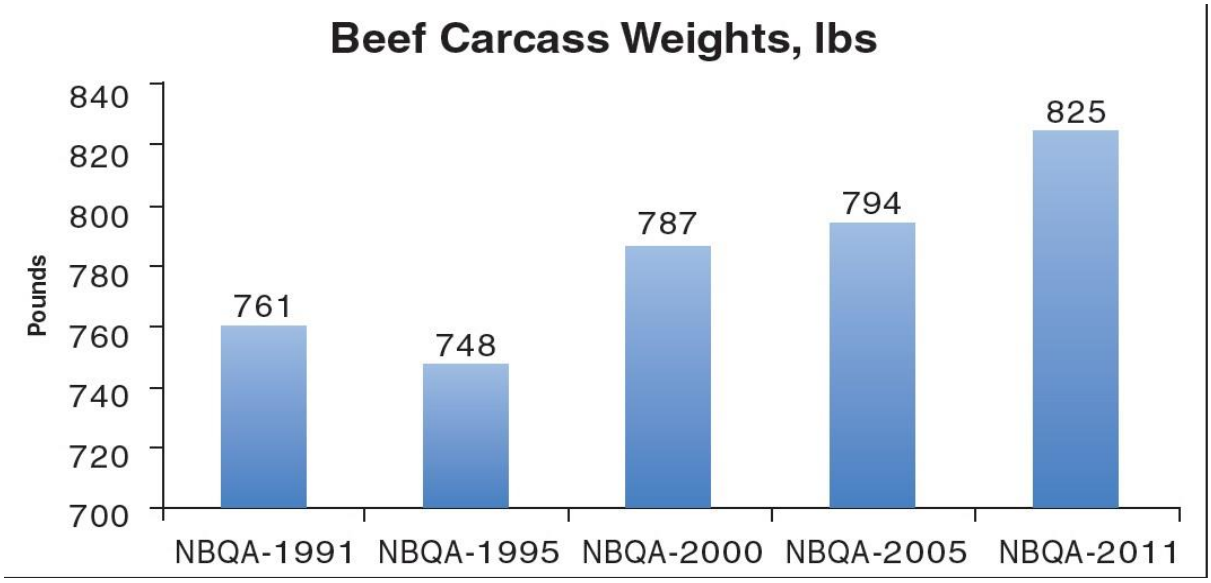


Figure 2.4 Beef carcass weight (pounds) increases as measured in the National Beef Quality Audits (USA)

(Savell, 2012)

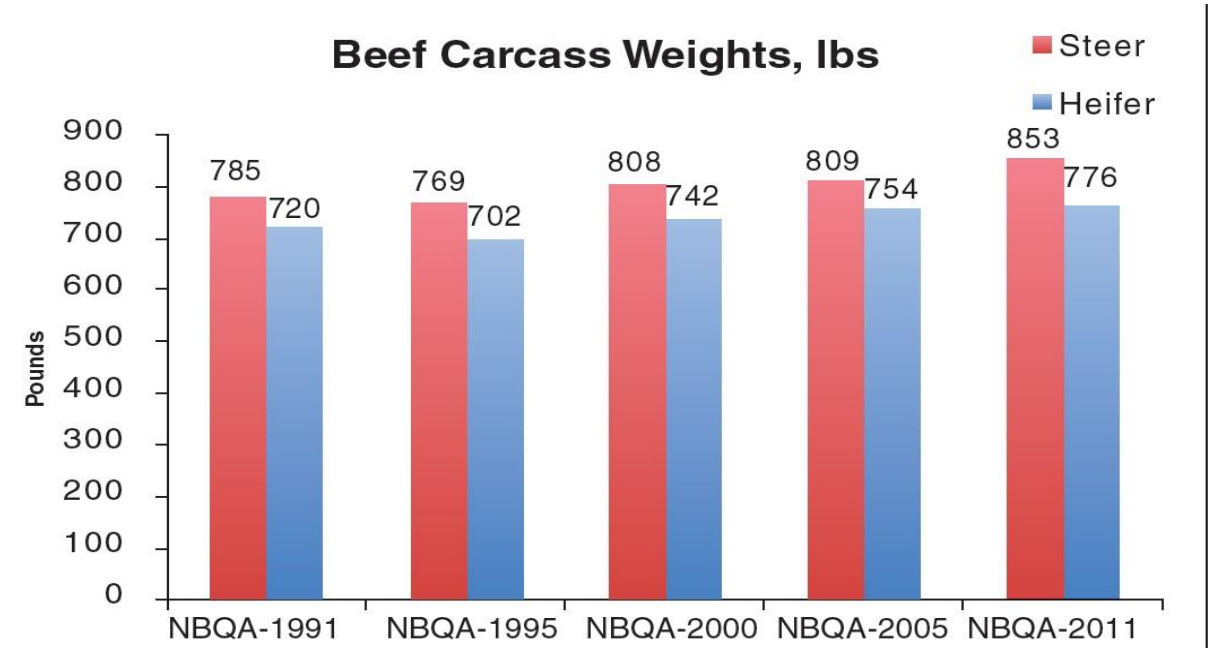


Figure 2.5 Beef carcass weight (pounds) increases, stratified by steers and heifers, as measured in the National Beef Quality Audits (USA)
(Savell, 2012)

According to a study conducted recently across the Australian meat industry, it was discovered that 72% of carcasses investigated had high temperature rigor (pH less than 6 when temperature is above 35 °C) (Warner et al., 2014). Most of the carcasses were from heavy feedlot cattle and the

percentage (72%) of carcasses that exhibited high temperature rigor was unacceptably high which calls for further investigations. A similar study showed faster pH decline and glycolysis in heavier carcasses even though they were not electrically stimulated (Hopkins et al., 2007). Heavier carcasses stand a higher risk of high temperature rigor which could result into carcasses entering the heat shortening window (Figure 2.3). Faster pH decline and glycolytic rate could lead to reduced tenderness and reduced ageing potential owing to early exhaustion of proteolytic enzyme activity at higher carcass temperature (Simmons et al., 2006). A recent review by Kim et al. (2014) indicated that there is still a gap in the understanding of the mechanisms by which proteolitic enzymes and other metabolic processes are influenced by high temperature rigor, which is common in heavier carcasses from commercial feedlot cattle. Studies on beef (Dolezal, Smith, Savell & Carpenter, 1982) and on lamb (Smith, Dutson, Hosteller & Carpenter, 1976) revealed that fat thickness played an important role in reducing cold shortening and, as a result, improved tenderness. The fat allowed the carcass to chill more slowly and to increase enzyme activity (Smith et al., 1976). These authors suggested that the chilling rate was decreased either by insulation or because of increased total mass. Smith and Carpenter (1973) suggested that 0.62cm at the 12^{th} rib is the minimum subcutaneous fat depth to prevent cold shortening in beef carcasses. Warner et al. (2014) attributed the increase in rigor temperature especially in the heavier carcasses, to higher blood plasma insulin, increasing number of days at the feedlot and fatness. Kim et al. (2014), however indicated that high rigor temperature affects tenderness, WHC and meat colour. According to these authors, high temperature rigor affects myofibrilar proteins and associated enzymes, which influence tenderness and WHC and sarcoplasmic proteins, which affects meat colour (These are discussed in more details in later sections).

2.3 Electrical stimulation, types of electrical stimulation and their merits and demerits

Electrical stimulation (ES) of carcasses implies running of an electrical current through the carcass to induce muscular contractions, thereby accelerating energy expenditure and pH decline (Locker & Daines, 1975; Swatland, 1981). Electrical stimulation accelerates glycolysis, thereby causing a faster onset of rigor, so that rapid chilling or freezing can be carried out at the shortest possible time after slaughter, without the` risk of cold shortening (Davey & Chrystall, 1980; Davey, Gilbert & Carse, 1976; Chrystall & Hagyard, 1976). Electrical stimulation can routinely drop the pH of beef carcass by 0.5 units over 60 seconds, a decline which may take up to three or more hours in un-stimulated carcasses (Ducastaing, Valin, Schollmeyer & Cross, 1985). According to Chrystal and Devine (1980), ES increases the rate of glycolysis resulting in immediate pH fall. The ΔpH

ranges from 0.6 pH units at 35 °C to 0.18 units at 15 °C. According to these authors, the energy of activation of ΔpH in stimulated beef *m.sternomandibularis* is calculated as 97 KJ/mol. The consensus is that, if carcass temperature falls too fast before rigor onset, cold shortening may occur (Bendall, 1973; Tornberg, 1996; Hwang et al., 2003; Devine, Hopkins, Hwang, Ferguson & Richards, 2004), which may bring about decreased tenderness. Meat tenderness is regarded by many authors as the most important and sought-after physical quality attribute of meat when eaten after some degree of cooking (Swatland, 1984; Miller, Carr, Ramsey, Crocket & Hoover, 2001).

Electrical stimulation helps in increasing the proportion of meat and carcasses that reaches the required pH-temperature window, and is fast becoming mandatory, especially in countries such as Australia and New Zealand, who are global leaders in meat production and export. Australia's Sheep Meat Eating Quality (SMEQ) programme identified that; for optimal eating quality, meat destined for overseas or domestic markets should attain pH 6 when carcass temperature is between 18 and 25 °C (Thomspon et al., 2005). However, there are discrepancies among reports in the influence of ES on meat quality. While some authors reported positive effects (Mckenna et al., 2003; Cetin & Topcu, 2009), some reported no effect (Wiklund et al., 2001; Kim et al., 2013), and others reported negative effects (Hector et al., 1992; Den-Hertog-Meischke et al., 1997). These paradoxes create further need to appraise reports, to create a balance in the effective application of ES to optimize meat quality and other gains that comes with it. According to Ferguson and Gerrard (2014) the effects of ES on carcass and meat quality depends on type, time and duration of application.

Electrical stimulation was first discovered by Benjamin Franklin in 1749. He found that the flesh of turkey was tenderized on applying ES shortly post-mortem (Petch, 2001). Electrical stimulation has now become a standard meat processing technology in commercial abattoirs for beef and lamb processing in most developed and developing parts of the world. It has found a widespread use, especially in the last two decades (Simmons et al., 2008). The use of ES as a means of reducing meat toughening was first reported by Harsham & Deatherage (1951) and Renschler (1951), although, it was first practically incorporated and adopted in New Zealand and then Australia in the late 1970's to reduce cold shortening and a resultant meat toughening in lambs that were frozen rapidly after slaughter. The need was driven by the high volumes of lambs that were exported to other countries (Simmons et al., 2008). The use of ES is also timely, because, recently, the meat industry has transformed from commodity based to consumer driven (Troy, 2006). This

implies that the consumers are now more quality conscious and their choices dictate the market. The use of ES is a significant intervention that helps in reducing variability in meat quality (Hopkins & Toohey, 2006). According to Hwang et al. (2003), electrical stimulation elicit changes in post-mortem muscle through three means: (i) prevention of cold shortening by ensuring that rigor takes place under optimal carcass conditions; (ii) physical disruption of muscle fibre/myofibrillar matrix (Ho, Stromer, Rouse & Robson, 1997); and (iii) acceleration of proteolysis (Uytterhaegen, Claeys & Demeyer, 1992), which is regarded as a secondary effect mediated through time-temperature-pH interaction, which affects enzyme activities and stability.

Based on his modelling of post-mortem (pm) calpain activity, Dransfield (1994) suggested that calpain activity in rapidly glycolysing muscle would be increased by a factor of 6 compared with muscle with normal glycolytic rate (un-stimulated). However, micro calpain is likely to undergo autolysis under this condition and that is why temperature and free calcium also play an important role. Electrical stimulation has now been adopted as a means of tenderisation in beef, lamb and goat carcasses (Chrystall & Hagyard, 1976; Savell, Smith, Dutson, Carpenter & Sutter, 1977; Davey, Gilbert & Carse, 1976; Geesink, van Laak, Barnier & Smulders, 1994). Electrical stimulation is also known to affect a range of other meat quality attributes including meat colour, colour stability (Ledward, 1992; Sleper, Hunt, Kropf, Kastner & Dikeman., 1983), water holding properties (Smulder et al., 1992; Simmons et al., 2008), drip loss (Kristensen & Purslow, 2001; Devine, 2009) and cooking loss (Marsh et al., 1981; Bouton et al., 1980). Electrical stimulation reduced tenderness variability compared with un-stimulated meat (Agbeniga & Webb, 2013; Rosenvold et al., 2008; Strydom, Frylinck & Smith, 2005) and was reported to reduce the microbial count on carcass and meat surfaces (Bawcom et al., 1995; Tinney et al, 1997; Mahapatra et al., 2008).

Without ES, most of the carcasses would not meet the FAO regulation that states that carcass destined for local or export market must attain a minimum of 7 °C while offal must attain a minimum of 3 °C. Electrical stimulation also reduces the processing time which would normally be around 48 hours following the traditional/conventional chilling regime (Munoz, 1991; James, 1996; EU parliament, 2004). However, ES has to be regulated and applied efficiently to obtain optimum results, in terms of objective carcass and meat quality. In some cases, the combination of high carcass temperature and low pH resulted in early exhaustion of calpain-1 activity, which led to reduced tenderization especially during extended ageing (Dransfield, Etherington & Taylor,

1992; Simmons, Singh, Dobbie & Devine, 1996). Thompson et al. (2005), cautions that ES must be controlled. If the pH declines too rapidly at high carcass temperature, protein may be denatured, which could result in meat toughness and increased DL. Devine (2009) also reported that rapid rigor by ES produces inevitable drip, which comes with economic implication owing to weight loss. Electrical stimulation can only be effectively carried out only with an understanding of the interaction between the electrical parameters, that is, pulse, amplitude, width, frequency and the extent of muscle contraction they produce (Simmons et al., 2008). To achieve the desired benefits from ES, it must be properly applied in conjunction with knowledge of various intricate antemortem, perimortem, and post-mortem management practices. Despite the extensive research on ES, the fundamental mechanism and the appropriate commercial applications remain obscure. There are still many paradoxical assumptions and speculations about ES.

There are three major types of ES. Extra low voltage (ELVES) which is < 100 V, high voltage stimulation (HVES), carried out at > 110 V, and low voltage (LVES), which is carried out at 100 V -110 V, which is also regarded as medium voltage ES (MVES) by some authors (Adeyemi & Sazili, 2014). Often referred to in literature is the low and high voltage ES. High variability still exists in impulse frequency, amperage, duration of application, type and position of electrode, pathways (direct or nerve) and the delay time between slaughtering and application. This variability accounts for the discrepancies in reports of the efficacy of ES. In addition, the variations in reports could be due to differences in animal age, size, species, breed, management and handling. However, both types of ES yield effective stimulation with merits and demerits, but the ideal method hinges on cost, type of animal, existing slaughter floor and throughput.

2.3.1 Low voltage electrical stimulation

Low voltage electrical stimulation (LVES) is mostly used when there is short time delay between slaughter and stimulation (up to 10 min. after bleeding) (Chrystall et al., 1980) and is usually applied before dehiding. LVES is considered more practical and more attractive to use under commercial conditions (Fabiansson & Laser Reutersward, 1985; Hawrysh, Shand, Wolfe & Price, 1987; Savell et all., 1977). It also attracts less stringent requirements from government regulatory agencies for accident prevention (Bouton, Ford, Harris & Shaw, 1980). The cost of installation is very low and it is safer to use in the workplace and can be easily installed in any existing abattoir (Bouton et al., 1978 & 1980b). In Australia, a new approach has been developed (Shaw et al., 2005), based on medium voltage electrical stimulation (MVES). Medium voltage stimulation is now favoured over traditional high voltage systems because of its compliance with power levels

and pulse width, occupational health and safety regulations (Australian Standard AS/NZS 60479, 2002). Abattoir audits by MSA indicate that grain-fed carcasses require less stimulation compared with grass fed carcasses to achieve a similar glycolytic rate, likewise heavy carcasses compared to lighter ones (Thompson, 2002). There has been speculation about the effectiveness of LVES in relation to the potency of the nervous system, especially after dressing. Some authors suggest that for LVES to be effective, it must be applied within a short time of slaughter (Chrystall et al., 1980). Fabianson & Laser Reutersward (1985), who administered LVES (85V) within 10 min of stunning, indicated the enhancement of energy consumption, which was reflected in improvement in tenderness of longissimus muscle. Some authors reported little or no effects of LVES on tenderness (Rdbotten, Lea, & Hildrum, 2001) and even negative effects (Unruh et al., 1986). Moreover, LVES has recorded larger variations in beef tenderness when used under industrial conditions (Hildrum et al., 1999).

Shaw, Eustace & Warner (1996) reported an effective pH drop using LVES applied 25-30 min pm. Simmon, Gilbert & Cairney, (1997) also reported a similar result in commercial lamb systems in New Zealand. These authors also re-evaluated the role of nervous system during LVES applied immediately after slaughter using sheep carcasses treated with curare. A 15 Hz waveform delivering 0.5A pulse amplitude was used. Carcasses were un-responsive for about 10 min after the stun but then, a response reappeared and increased in strength over 5-10 min. This suggests that LVES is mediated by the nervous system while it is functional but, afterwards, can evoke direct muscle stimulation through changes in muscle membrane excitability (Simmons et al., 2008). Figure 2.6 shows the response of curare-treated lamb carcasses to ES.

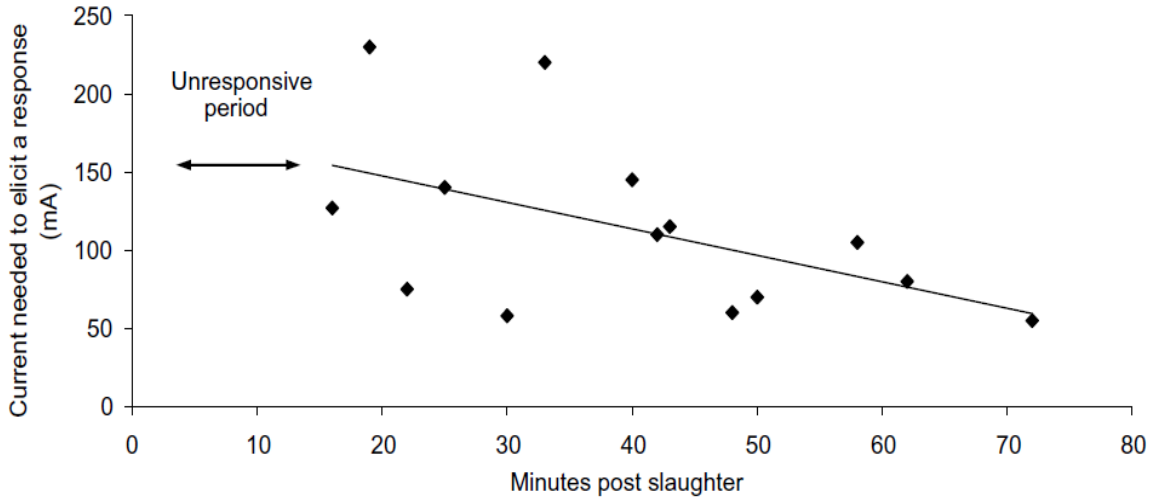


Figure 2.6 Emergence of responsiveness of stimulation in *m. longissimus* of curare-treated lamb carcasses
(Simmons et al., 2008)

Low voltage electrical stimulation at 40 min pm produced juicier meat than the un-stimulated meat (Hwang & Thompson, 2001). The work of Aalhus, Jones, Tong, Jeremiah, Robertson & Gibson, (1992) and Olsen et al. (1994) supports this. According to Hwang and Thomspon (2001), LVES has a tendency to produce higher calpain-1 level post-mortem, however, this was not reflected in tenderness improvement, probably due to simultaneously higher calpastatin level. In the same study, significantly lower tenderness scores for HVES at 3 min pm coincided with a significantly lower level of micro-calpain at 24 h post-stimulation. This was partly supported by Simmons et al. (1996) and Geesink et al. (1994). The major demerit of LVES is that it is labour intensive since the electrode has to be applied manually. Some researchers have reported that its consistency in effect is lower, especially on tenderness, compared with HVES (500-1000V), especially when used under industrial conditions (Bendall, 1980; Bouton et al., 1980; Hildrum et al., 1999).

2.3.2 High voltage electrical stimulation

High voltage electrical stimulation gives room for a longer delay time between bleeding and stimulation (up to 60 min after exsanguination). It requires huge capital expense and adequate precautionary needs to ensure the safety of workers, because it produces lethal amount of electricity (Savell, 1982). HVES has been reported to cause disruption of muscle fibres to bring about meat tenderization (Marsh et al., 1986; Takahashi et al., 1987). However, some studies indicated indirectly that physical disruption had less effect on tenderization than proteolysis (Pommier, Postes & Butler, 1987; Unruh, Kastner, Kropft, Dikeman & Hunt, 1986). Studies have also shown that similar rates of pH fall are achieved by LVES and HVES (Chrystall, Devine, Ellery & Wade, 1984). McKeith et al. (1981) compared the efficacy of HVES and LVES in terms of meat quality at different time post-mortem using 390 calves and young bullocks. Carcasses were stimulated at 550 V or 150 V for 1 or 2 min, at 16 pulses/min compared with NES carcasses. The authors reported that carcasses stimulated at 550 V had more tender, brightly coloured and better flavoured and leaner meat than those stimulated at 150 V. It was concluded that HVES (550 V) and a shorter stimulation time (1 min) are ideal for improving meat palatability. Roeber et al. (2000) also reported that duration and voltage intensity of ES affected beef colour and tenderness. Regarding ATP decline post-mortem, Morton and Newbold (1982) reported that time taken for ATP to decline to half of the initial content in sheep was about 20 min (in HVES), 2-3 h (in LVES) and 5-6 h in control carcasses. The use of HVES has been marred by the tendency to cause over-stimulation because of fast pH decline pre-rigor. This could result in PSE (pale soft

exudative) conditions in meat, thereby causing adverse effects on tenderness, WHC, colour stability and overall eating quality (Geesink et al., 2001; Koh et al., 1987; Unruh et al., 1986). Most of the effects of over-stimulation could be attributed to denaturation of muscle structural proteins and enzymes.

2.4 Time of application of electrical stimulation on carcasses and its effect on meat quality

There are two major types of ES, namely LVES, which is usually applied immediately after bleeding (up to 10 min after bleeding), and before dehiding, and HVES, which is usually applied after evisceration (up to 60 min pm). According to Hwang and Thompson (2001), stimulation at 40 min pm resulted in lower meat shear SF and a slower pH decline compared with ES at 3 min pm. This is in accord with the report of Unruh et al. (1986) and Pommier et al. (1987), who found that over- rapid pH decline caused a small increase in meat toughness. Rigor shortening was also evidenced by shorter sarcomeres for ES at 3 min pm compared with ES at 40 min pm (Hwang & Thomspon, 2001). Similarly, Wahlgren, Devine & Tornberg (1997) reported that if there is a rapid pH decline as a result of ES within 2 min of slaughter, the meat is not as tender after 3 days of ageing at 4 °C compared with when ES takes place 30 min after slaughter (rigor mortis at approximately 15 °C). These authors reported, however, that both meats were more tender than NES (non-ES) meat. They indicated that after 14 days of ageing, all the meat became tender but the ones stimulated at 30 min pm was still the most tender. This report suggests that meat attaining rigor mortis close to 15 °C would be more tender compared with the ones entering rigor at other temperatures. In the same vein, Hwang and Thompson (2001) reported that, no matter the type of ES used, when it is applied at 3 min pm, its tenderizing effect would be relatively less compared with about 40 min pm. This was attributed to the initial decrease in calpain activity combined with relatively high calpastatin level immediately after slaughter. The work of Hwang and Thompson (2001) also showed that LVES applied 40 min pm produced juicier meat compared with control (NES) sides.

Recent studies on sheep showed that there could be significant improvement in the efficiency of ES with the timing of application, duration, pulse width and the type of voltage (Toohey, Hopkins, Stanley & Nielson, 2008, Pearce et al., 2009). Bendall (1976) suggested that the response of bovine to ES reduced significantly after 50 min pm and sooner for lamb carcasses. Kim et al. (2013) also reported that LVES at 90 min after exsanguination had no effect on proteolysis and tenderness development in *longissimus dorsi, semimembranosus and abductor* muscles in beef. However, in recent times, ES has been carried out immediately after bleeding which has been

reported to enhance bleed-out, lighter and redder colour and meat tenderness (MLA & AMPC, 2011).

2.5 Durations of electrical stimulation and their effects on carcass and meat quality

There are various reports on the duration of application of electrical stimulation, be it HVES or LVES, and, there is no consensus on optimum time for the duration of application. Currently in South Africa, a number of abattoir operators are worried about over-stimulation which has negative effects on carcass and meat quality. According to observations at some of the abattoirs, some operators stimulate for over 2.5 min (Agbeniga & Webb – (unpublished)). This is considered too long, especially with high voltage stimulation systems. Apart from the detrimental effects on the carcass and meat, more time is being spent and a subsequent slow down of line movement at the abattoirs is experienced. Some studies show that ES posed the risk of over- or under- stimulation on carcasses and these could lead to reduction in meat quality such as colour defect, reduced tenderness, increased drip loss and diminished shelf-life, instead of improved quality (Hildrum, Solvang, Nilsen, Froystein & Berg, 1999; Hwang et al., 2003). Gursansky, O'Halloran, Egan and Devine (2010) acknowledged that LVES could be just as advantageous as HVES in terms of tenderness as long as the duration is not less than 40 seconds as opposed to 10. Strydom and Frylinck (2014) also recorded that shorter duration (15 s) of medium voltage stimulation (150 V) on beef carcasses was more beneficial to initial tenderness, tenderness development, colour and drip loss, compared with stimulation of 45 and 90 s during prolonged ageing when pre-slaughter stress was minimized. Additional advantages of shorter duration were reflected in better colour attributes and lower drip loss when meat was aged for shorter period. Smulders et al. (1990) suggested that for ES (380 V-50 V at 60 Hz) to be effective, it must be for a sufficient time to lower the loin pH 3 h pm to 6.3 or below. In their work, a stimulation time of 20 s or more brought about pH 3 h below 6.3 in all carcasses and this produced an acceptable tenderness score. They also recognized that prolonged stimulation might result into excessive glycolysis (pH 3 h < 5.9), leading to reduced tenderness and paler colour (higher L* value) (Devine et al., 2002), which was confirmed by the work of Marsh et al. (1987). It could also lead to reduced fluid retention (Eikelenboom & Smulders, 1986; Strydom & Frylinck, 2014). McKeith et al. (1981) concluded in their work that shorter time (1 min) with HVES (550 V) was ideal for improving meat palatability. Roeber et al. (2000) also reported that duration and voltage intensity of ES affected beef colour and tenderness. The work of Pearce et al. (2006) reported that a setting of 300 V, 1A, 2.5 ms pulse width, 15 Hz at 34 s was the best, resulting in 60% of the carcasses hitting the pH/temp window (pH 6 between 18 and 25 °C).

2.6 Effects of carcass weight on the efficacy of electrical stimulation and the resultant carcass and meat quality attributes.

There have been few studies on the effects of carcass weight on the efficacy of ES in relation to carcass and meat quality. A recent finding by some authors is that heavier carcasses, especially from feedlot, exhibited faster glycolytic rate and post-mortem (pm) muscle metabolism, especially when electrically stimulated (Warner et al., 2014; Jacob & Hopkins, 2014; Strydom & Rosenvold, 2014). This leads to a phenomenon known as high rigor temperature or high temperature low pH, whereby the pH decreases at a faster rate and rigor sets in when the temperature is still high (pH 6 at temperature above 35 ^{o}C) (Jacobs & Hopkins, 2014). This was attributed to factors such as increased slaughter weight, plasma insulin level, increased electrical inputs, and more days on the feedlot (Warner et al., 2014a). This condition affects final tenderness, tenderization when aged, initial colour and colour stability and water holding capacity (Kim et al., 2014; Savell et al., 2005). Apart from ES, electrical inputs on carcasses pm have increased in most of commercial slaughter houses and these includes immobilizers, hide pullers and bleeding rails (Warner et al., 2014c). The high electrical input increases the incidence of high rigor temperature by elevating the glycolytic rate and muscle metabolism thereby causing the carcasses to miss the MSA (Meat Standard Australia) pH/temperature window (temperature below 35 ^{o}C at pH 6- Thompson 2002). Muscles under these conditions may be tough due to 'hot rigor' or fail to tenderize owing to denaturation of contractile proteins (actin and myosin) and the early exhaustion/autolysis of proteolytic enzymes, especially calpains (Kim et al., 2014). According to Offer (1991), denaturation of myosin induces the shortening of the myosin head and the reduction of filament space thereby forcing water from the muscle cells into the extra-cellular space, causing reduced WHC. Colour stability is also affected by high rigor temperature and could lead to PSE-like condition (Jacob & Hopkins, 2014).

2.7 Effects of electrical stimulation on some carcass and meat quality attributes

2.7.1 Effects of electrical stimulation on pH/temperature decline

The effects of ES on pH decline cannot be over-emphasized. In most research, carcass pH, which is a good indicator of the resultant meat and carcass quality attributes, is measured at 45 min or 1 (initial pH), 3, 6, 12 and 24 h pm (ultimate pH (pH_u)). As stated and as recorded in many of the literature, pH and its decline post-mortem determines the overall carcass and meat eating quality (Hwang and Thompson, 2001). One of the main reasons for applying ES to carcasses is to bring about a faster pH decline by the process of glycolysis which causes a faster rigor onset, so that rapid chilling can take place without the risk of cold shortening (Davey & Chrystal, 1980; Davey,

Gilbert & Carse, 1976; Locker & Daines, 1975, Swatland, 1981). Glycolysis brings about the production of lactic acid, while reducing ATP re-synthesis thereby bringing about pH decline. According to Ducastaing et al. (1985), ES can routinely drop the pH of beef carcass by 0.5 units over 60 seconds. This process could require three or more hours in un-stimulated carcasses. Electrical stimulation helps in increasing the proportion of carcasses reaching the required pH and temperature window (Thompson et al., 2005) (pH 6, when carcass temperature is below 35°C). The pH fall range from 0.6 pH units at 35 °C to 0.18 units at 15 °C. The energy of activation of change in pH in stimulated beef *m.sternomandibularis* is calculated as 97 KJ/mol, according to Chrystall & Devine (1980). Figure 2.7 shows the pH decline from ES and NES carcasses.

Toohey et al. (2008) modelled the rate of pH decline in ES muscle, using Genstat 7.1 (2004) thus: $pH_t = pH_f + (pH_i - pH_f) \times e^{-kt}$ where pH_t is the pH at time t; pH_f is the final pH; pH_i is the pH at t = 0; k is the rate constant of pH decline and t is the time in hours. The same model goes for temperature decline. Figure 2.7 shows the pH decline over time in ES and NES beef carcass.

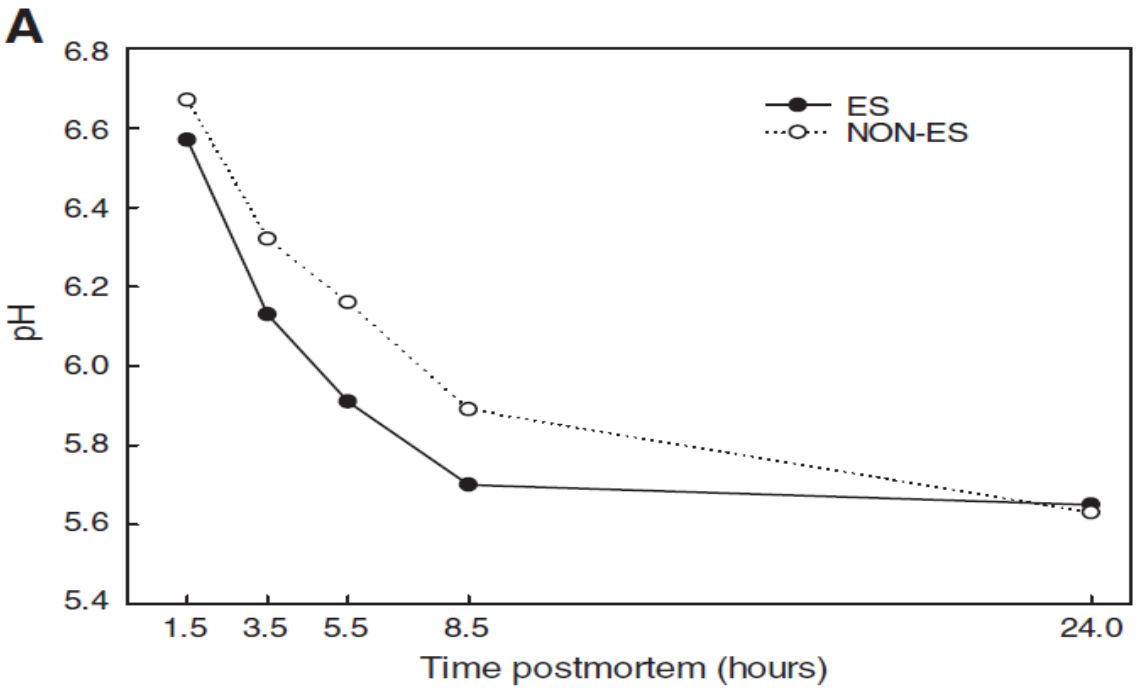


Figure 2.7 pH decline post-mortem in electrically and non-electrically stimulated beef *m. longissimus dorsi* muscle
(Kim et al., 2013)

Temperature has been shown to exert some effects on the efficacy of ES. However, Toohey et al. (2008) on lamb carcasses and Hollung et al. (2007) on beef carcasses showed that ES did not

affect temperature decline. Also, Den Hertog-Meischke et al. (1997) reported that muscle temperature was not affected by ES in either LT or SM. However, McKeith, Savell, Murphey, & Smith (1982) observed that the initial (1 h) rib and round temperatures were significantly higher for the electrically stimulated sides compared with the control (NES) sides but they however stated that, at 24 h pm, neither the round nor the rib temperatures differed between the ES and control (NES) sides. The work of Marchello et al. (1999) reveals that carcasses showed less resistance to ES input with warmer temperatures. Jeacocke (1977) also shows that the rate of glycolysis was faster at high temperatures but fell to a minimum at 12 °C and then rises again. The latter increase arose because of the remaining ATP required for a cold contracture. The work of Hwang & Thompson (2001b) shows that most tender beef, after 14 days of ageing was achieved when temperature at pH 6 was between 29-30 °C under in-situ conditions. However, in several studies, optimum tenderization was achieved when optimum stimulation and chilling rate brings about rigor at 15 °C. Under commercial conditions, stimulated carcasses enter rigor at higher temperature and so, tenderization could be attributed to faster rate of pH decline at higher temperatures (Hwang et al., 2003). Figure 2.8 shows temperature decline over time in ES and NES carcasses.

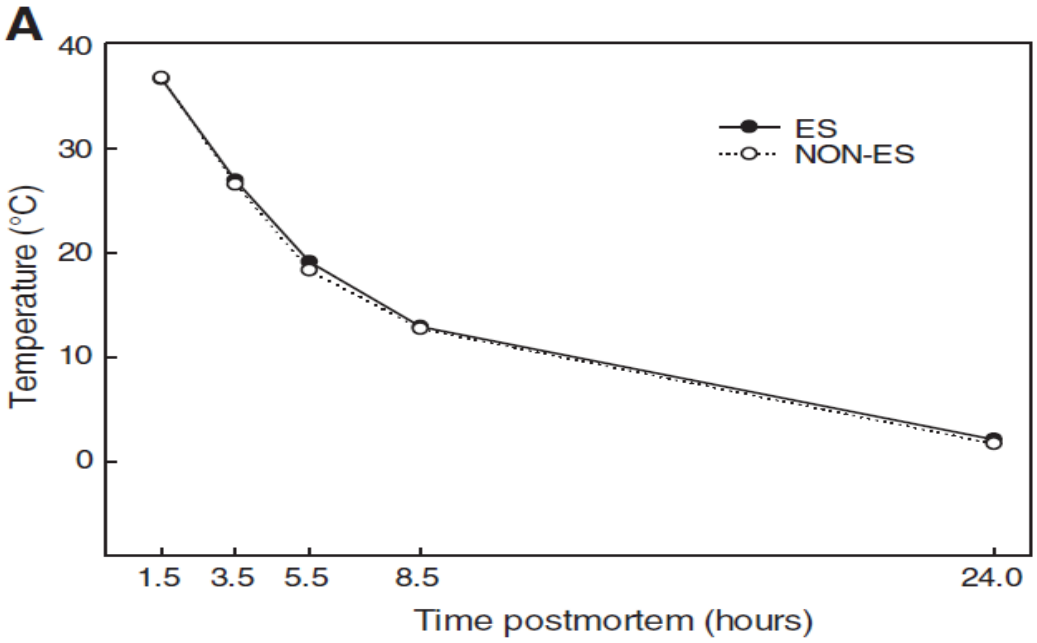


Figure 2.8 Temperature decline post-mortem in electrically and non-electrically beef *m. longissimus dorsi* muscle

(Kim et al., 2013)

On the other hand, NES brings about elevated pre-rigor temperature which reduces tenderness (Devine et al., 1999) through reduced enzyme activity (Simmons et al., 1996).

Figure 2.9 shows pH and temperature decline coordinates of Zilmax treated ES and NES carcasses at various times, represented by the data points.

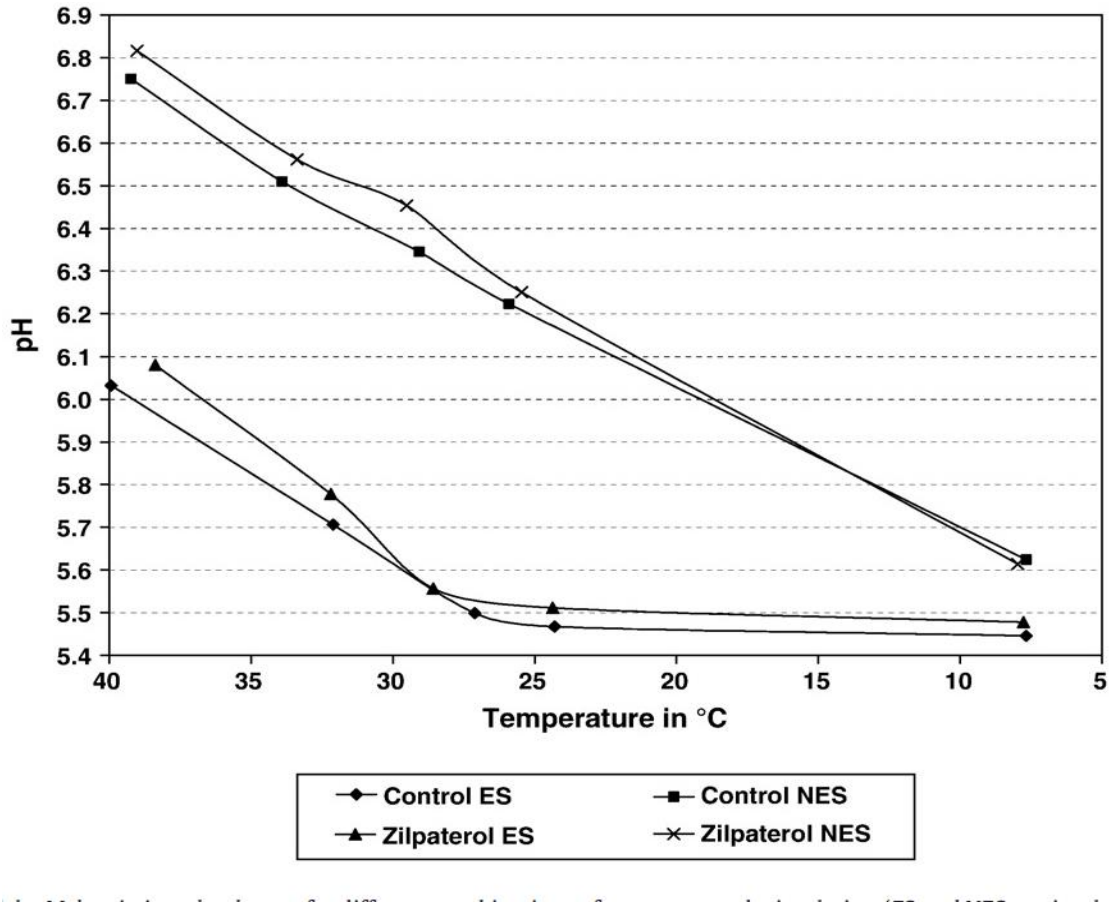


Figure 2.9 pH and temperature plot of *m. longissimus lumborum* for electrically and non-electrically stimulated carcasses from cattle fed with zilpaterol at 1, 2, 3, 4, and 18 h post-mortem
(Hope Jones et al., 2010)

2.7.2 Effects of electrical stimulation on meat shear force

Meat Shear force (SF) which is an indication of tenderness, is a function of background toughening, the toughening phase and the tenderization phase (Koohmaraie, 1996). Managing the toughening and tenderization phase are a responsibility of meat processors. One of the key tools in doing this, is the use of ES (Hwang et al., 2003). Electrical stimulation was originally developed in New Zealand to manage toughening and cold shortening in lambs that were frozen rapidly in extreme cooling conditions after slaughter (Simmons et al., 2008). Several research has been conducted on the effects of ES on meat SF and the principles are well covered (Hwang et al., 2003). Variation in meat tenderness and consumer concerns over tough meat poses a great deal of

concern to the meat industry worldwide. Some researchers have shown that consumers are able to detect tender and tough meat with different Warner Bratzler Shear Force (WBSF) readings and are willing to pay more for tender meat (Miller, Carr, Ramsey, Crockett & Hoover, 2001). Perry et al. (2001) suggested an acceptable 40 Newton (N) or 4 Kg threshhold as instrumentally acceptable tenderness for beef. Shorthose, Powell and Harris (1986) also suggested that a SF threshold of 4.9 kg is the optimal threshold for tenderness/toughening of meat, using Australian consumers. Eelectrical stimulation is known to elicit changes in post-mortem muscle which enhances tenderization in three ways: first, by preventing cold-induced shortening thereby ensuring that rigor-mortis takes place under optimum conditions; second, by physical disruption of muscle fibre/myofibrillar matrix, and third, by acceleration of proteolysis, mediated through time-temperature-pH interaction, which affects enzyme activities and stability (Hwang et al., 2003).

Currently, some of these findings are being debated and research is still being carried out to fully ascertain the mechanisms of these findings. Electrical stimulation is known to hasten rigor and cause the tenderization process to start earlier at a higher temperature, compared with un-stimulated carcasses, thereby reducing ageing time (Dransfield, Etherington & Taylor, 1992). Electrical stimulation has been shown to advance rigor onset by activation of calpain-1 which is initiated by Ca+ ion release which enhances proteolysis, assisted by higher temperature, which leads to tenderization (Dranfield et al., 1992; Hwang & Thompson, 2001a). Some researchers also showed that calpastatin activity decreased more, over time in ES samples compared with NES samples (Ducastaing et al., 1985; Hope-Jones, Strydom, Frylinck & Webb, 2010).

Studies have revealed that there is a strong relationship between physical disruption of the myofibrillar complex and an increase in tenderness (Dutson, Smith, Savell & Carpenter, 1980; Ho, Stromer & Robson, 1996). Similarly, other studies have advocated the link between physical disruption and improved tenderness for HVES (300-500 V) (Takahashi, Wang, Lochner & Marsh, 1987; Will, Ownby & Henrickson, 1980) and for intermediate voltage systems (145-250 V) (Ho et al., 1996; Sorinmade, Cross, Ono & Wergin, 1982). Earlier studies of Dutson, Yates, Smith, Carpenter and Hosteller (1980), hypothesized that physical disruption per se lowers resistance of fibres to chewing or mechanical shearing force. However, other studies speculated that physical disruption facilitated ageing in other ways. It was indicated that contracture bands were not a result of ES current passing through the muscle, but of supercontracture caused through localized excessive calcium ion release from sarcoplasmic reticulum. It was speculated that this extra calcium could cause tenderization to proceed (Hwang et al., 2003).

Despite widespread report on the positive effects of ES on meat SF, some researchers showed that ES could produce an adverse effect on tenderness, especially when intense stimulation was combined with slow chilling (Koh et al., 1987; Marsh et al., 1987; Takahashi, Lochner & Marsh, 1984; Unruh et al., 1986). Geesink, Mareko, Morton & Bickerstaffe (2001) also showed that intensely stimulated carcasses produced significantly tougher loins at 7 d pm, compared with mildly stimulated carcasses. Figure 2.10 shows the plot of meat SF with ES and NES samples.

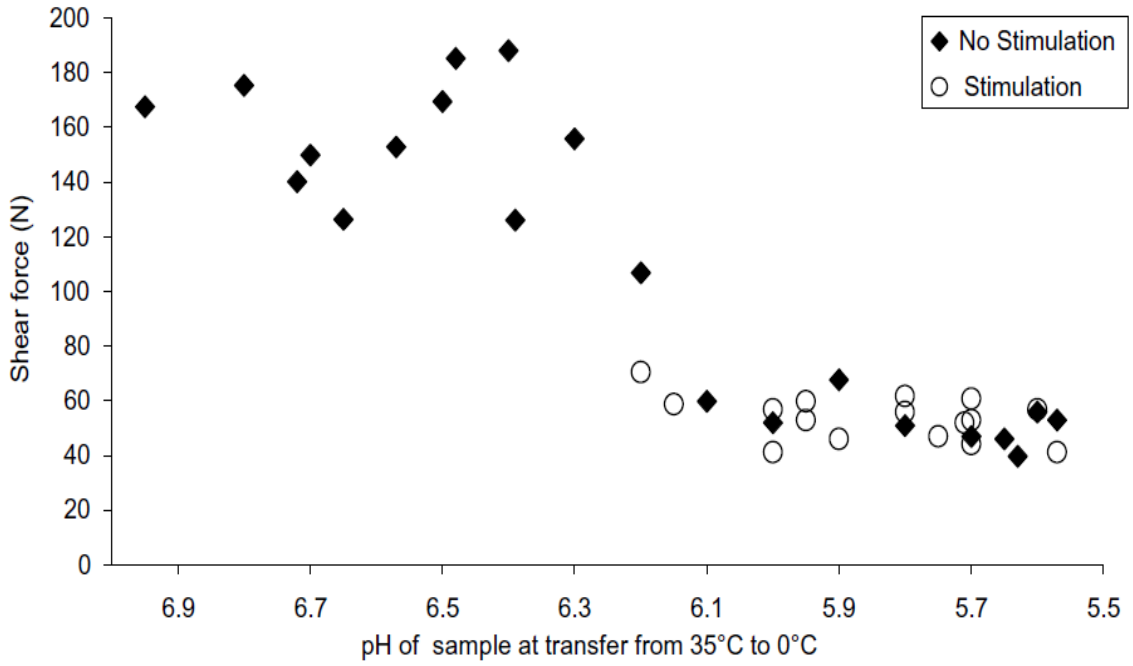


Figure 2.10 Shear force of beef *m. longissimus* samples following pre-rigor incubation at 0 ^{O}C and 3 weeks of ageing

(Simmons et al., 2008)

Figure 2.11 shows the calculated tenderness curves for ES and NES carcasses.

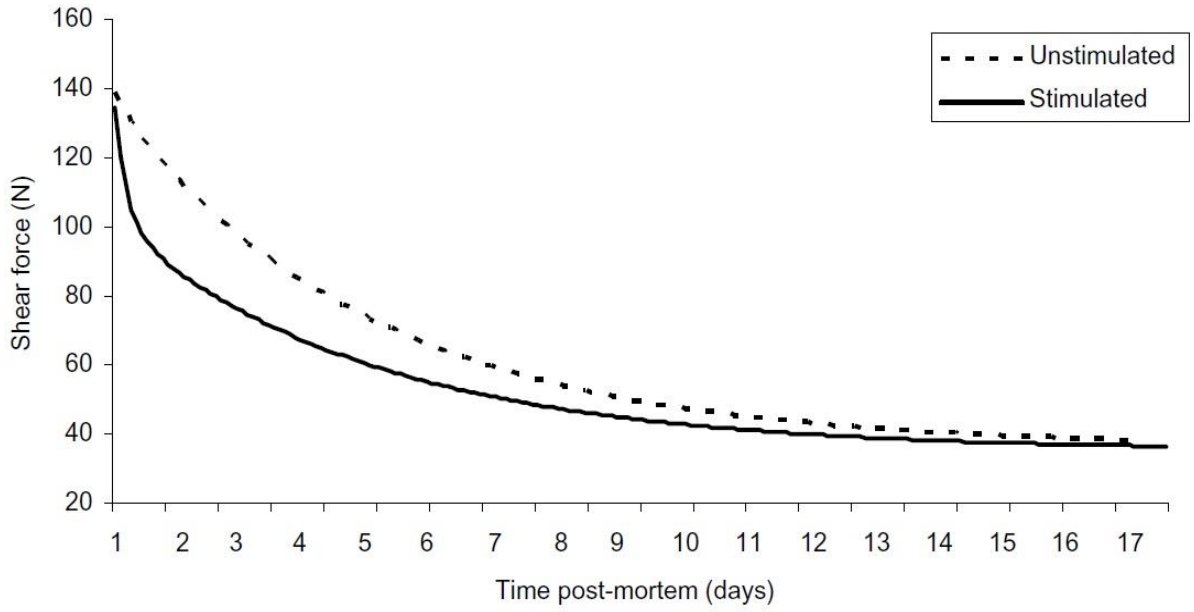


Figure 2.11 Calculated tenderness curves for electrically and non-electrically stimulated carcasses

(Simmons et al., 2008)

2.7.3 Effects of electrical stimulation on meat water holding capacity

Electrical stimulation has been shown to affect meat WHC, which is one of the most important commercial attributes of meat (Li, Jing, Xin, Marchen & Kerstin, 2011). Unruh et al. (1986) revealed that LVES (50 V) during exsanguination had a negative effect on cooking loss, sensory attributes and colour stability. However, few data are currently available on the effects of LVES after dressing on WHC, meat colour and colour stability of beef muscle. Water holding capacity has been shown to be influenced by early pH decline at higher temperature and the resultant rigor mortis (Schafer, Rosenvold, Purslow, Anderson & Henckel, 2002). An important factor when evaluating WHC is the protein solubility, especially in PSE (pale soft exudative) and DFD (dark firm dry) meat (Joo, Kauffman, Kim & Park, 1999). It is a good indicator of the muscle protein response to fast pH decline induced by ES. Li *et al*. (2011) showed that LVES applied after dressing did not affect purge, thaw and cooking loss. These authors, however, noted that the fast pH decline owing to ES would accelerate the decrease in net negative charges on the surface of myofilaments, and the lactate formed by glycolysis acting as an anionic chaotrope would impair the interaction between water and protein, leading to drip loss (Fujita et al., 2007; Puolanne & Halonen, 2010). These authors also reported that thawing loss increased significantly with ageing in ES meat. In addition, ES did not affect the solubility of sarcoplasmic or myofibrillar proteins.

However, the solubility of sarcoplasmic proteins increased up to 72 h, but decreased afterwards in ES muscles. Low pH_u of around 5.4 is near isoelectric point of major muscle proteins. This low pH_u has the ability to reduce the net charge that decreases myofilament spacing and protein solubility, which leads to reduced WHC and poor processing yield (Irving, Swatland & Millman, 1989; Joo et al., 1999).

Low pH and high temperature in post-mortem muscle reduces the WHC of meat, which is attributed to the denaturation of muscle proteins, especially myosin (Offer, 1991). This is supported by the work of Micklander et al. (2005), who suggested that the faster pH decline coupled with high rigor temperature could lead to increased myofibrillar shrinkage and protein denaturation, which reduces WHC. The same effect was pointed out by Buts, Casteels, Claeys & Demeyer (1986) on veal carcass using LVES immediately pm followed by and moderate cooling (85 V max, 14 Hz, & 5 ms pulse width at 6 °C and after 2 h, temperature was reduced to 3 °C for the remaining cooling time) pm. This resulted in reduced WHC in terms of press loss. The authors suggested it was because of denaturation of proteins owing to rapid pH decline. This denaturing condition is more severe when pH drops rapidly as in PSE conditions in pork and especially from HVES, combined with very slow chilling (Babiker & Lawrie, 1983). The pH decline from ES, depending on the chilling rate contributes to the reduced WHC in beef (Babiker & Lawrie, 1983; Den Hertog-Meiscke, Smulders, van-Logtestijn & van Knapen, 1977; Martin et al., 1983). However, the work of Wiklund et al. (2001) on red deer shows that ES and the resultant faster pH decline had no appreciable effects on drip loss. On the contrary, Bekhit, Farouk, Cassidy and Gilbert (2007), whose work was on venison quality as a result of ES and rigor temperature reported that the percentage of expressible water increased significantly, with rise in rigor temperature. This was also attributed to protein denaturation as a result of rapid pH decline, coupled with high muscle temperature especially above 35 °C (Hamm, 1961; Scopes, 1964). On the other hand, the work of Pearce et al. (2009) reveals that, there was a significant effect ($P < 0.05$) of stimulation on drip loss of loin after 10 days of ageing, using MVES (300 V peak) and modulating frequencies. Hopkins et al. (2014) reported increased drip loss in strip loin of ES beef that went through high rigor temperature, compared with the NES counterpart. Honikel et al. (1980) also discovered that drip loss was high in contracted and shortened muscle compared with un-shortened muscles. Figure 2.12 shows the effects on ES applied after dressing and pm ageing on thawing loss, cooking loss and protein solubility of bovine *longissimus* muscle.

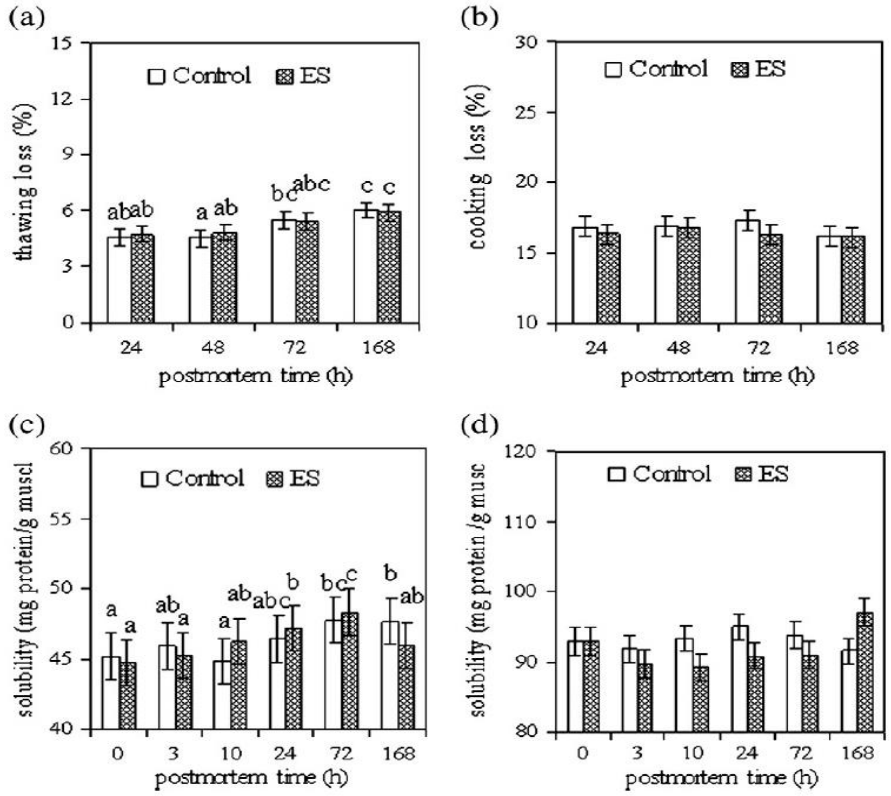


Figure 2.12 Effects of electrical stimulation and post-mortem ageing on thawing loss, cooking loss and protein solubility of bovine longissimus muscle (means ±)

N:B: (a) thawing loss; (b) cooking loss; (c) sarcoplasmic protein solubility; and (d) myofibrillar protein solubility. [abc] Different letters indicate significant differences ($P < 0.05$) between all groups (Li et al., 2011).

2.7.4 Effects of electrical stimulation on sarcomere length

For over 50 years, the state of muscle contraction after rigor mortis has been studied extensively. Knowledge of muscle shortening as a major cause of meat toughness led to the realization that post-mortem treatments far out-weighed live animal factors such as age, breed, and ante-mortem (pre-slaughter) factors in determining palatability (Polidori et al., 1999). Sarcomere length (SL) was known to be one of the major determinants of meat toughening or tenderness (Locker, 1960; Marsh & Leet, 1966; Locker & Daines, 1975; Locker & Hagyard, 1963; Herring et al., 1965). These researchers have demonstrated that there is a strong and positive correlation between SL and tenderness. They reported increased toughness in shortened muscles. Devine et al. (1999) confirmed that the longer the SL, the more tender the meat, even after 14 days of ageing. They concluded that a rigor temperature of 15 °C gave the best tenderness value. Wahlgren et al. (1997) hypothesized that when ES was applied so that meat entered rigor close to 15 °C, the meat

tenderized faster and reached the highest ultimate tenderness faster, compared with other temperatures.

Meat tenderness is known to be affected by the extent of muscle contraction at rigor, which is a function of SL (Smulders, Marsh, Swartz, Russell & Hoenecke, 1990). It is known that, manipulating the rate of glycolysis, which is achieved by ES in conjunction with an appropriate chilling regime, can produce a wide range of SL (Smulders et al., 1990). However, some researchers indicated that there was no difference in SL between stimulated and un-stimulated meat using MVES (300 V peak) and different modulating frequencies (Pearce et al., 2009). This is in accord with the work of Smulders et al. (1989) on veal carcasses who found no significant difference in sarcomere length of *longissimus dorsi* of ES and NES controls. The work of Wiklund et al. (2001) on red deer also supported these findings. They found no significant difference in sarcomere length between ES and NES meat. Also, the work of Toohey et al. (2008) on lamb meat revealed that SL was in a normal range (Hwang & Thompson, 2001) between ES and NES carcasses. According to the threshold grouping of Marsh and Leet (1966), SL in the range of 1.7-2.0 μm is moderately tender or moderately tough. Also, according to the threshold grouping of Starkey, Geesink, Collins, Oddy and Hopkins (2016) and Hopkins, Stanley, Toohey et al. (2007), SL of 1.2 μm is regarded as extremely tough while SL of 2.2 μm is regarded as extremely tender.

Some researchers indicated that ES could bring about a negative effect on SL. Buts et al. (1986) revealed that ES brought about reduced sarcomere length. According to these authors, this finding is in accord with the general theory that a fast glycolysing post-mortem muscle results in shorter sarcomeres and tougher meat (Marsh et al., 1981). Marsh et al. (1981) however, indicated that the net effect could thus result in tenderization, despite the sarcomere shortening, probably owing to the mechanical effect of disruption of fibres. Buts et al. (1986) also reported a mixed concomitant effect of ES on tenderness, despite of shortened SL, whereby some groups were tender and some exhibited tough meat. Furthermore, Smulders et al. (1990) indicated that tenderness was less dependent on SL in fast glycolysing muscles with rapid pH decline.

Devine, Ellery & Averill (1984), showed that red muscles (e.g. masseter) did not exhibit an increase in the rate of pH decline but showed evidence of super-contracture when stimulated. The red muscle was also found to be more susceptible to cold shortening and had few super-contracture bands and increased rate of pH fall after ES. White muscles (e.g. cutaneous), on the other hand, were not so susceptible to cold shortening, had few super-contractures and increased

pH decline after ES. Figure 2.13 shows the contracture bands in ES beef *m. Longissimus* compared with controls and changes during ageing.

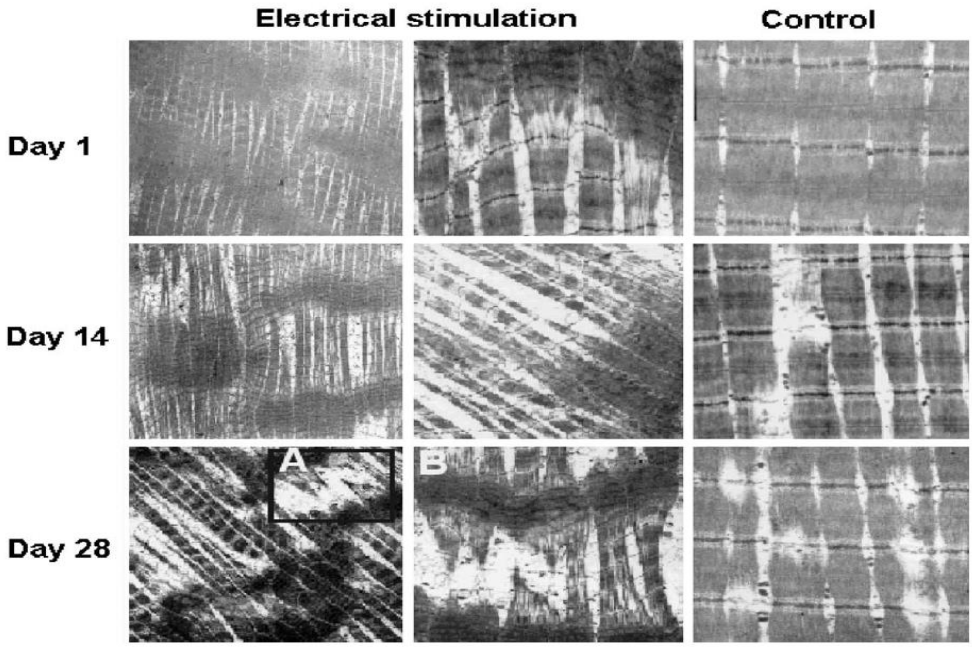


Figure 2.13 Characteristics of contracture bands in electrically stimulated beef *m. longissimus* compared with non-electrically stimulated controls and changes during ageing

N.B: Box "B" of Day 28 is a higher magnification of square "A" of Day 28 (Hwang and Thompson, 2002)

2.7.5 Effects of electrical stimulation on myofibrillar length

Myofibril fragment length (MFL) refers to the length of the myofibrillar fragments that remain after a defined homogenization procedure. The value denotes the degree of proteolytic activities that have taken place over the ageing period (Devine, Wahlgren & Tornberg, 1999). It is widely accepted that degradation of myofibrillar proteins which includes contractile elements, actin and myosin and cytoskeletal proteins (Titin, nebulin, desmin, and troponin-T) during ageing, plays a major role during tenderization (Ho, Stromer & Robson, 1996; Koohmaraie, Seideman, Schollmeyer, Dutson & Crouse, 1987). These proteins and their degradation represent a major structural alteration during meat ageing (Wang, McClure & Tu, 1979; Koohmaraie, Whipple, Kretchner, Crouse & Mersmann, 1991). Rhee, Ryu, Imm and Kim (2000), using LVES showed that the combination of rapid pH decline owing to ES with a high temperature early pm, effectively hastened cytoskeletal protein degradation. Hwang et al. (2003) also pointed out that since ES alters pH and temperature interactions, it is only reasonable to expect that proteolysis would be affected. This includes the degradation of myofibrillar protein. A number of studies indicated that ES provided suitable conditions for the activation of calpain enzymes through Ca^{2+}

activation early pm (Hwang et al., 2003; Rhee and Kim, 2001; Kim et al., 2014). According to Geesink et al. (2006), calpain is responsible for most of the proteolysis and tenderization in the first 72 h pm.

Myofibrillar protein degradation has been studied with various techniques, including myofibril fragmentation (Olson, Parrish & Stromer, 1976), 'free' amino acid determination (Field & Chang, 1969), protein solubility measurement (Claeys, Uytterhaegen, Demeyer & DeSmet, 1994), measurement of non-protein nitrogen (Davey & Gilbert, 1966) and gel electrophoresis (Olson, Parrish, Dayton & Goll, 1977; Penny & Ferguson-Pryce, 1979). These different types of measurement have brought about conflicting reports on the effects of proteolysis and ES on myofibrillar proteins. This disparity could give a plausible explanation for ES being reported in some studies as having no effects on protein solubility (Den Hertog-Meischke, Smulders, Van Logtestijin, & Van Knapen, 1997). It was speculated that, the rate of change in the myofibrillar proteins might have been different between death and subsequent measurements, which was obvious in the work of Hopkins and Thompson (2001b) and that of Geesink, Smulders, Van Laack, Van der Volk, Wensing and Breukink (1993) on *m. Longissimus dorsi*. In the past, the most common technique for measuring changes in myofibrillar proteins was electrophoresis which had potential limitations as demonstrated in the work of Ho et al. (1996, 1997). In one set of results, (Ho et al.) found that ES caused faster degradation in proteins such as titin and troponin-T in some muscles. In another trial (1997), they found no such effects. This was speculated to be a technique problem. Sonaiya et al. (1982) found no significant effect of ES on the rate of change in MFI's (mofibrillar index) in three muscles. Geesink et al. (1993), however, pointed out that the decrease in MFI values after rigor in the work of Sonaiya et al. (1982) suggested a problem in technique because the effect of proteolysis becomes more marked as meat ages, which should drive MFI values up (Hwang et al., 2003).

Dutson, Smith, Savell & Carpenter (1980) and Ho, Stromer & Robson (1996) suggested that there was a potentially strong relationship between physical disruption of the myofibrillar complex owing to ES and increase in tenderness. However, it was not clear whether it was the physical disruption per se or whether the physical disruption hastened ageing by other means. Hwang et al. (2003) suggested that it is possible that physical disruption or stretching and tearing could lead to an acceleration of proteolysis as a result of greater exposure of proteolytic substrates in the muscle fibres in addition to the physical effect of muscle tearing. They concluded, however, that there was no direct evidence to support this. When carcasses are subjected to cooling rigor temperature, those fibres at elevated temperatures enter rigor earlier and experience initial faster tenderization

(Grafhuis, Lovatt & Devine, 1992). This shows that tenderness that was measured at the completion of rigor mortis will be substantially different for ES muscles compared with NES muscles. Li et al. (2011) found that ES did not significantly increase myofibrillar protein solubility in response to pm ageing. Toohey et al. (2008) using MVES (300 V, 15 Hz, 0.5 m/s pulse width, 800 mA, applied for 60 s at exsanguination, (while the wool was on) on lamb carcasses partially supported this. Electrical stimulation did not affect the MFI, although there was a higher mean MFI at both 1 and 5 days for ES compared with NES, but after 5 days of ageing, there were significant differences in the ES and NES samples compared with day 1 ageing. This theory was also supported by Sonaiya et al. (1982) and Martin et al. (2006). The work of Strydom, Hope-Jones, Frylinck & Webb (2011) on the effects of ES on Zilmax-treated carcasses, stimulated by HVES (400 V, 30 min pm, after evisceration) revealed that ES did not reduce MFI, but the control sides recorded shorter MFL (more myofibrillar degradation) after ageing (3 & 14 d pm). They indicated less myofibrillar breakdown in the ES samples and this was contrasted by the lower SF (more tender meat) in the ES samples. They suggested other tenderizing mechanisms were involved.

An earlier study by Marsh, Leet & Dickson (1974) revealed that extreme fibre disruption due to ES resulted in an increase in tenderness. Likewise, other studies showed the link between physical disruption of fibre and improved tenderness for HVES (300-500V) (Takahashi, Wang, Lochner, & Marsh 1987; Will, Ownby & Henrickson, 1980) and also for MVES (145-250 V) (Ho et al., 1996; Sorinmade, Cross, Ono & Wergin, 1982). Dranfield (1994) also indicated that there was no doubt that a few breaks in myofibrils which could be caused by ES, could affect meat tenderization significantly.

2.7.6 Effects of electrical stimulation on meat colour attributes

Meat colour is one of the most important qualities of meat, especially at the point of sale. Colour is the most influential attribute, more than any other quality factor, in the customer's decision to buy meat. Consumers use the degree of discoloration of meat as an indication of freshness and wholesomeness (Mancini & Hunt, 2005). For this reason, it is economically important for meat retailers and merchants to ensure that meat colour is desirable and attractive to consumers. An important key element in prolonged meat colour stability is the rate of the formation of metmyoglobin, which begins in a band between oxygenated and deoxygenated layers. As meat ages, the thickness of the band (metmyoglobin) increases. Eventually, it extends all the way to the meat surface, resulting in a brownish colouration on the surface (Channon et al., 2005). Nearly

15% of retail beef in the USA is discounted as a result of surface discoloration. This amounts to an annual revenue loss of US 1 billion dollars (Smith, Belk, Sofos, Tatum & Williams, 2000). Therefore, improvements in achieving a quality colour life potential depend on our knowledge of pre- and post-mortem myoglobin chemistry. The rate at which meat colour progresses from red to brown is thought to be determined by three factors: (i) the rate of oxygen diffusion and consumption, (ii) the rate of auto-oxidation of myoglobin pigments into metmyoglobin, and (iii) the rate of metmyoglobin reductace activity (MRA) (Faustman & Cassens, 1990). The attributes of meat colour that are usually measured instrumentally are L^* (lightness), a^* (redness), and b^* (blue to yellow) (O'Sullivan et al., 2003b; Gregory, 1998). Other measurements of colour are chroma (C) which is derived as: $C = (a^{*2} + b^{*2})^{1/2}$ and hue angle (H), derived as: $H = \tan^{-1} b^*/a^*$ (AMSA, 1991).

An important intrinsic factor that links meat colour to other carcass traits is the muscle oxidative or glycolytic capacity. O'Keeffe and Hood (1982) and Renerre and Labas (1987) showed that the more oxidative muscles such as *semimembranosus* are more prone to rapid discoloration after slicing for display compared with the more glycolytic muscles such as *semitendinosus* owing to higher myoglobin and iron concentration and higher oxygen consumption rate. Increased muscle weight has been demonstrated to increase the expression of type IIX glycolytic myofibres (Greenwood et al., 2006; Wegner et al., 2000). This could influence meat colour by changing its myofibre composition. Calnan, Jacob, Pethick and Gardner (2014) reported that lambs with heavier muscle and heavier carcass weight with higher growth rate produced loins with better reflectance (R630/R580) after three days of simulated display.

Over the years, contrasting reports have described the effects of ES on meat colour and its stability. Some researchers reported favourable effects of ES on meat colour, some recorded no significant effects of ES on meat colour, and some others registered detrimental effects. Electrical stimulation is known to affect meat colour in beef, but the extent of colour change depends on the pH-temperature relationship, ES intensity (Mareko, 2000) and the type of muscle (Ledward, 1985). Ledward (1985) demonstrated that, while a reduction in colour stability of deep muscle, such as *semimembranosus* was caused by ES, surface muscle such as *longissimus dorsi* was unaffected. Currently, there is a dearth of data about the effect of ES after dressing on meat colour and colour stability. Generally, ES is known to produce a paler colour in meat (Hector, Brewgraves, Hassen & Ledward, 1992; Ledward, Dickson, Powell & Shorthose, 1986; Martin et al., 1983; Sleper, Hunt, Kropf, Kastner & Dikeman, 1983). This is attributed to protein

denaturation, myofibrillar lattice shrinkage and altered oxygen consumption (Offer & Trinick, 1983; Swatland, 1993). The rapid pH decline at higher carcass temperature that is common in ES meat results in brighter and redder color (higher L* and a*) in the initial display period pm, but as meat ages, discoloration (higher b* and hue angle) increases (Kim et al., 2014). This was referred to by Kim et al. (2014) as transient color improvement. Some researchers showed that ES treated meat accumulated less MetMb (metmyoglobin) (Renerre, 1984) and displayed a more appealing lighter bright red colour (Eikelenboom, Smulders & Ruderus, 1985; Savell, Smith & Carpenter, 1978; Smith, 1985; Tang & Henrickson, 1980; Unruh, Kastner, Kropf, Dikeman & Hunt, 1986). Studies have also shown that at 24 h pm, ES beef displayed a redder colour (higher 'a' value) compared with NES beef (Eikelenboom *et al*., 1985; Sleper et al., 1983). This effect is attributed to the damage to enzyme systems, which are responsible for oxygen consumption, reduced oxygen consumption rate and as a result, higher concentration of oxymyoglobin on the surface layer of the meat (Ledward, 1992). Some authors indicated that meat ageing reduced the rate of oxygen consumption (Ledward et al., 1986; Madhavi & Carpenter, 1993).

Stiffler et al. (1984) demonstrated that LVES (35 V) applied to the whole carcass during bleeding and HVES (550 V) could produce a brighter, more youthful appearance of colour of lean meat. Li et al. (2011), using LVES (80 V, 253 mA, 5 ms pulse width for 35 s) after dressing (about 30 min pm) on bovine *longissimus* showed that ES produced a brighter red colour at 24 h pm by increasing the oxygenation capacity of myoglobin ($P < 0.01$), which was reduced by post-mortem ageing. This is supported by the finding of Toohey et al. (2008) who established that ES lamb had a higher a^* value at 24 h pm. According to these authors, ES had a significant ($P < 0.05$) effect on L^* and a^* and percentages of myoglobin derivatives (MbO_2 (oxymyoglobin) and deoxyMb deoxymyoglbin)). Electrical stimulation increased L^*, a^*, b^* and MbO_2 but reduced deoxyMb percentage at 24 h pm ($P < 0.05$). However, as the meat aged, the differences disappeared except for L^* at 168 h pm and deoxyMb percentage at 72 h pm. Figure 2.14 shows the effects of ES on post-mortem ageing time on colour attributes and percentages of myoglobin species on bovine *longissimus* muscle.

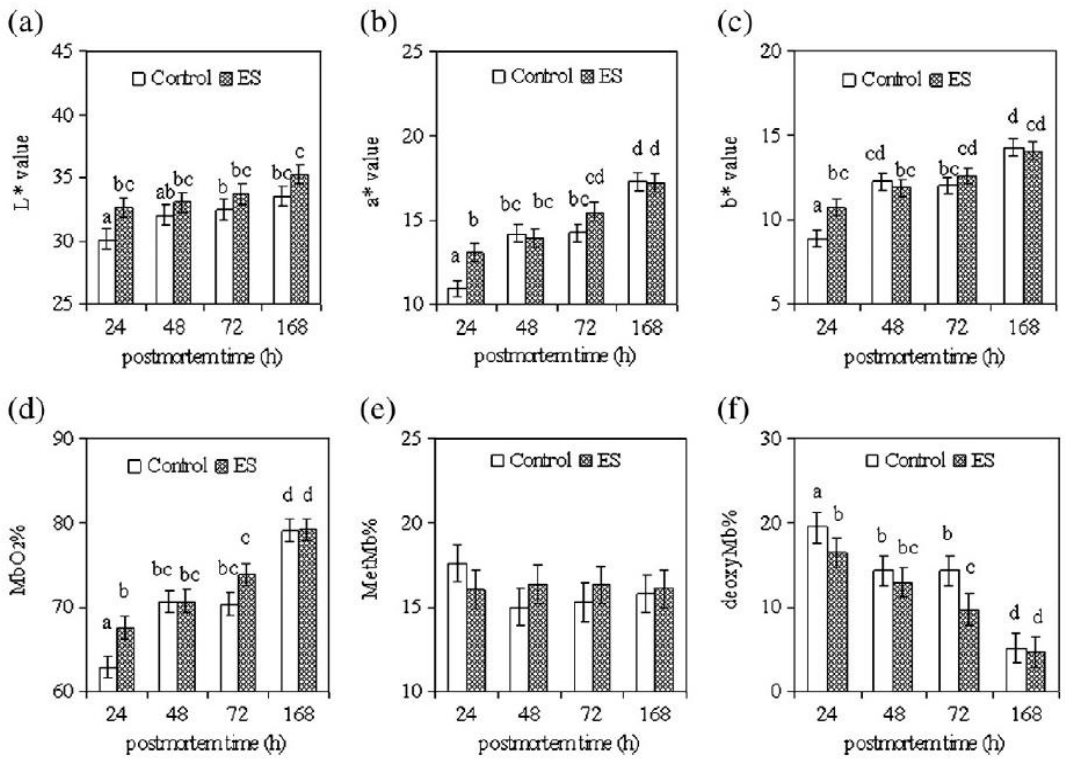


Figure 2.14 Effects of electrical stimulation and post-mortem ageing on colour attributes and percentages of myoglobin species of bovine longissimus (means ± s.e)

Key: (a) L^* value; (b) a^* value; (c) b^* value; (d) MbO_2%; (e) metMb%; and (f) deoxyMb%. [a,b,c,d,] Different letters indicate significant differences ($p < 0.05$) between all subgroups (Li *et al*., 2011)

Color stability, is defined as the rate of metmyoglobin accumulation on the surface of meat (Wiklund et al., 2001). Some researchers postulated that colour and its stability were linked to the inter-conversion of three myoglobin derivatives: deoxymyoglobin (deoxyMb), oxymyoglobin (MbO_2) and metmyoglobin (metMb) which are affected by various ante- and pm conditions (Mancini & Hunt, 2005). Ledward (1992) argued that oxygen consumption rate defined colour stability in the early pm period and that metmyoglobin-reductase activity (MRA) takes over during ageing as the oxygen consumption rate is reduced. The work of Wiklund et al. (2001), on red deer (using LVES- 90 V, 7.5 ms pulse width for 55 s) suggests that ES accelerated the loss of MRA so that, after one week of ageing, metmyoglobin accumulation was increased. Colour stability is generally reduced by ES post-rigor and after ageing (Sleper et al., 1983; Unruh et al., 1986). In contrast to beef, ES did not affect the 'a'*- values of red deer, according to Wiklund et al. (2001), in which colour was measured at one week of ageing. These authors explained that, at one week pm, the reduced oxygen consumption rate attributed to pm storage may have over-shadowed any immediate effects of ES. Moreover, Li et al. (2011) (on bovine longissimus, stimulated 30 min pm) showed that ES muscles had significantly higher ($P < 0.05$) $L^*$and a^* and

decreased (P < 0.05) deoxymyoglobin values early pm (24 h pm) compared with NES controls. After ageing for seven days, there were still significantly higher (P < 0.001) L* and a* (at 72 h pm), while b* values did not show any significant difference at these times in ES meat compared with the NES meat. This confirms that colour stability decreases in ES meat as meat ages, despite the advantages of ES in brighter red colour in the early pm period. Figure 2.15 shows the effects of ES and storage time on colour stability of bovine *longissimus* muscle stimulated after dressing.

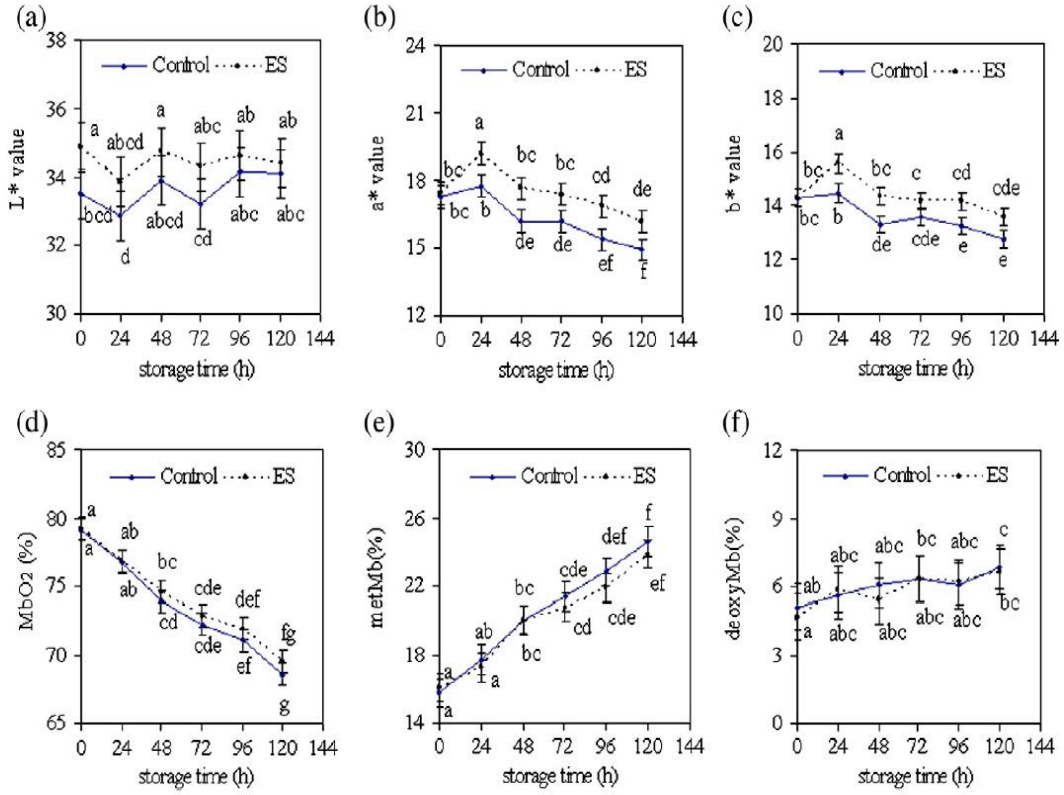


Figure 2.15 Effects of electrical stimulation and storage time on colour stability of bovine longissimus muscle (mean ± s.e.)

Key: (a) L^* value; (b) a^* value; (c) b^* value; (d) MbO_2%; (e) metMb% and (f) deoxyMb%. ^{a,b,cd,} Different letters indicate significant differnces ($p < 0.05$) between all subgroups (Li et al., 2011)

Bekhit (2000) found that ES did not affect metmyoglobin reduction. Sammel *et al*. (2002b) found that traditional chilling of large beef carcass muscle was damaging to colour stability, especially in the round muscles. Sammel et al. suggested that the rapid pH decline especially after ES, coupled with higher meat temperatures in the deep muscle caused protein denaturation and less colour stability. Pearce et al. (2009) showed that modulating frequency using MVES had effect on meat colour, but only in early pm period and not at 60 h pm or later. Higher a^* and b^* values were observed in the loin and topside at 0 h pm compared with 60 h pm in the 10 day-aged samples. A higher reflectance ratio (630/580, nm), early pm, was also observed, which indicated less

metmyoglobin development and hence redder meat colour. This increased ratio could be as a result of faster rate of glycolysis, as indicated by the initial lower pH and temperature at pH6 as meat has a more open muscle structure, and hence, higher tendencies for oxygenation of myoglobin to oxymyoglobin which results in redder meat (Van Laack & Smulders, 1990). The authors, however, stated that the result of increasing the formation of oxymyoglobin was a greater potential for metmyoglobin formation and therefore, a shorter display life. Figure 2.16 shows the visible myoglobin redox inter-conversion on the surface of meat.

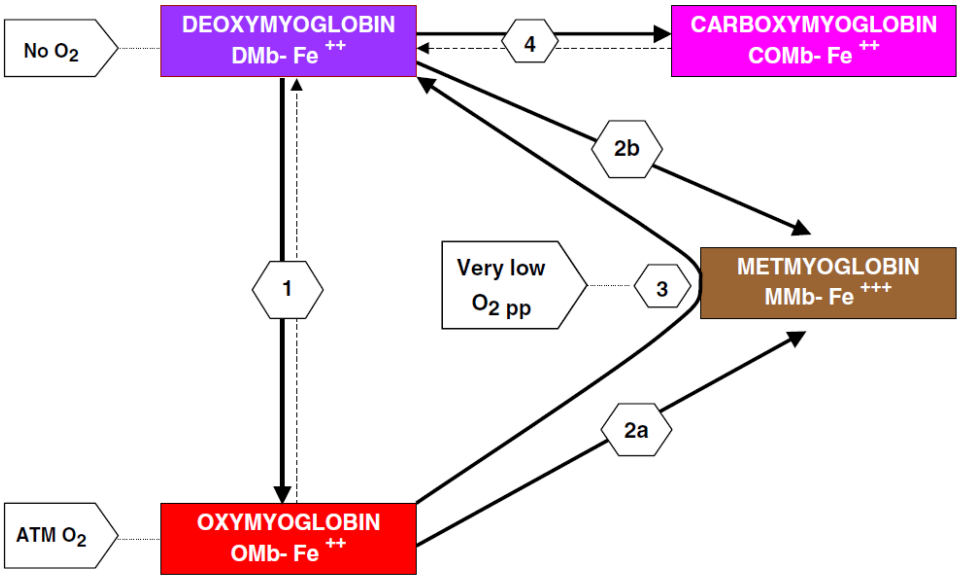


Figure 2.16 Visible myoglobin redox inter-conversions on the surface of meat
(Mancini & Hunt, 2005)

2.7.7 Effects of electrical stimulation on muscles and energy metabolites: lactate, glucose, glycogen, creatine phosphate, adenosine-tri-phosphate, & glucose-6-phosphate

There are three important energy substrates for muscles, namely glycogen, glucose, and fatty acids (Gregory, 1998). Glycogen is known in pm muscle as the primary source of store of energy for the synthesis of ATP (adenosine-tri-phosphate). In cattle and sheep muscles, glycogen concentrations usually range from 75-120 mmol/g (Immonem, Kauffman, Schaefer & Puolanne, 2000; Lambert, Knight, Cosgrove, Anderson, Death & Fisher, 1998). It is known that when pre-slaughter muscle

glycogen reserves fall below the critical threshold level of 45-55 mmol/g, the normal ultimate meat pH (5.5-5.6) would not be attained (Howard, 1963; Tarrant, 1989). There are two forms of glycogen, based on the protein to carbohydrate ratio. They are identifiable based on their solubility in perchloric acid (PCA) and size (Alonso, Lomako, Lomako & Whelan, 1995). The smaller glycogen (up to 400 KDa) known as proglycogen is not soluble in PCA because of its higher protein/carbohydrate ratio. Contrarilly, the "mature" macroglycogen is soluble in PCA by reason of its size (*ca* 10^7 KDa) and is therefore, considered to have smaller protein ratio.

Before slaughter, glycolysis, which is the breakdown of glycogen, draws energy from glucose in the bloodstream but after slaughter, glycolysis is no longer fuelled by glucose derived from blood-stream. Instead, it relies mainly on the glycogen that is stored in the muscle (Gregory, 1998). Also, after slaughter, blood supply to the muscles stops. The muscle's oxygen supply also stops after a while. The electron transport chain ceases to function and ATP re-synthesis continues for a short while from the store of creatine phosphate (CP). At this time, the anaerobic condition causes the TCA (tri-carboxylic-acid) cycle to stop, while glycolysis carries on. After a while, glycolysis is also halted, either through the depletion of substrates (glycogen, glucose and hexophosphates) or the build-up of lactic acid, which inhibits the enzymes in the glycolytic pathway.

The muscle contraction phenomenon is usually initiated by Ca^{2+}, which is released into the sarcoplasm when the muscle is stimulated by a nerve impulse. When the nerve impulse reaches the sarcolemma of the muscle, it goes through the tubules and depolarizes the sarcoplasmic reticulum of the cells. The sarcoplasmic reticulum then releases Ca^{2+} into the sarcoplasm, which then binds to troponin molecule in the actin myofilaments. The bound Ca^{2+} then triggers the side branches of myosin to bind to actin to form actomyosin (Gregory, 1998). The activation of Ca^{2+} is known to be enhanced by ES (Bendall, 1980; Daly et al., 2006). These authors suggested that ES increased the pump activity of the sarcoplasmic reticulum, thereby releasing more Ca^{2+} in the cytoplasm. Ferguson and Gerrard (2014) showed that other intrinsic muscle properties such as pre-stimulation pH and muscle fibre type also influenced the glycolytic response to ES. According to these authors, the change in pH due to ES was contingent on several factors including the amount of voltage, frequency, current and waveform, duration and time of stimulation. Contrasting findings have also been reported on ATPase activity due to ES. Tume (1979) and Ferguson (2003) reported a reduction in sarcoplasmic reticulum Ca^{2+} activity following ES, while Horgan and Kuypers (1985) recorded the opposite. In addition, the findings of Ferguson et al. (2008) showed that the glycolytic response to ES as measured by change in pH was not influenced

by glycogen concentration. Instead, it was positively correlated with pre-stimulation pH. Figure 2.17 illustrates the sliding filament theory of actomyosin formation.

Actin + myosin ⟷ Actomyosin

Myosin ATP_{ase}

ATP ⟷ ADP + P_i

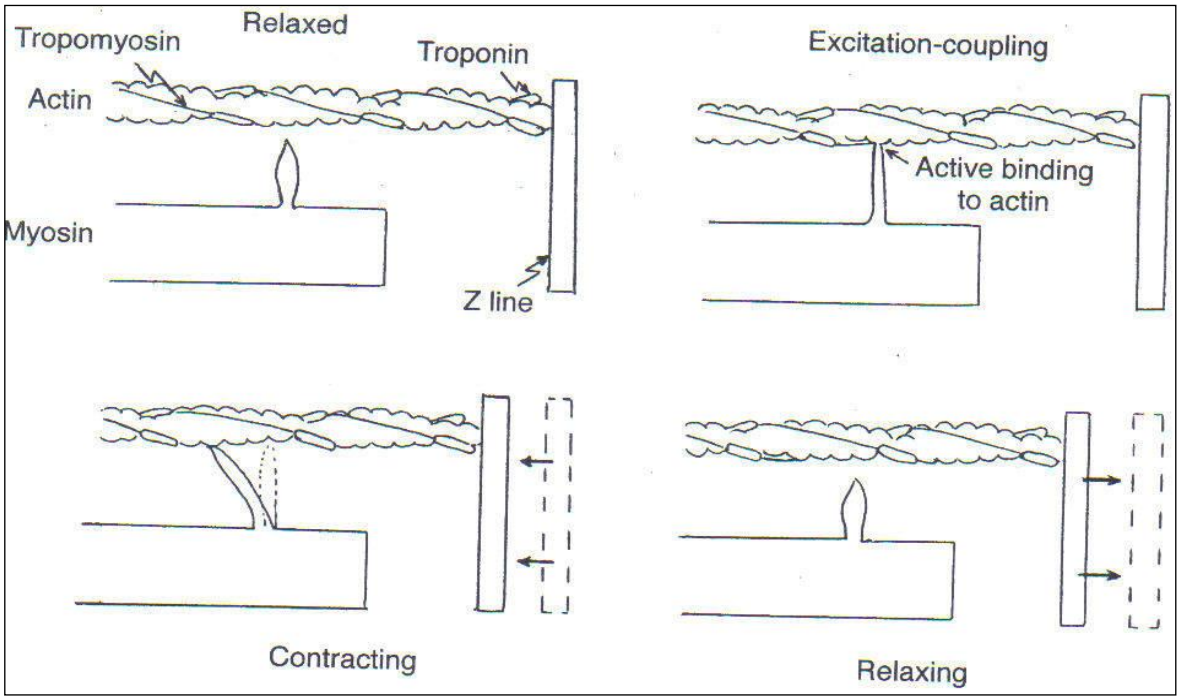


Figure 2.17 Muscle contraction according to the sliding filament theory

(Gregory, 1998)

During the binding process, a side of the myosin filament is exposed, which bears the ATPase enzyme. The enzyme then causes the terminal ATPase to be broken down to ADP + P, with the resultant release of a large amount of energy. With the release of energy, the actin filament slides over the myosin in one direction or the other. If the actin slides towards the Z- line, the myofibres shorten but if it slides away from the Z-line, the myofibres lengthen. This process uses energy in form of ATP. When nerve impulse ceases, Ca^{2+} is unbound from the troponin and passes back to the SR (Gregory, 1998).

In the ante-mortem state, during exercise, the TCA cycle and the glycolytic pathways are activated. These pathways show the breakdown of glycogen and the production of lactic acid through the glycolytic pathway. Figure 2.18 shows the glycolytic pathway.

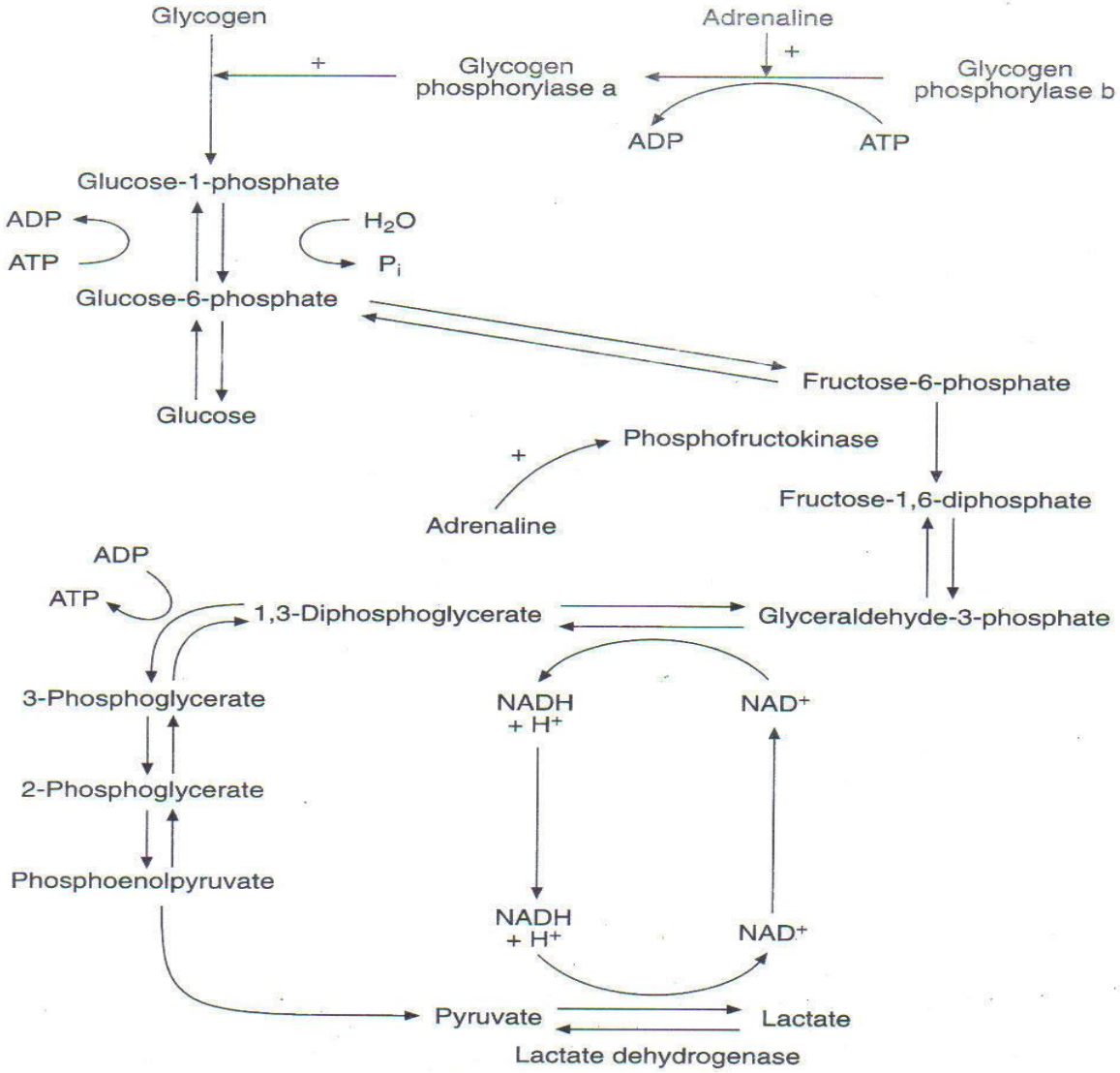


Figure 2.18 Glycolytic pathway

(Gregory, 1998)

2.7.7.1 Types of muscle fibre and their glycolytic abilities

There are three main types of muscles according to their contractile and metabolic characteristics; (i) β-red, that is, slow twitch oxidative, (ii) α- red, that is, fast twitch oxidative, and (iii) α- white, fast twitch glycolytic. The α-fibres contract rapidly and are involved in phasic contraction, which occurs during a burst of activity. The β- fibres contract tonically in a continuous way and are used in posture. The red fibres are fashioned for oxidative metabolism, whereas the white fibres rely more on the glycolytic pathway. The red fibres possess high myoglobin content and a relatively high capillary blood flow. This capillary blood flow helps in maintaining oxygen supply. The red fibres possess the ability to utilise fatty acids more effectively compared with the white fibres, and they store more lipid (Gregory, 1998).

2.7.7.2 Glycolytic potential

Glycolytic potential (GP) is a term that is used to estimate the ability of muscle to generate lactic acid (Hanson & Calkins, 2001). Knowledge of GP helps in identifying meat quality defects such as dark cutting beef (DCB), dark firm dry meat (DFD) and pale soft exudative meat (PSE). Glycolytic potential is calculated as; GP = 2 x (glucose + glycogen + G-6-P) + lactate (Monin & Sellier, 1985; Van Laack & Kauffman, 1999). Each molecule of glycogen generates two molecules of lactic acid. Measuring GP before rigor underestimates glycogen by 10 to 15% according to Hanson & Calkins (2001). Wulf, Emnett, Leheska, & Moeller (2002) related higher GP to increased tenderness and low GP was associated with DFD conditions which resulted in less palatability of cooked steaks. Studies on porcine muscles found relationships among GP, pH 24 h and tenderness (Hamilton et al., 2000; Van Laack et al., 2001). Wulf et al. (2002) revealed a curvilinear relationship between GP and pH 24 h and GP and colour attributes in beef *longissimus dorsi*. These authors also pointed out that there is a variation in GP between unstressed and stressed animals, ante-mortem and post-mortem. Wulf et al. (2002) suggested that genetics, pre-slaughter and environmental factors that increases GP in live cattle might improve meat tenderness. Electrical stimulation is known to speed up the glycolytic rate in muscles thereby allowing the carcasses to be chilled faster to avoid cold shortening (Simmons et al., 2008). Thompson (2002) reported that MSA audit indicated that heavy carcasses require less ES compared with light carcasses. Likewise, grain-fed carcasses require less ES than grass-fed carcasses. Hopkins et al. (2007) and Warner et al. (2014) found that heavier carcasses exhibited faster glycolytic rate and many heavy carcasses missed the ideal MSA pH/temperature window (Thompson, 2002). According to Jacob and Hopkins (2014) and Warner et al. (2014), the common factors that influence the glycolytic rate in heavier carcasses were carcass fatness (which affects the cooling rate), plasma insulin content, carcass size, and number of days on feed at feedlot. The rate of glycolysis in muscle was also affected by the fibre type (Ferguson & Gerrard, 2014). Table 2.1 summarizes the main properties of the three muscle fibre types.

Table 2.1 Main Properties of the three types of muscle fibre

Properties	β-red	α –red	α - white
Colour	red	Red	white
Contraction speed	slow	Fast	fast
Contraction action	tonic	Phasic	phasic
Myoglobin concentration	high	High	low
Capillary density	high	High	low
Fibre diameter	small	small/intermediate	large
Number of mitochondria	high	Intermediate	low
Glycogen storage	low	Intermediate	high

(Gregory, 1998)

The muscles in the carcass have different proportions of the three types of muscle fibres. The postural muscles for example, the trapezius, have a high proportion of beta red fibres, whereas muscles involved in breathing movement, for example cutaneous trunci, and running, for example semitendinosus, have higher proportions of alpha white fibres. Table 2.2 shows the proportion of fibre types in some muscles.

Table 2.2 Proportion of fibre types in some muscles

	β -red	**α –red**	**α – white**
Pig			
Longissimus	10	14	76
Semispinalis capitis	39	20	41
Trapezius	43	34	23
Cattle			

Semitendinosus	8	26	66
Cutaneous trunci	7	28	65
Semimembranosus	13	31	56
Gluteous medius	26	22	52
Longissimus	25	25	50
Triceps longus	22	32	46
Psoas major	52	15	33

(Gregory, 1998)

Predominantly white fibred muscles are better equipped to obtain energy by anaerobic metabolism compared with the red muscles. They store energy in more utilizable form such as CP and ATP, and during post-mortem metabolism, they produce more lactate and lower ultimate pH than red fibres. Predominantly white fibred muscles are more likely to exhibit rapid post-mortem glycolysis compared with muscles with predominantly oxidative type of metabolism. White fibred muscles are also able to exert strong tensions and can contract and relax rapidly while red fibres can maintain a sustained but weaker contraction for a longer time (Gregory, 1998). Generally, the importance of post-mortem muscle metabolism hinges on the following meat quality defects, namely PSE meat, DCB, dark, DFD pork, tough meat, abnormal meat colour and excessive drip loss (Gregory, 1998).

2.7.7.3 Adenosine tri-phosphate resynthesis

There are two main ways in which ATP is resynthesized. The first is from an energy store that is in the form of creatine phosphate (CP), which transfers a high energy phosphate group to ADP to form ATP in a reaction catalysed by the enzyme creatine phosphokinase (CPK), as shown in the following reaction: CP + ADP ↔ ATP + C (Gregory, 1998).

Creatine phosphate (CP) is usually present in large amounts in muscle, and its leakage into the bloodstream in live animals could be a good indicator of damage to muscle membranes or muscle excessive activities. The second way is through the mitochondrial respiratory chain, which involves an electron transport system that is catalysed by NAD-linked dehydrogenases, flavoprotein dehydrogenases and cytochromes. Each time the electron chain is activated, three

molecules of ATP are produced from three molecules of ADP and P_i, and one atom of oxygen. This process requires oxygen (Gregory, 1998).

Electrical stimulation is known to speed up the use of glycogen and accelerates pH decline, thereby producing more lactic acid through glycolysis (Kim et al., 2013; Hwang et al., 2003; Simmons et al., 2008)). The depletion of ATP by ES reduces the chill storage time necessary to deplete ATP. Low voltage ES (28 V, 60 Hz for 60 s, immediately after slaughter on lamb) was found to significantly ($P < 0.05$) reduce the ATP content of stimulated carcasses at 3 and 6 h post-stimulation compared with controls (Polidori et al., 1999).

During rigor onset, depletion of ATP to ADP takes place. ADP is then further depleted to ionosinemonophosphate (IMP) (Savell et al., 2005). ES ensures full conversion of ATP to IMP (Prandl et al., 1994). Dutson, Savell & Smith (1981) emphasized that there was no improvement in meat quality as a result of ES unless it markedly accelerated post-mortem glycolysis. In the study by Polidori et al. (1999) using LVES, ES produced a more rapid glycolysis early post-mortem in both *longissimus* and *semimembranosus* muscles over 24 h pm as seen in the pH and ATP ($\mu mol/g^{-1}$) concentration decline in Tables 2.3 and 2.4 below.

Table 2.3 pH and temperature (mean ± s.e) for *longissimus* and *semimembranosus* muscles in electrically stimulated and control groups

Muscle	Time pm	pH		Temperature	
Longissimus		Stimulated	Control	Stimulated	Control
	45 min	6.29 ± 0.04a	6.67 ± 0.05b	37.8 ± 0.04a	37.5 ± 0.94a
	3 h	5.91 ± 0.03a	6.16 ± 0.04b	15.1 ± 1.1a	17.2 ± 1.1a
	6 h	5.77 ± 0.02a	5.95 ± 0.02b	4.60 ± 0.5a	4.68 ± 0.5a
	10 h	5.68 ± 0.02a	5.80 ± 0.02a	0.90 ± 0.2a	0.11 ± 0.04a
	24 h	5.60 ± 0.01a	5.63 ± 0.01a	0.09 ± 0.01a	0.11 ± 0.04a
Semimembranosus					
	45 min	6.37 ± 0.04a	6.72 ± 0.05b	37.8 ± 0.4a	37.1 ± 0.4a
	3 h	6.01 ± 0.05a	6.22 ± 0.05b	15.2 ± 0.8a	17.0 ± 0.8a
	6 h	5.87 ± 0.03a	6.00 ± 0.03b	5.21 ± 0.5a	5.09 ± 0.5a
	10 h	5.75 ± 0.02a	5.85 ± 0.02a	1.33 ± 0.2a	1.31 ± 0.2a
	24 h	5.66 ± 0.01a	5.69 ± 0.01a	0.16 ± 0.1a	0.22 ± 0.1a

(Polidori et al., 1999)

Table 2.4 shows the shows the values of ATP in longissimus muscle for electrically stimulated and non-electrically stimulated (control) carcasses at various times post-mortem.

Table 2.4 Mean values and standard errors for adenosine-tri-phospate (µmol g^{-1}) in *longissimus* for electrically stimulated and control groups

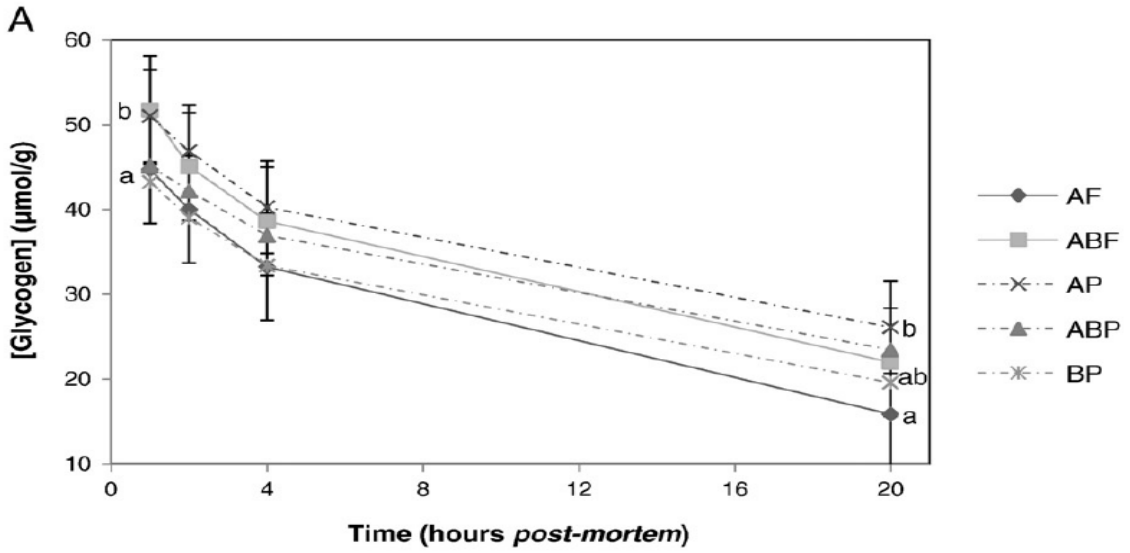
Mean values and standard errors of ATP (µmol g^{-1}) in *Longissimus* muscle[a]

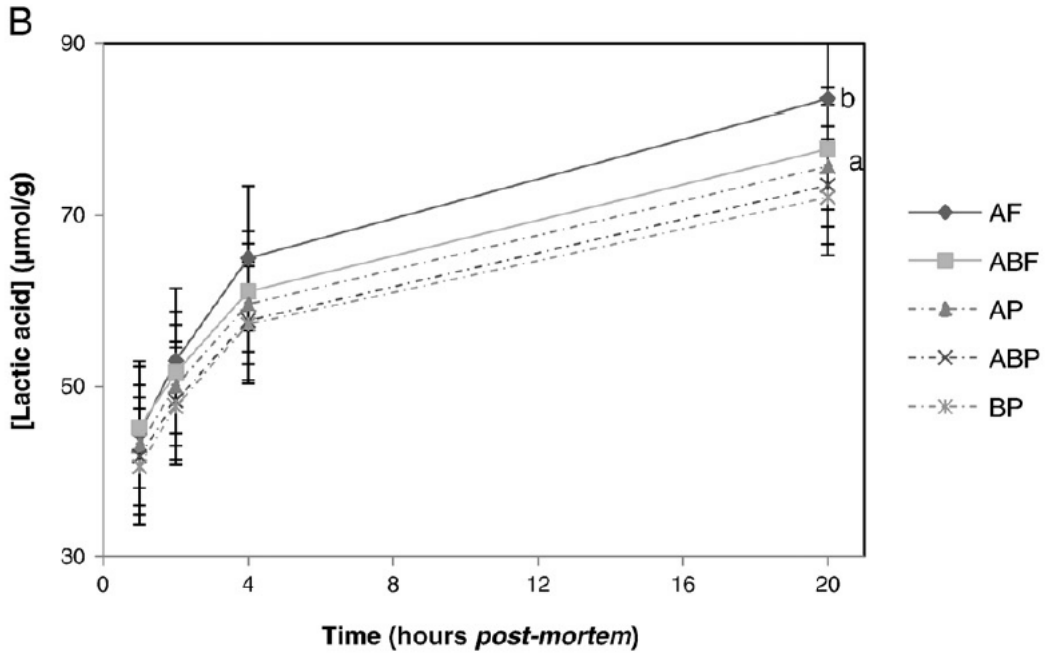
Muscle	Time post mortem	Stimulated	Control
Longissimus	3 h	4.82 ± 0.51a	5.77 ± 0.45b
	6 h	4.04 ± 0.52a	4.90 ± 0.77b
	10 h	3.91 ± 0.56a	4.54 ± 0.66a
	24 h	3.78 ± 0.61a	4.02 ± 0.63a

[a] Same letters in the same row indicate no significant difference ($P < 0.05$); $n = 12$ for treatment and control group.

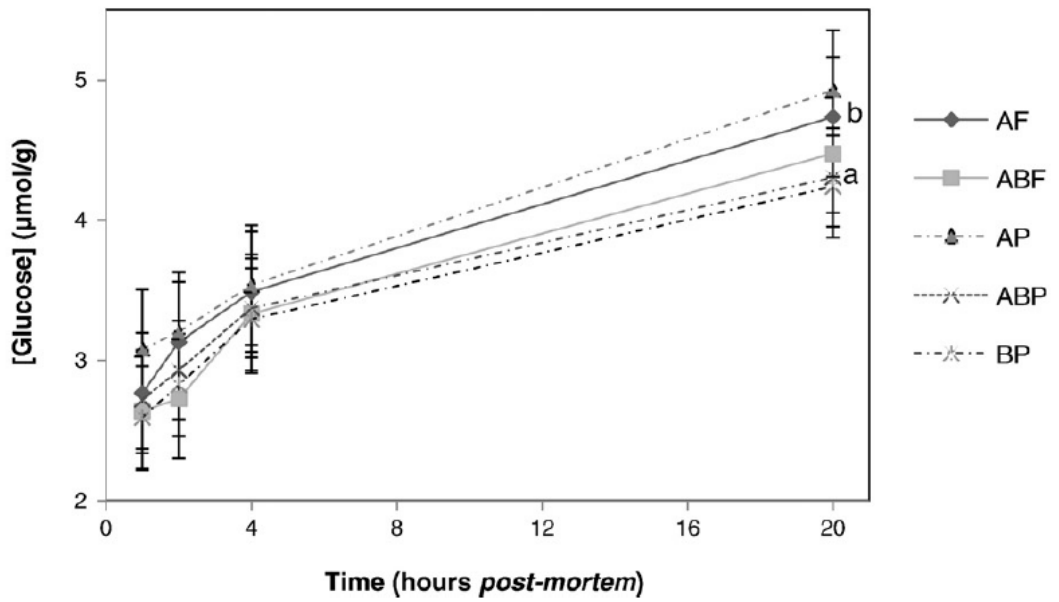
(Polidori et., 1999)

The energy status of muscle immediately post-mortem affects meat tenderness and colour (Monin & Sellier, 1985; Scheffer, Park & Gerrard, 2011). Also, there is a good chance of DFD in beef with a muscle glycogen level below 50 µ mol/g (Immonen, Ruusunen & Puolanne, 2000). Thompson (2002) sets the critical threshold at between 45-57 µmol/g below which the normal ultimate pH of 5.5-5.6 wouldl not be reached (Tarrant, 1989). Frylinck, Strydom, Webb and Du Toit (2013) shows the rate of decline or increase in each of the energy metabolites after ES (400 V for 15 s, 5 ms pulse at 15 pulses/s) applied immediately after slaughter on Brahman, Simental and Nguni crossbreeds of cattle, raised on different production systems. Samples were taken at 1, 2, 4 and 20 h pm. The animals were finished on natural pasture or on grain concentrate at a feedlot. Figure 2.19 (A-G) shows the rate of increase or decrease in each metabolite in the *longissimus* muscle over time.

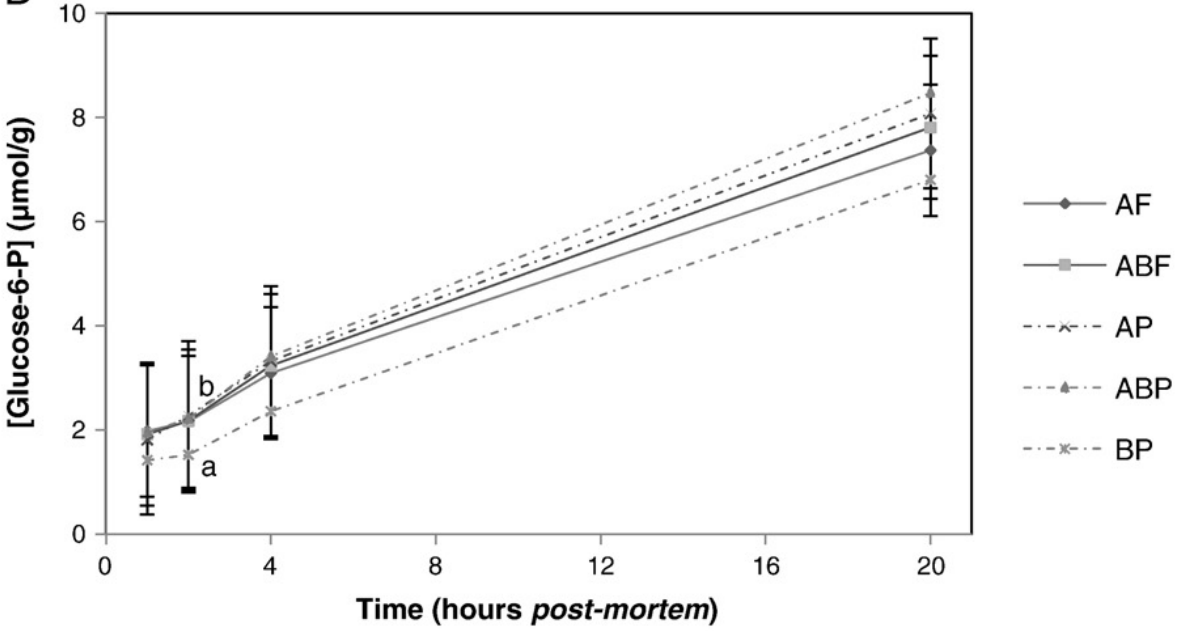

A
60
50
40
30
20
10
[Glycogen] (µmol/g)
b
a
b
ab
a
0
4
8
12
16
20
Time (hours post-mortem)
AF
ABF
AP
ABP
BP


B
90
70
50
30
[Lactic acid] (µmol/g)
b
a
0
4
8
12
16
20
Time (hours post-mortem)
AF
ABF
AP
ABP
BP

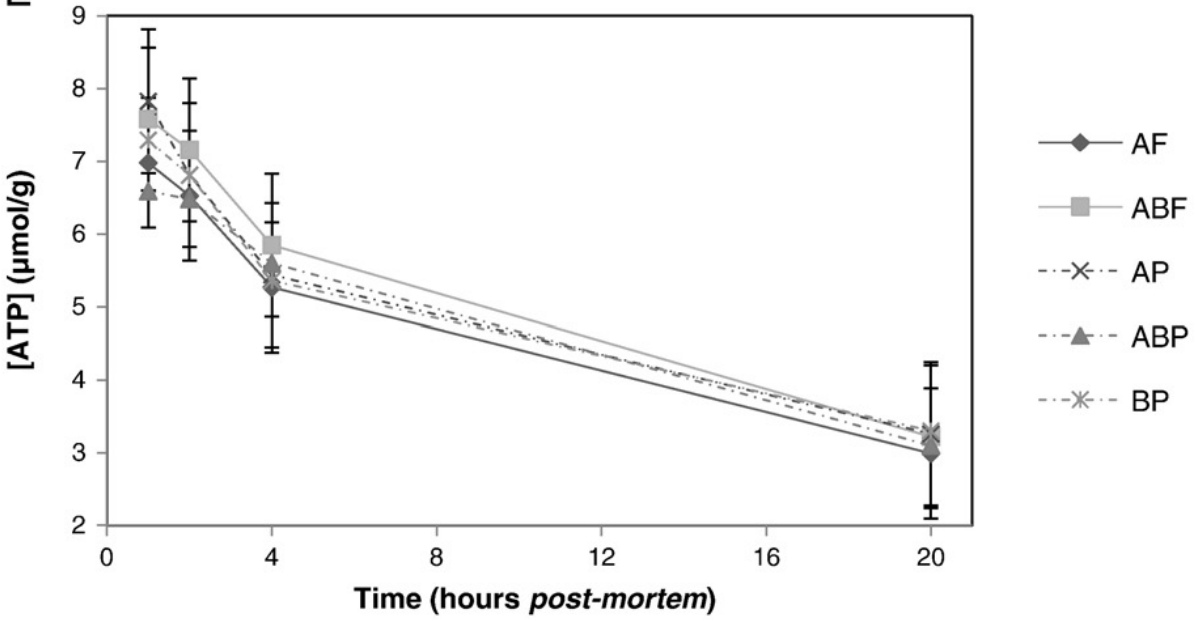
C


[Glucose] (µmol/g)
5
4
3
2
0
4
8
12
16
20
Time (hours post-mortem)
b
a
AF
ABF
AP
ABP
BP

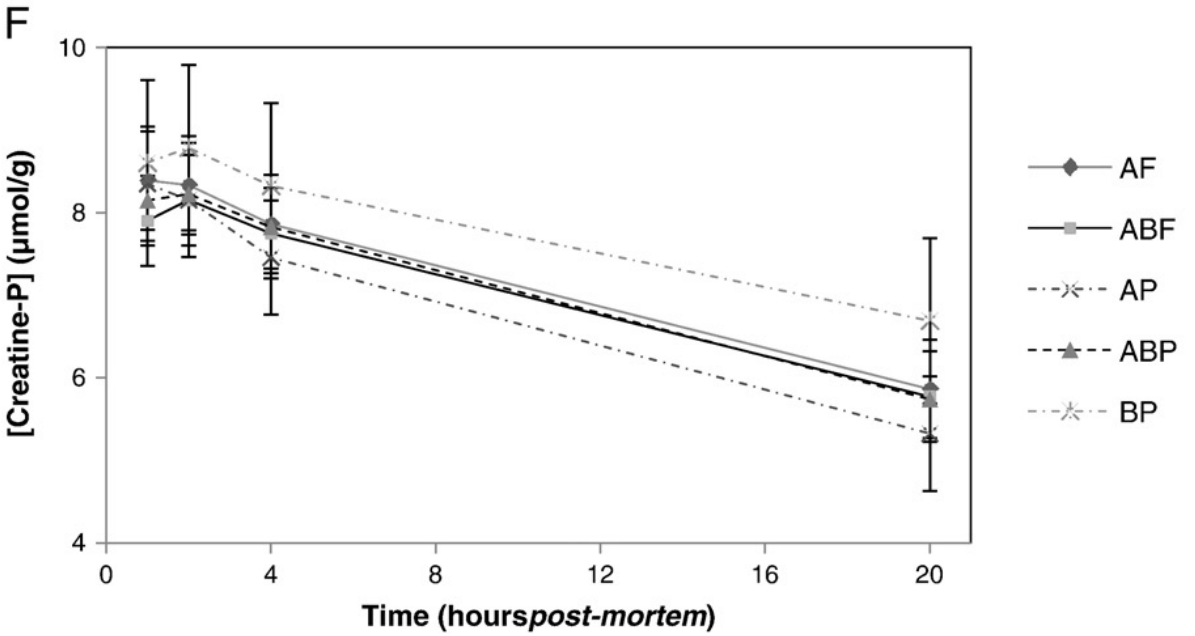
D


[Glucose-6-P] (µmol/g)
10
8
6
4
2
0
0
4
8
12
16
20
Time (hours post-mortem)
b
a
AF
ABF
AP
ABP
BP

Ε


[ATP] (µmol/g)
9
8
7
6
5
4
3
2
0
4
8
12
16
20
Time (hours post-mortem)
AF
ABF
AP
ABP
BP

Τ


[Creatine-P] (µmol/g)
10
8
6
4
0
4
8
12
16
20
Time (hourspost-mortem)
AF
ABF
AP
ABP
BP

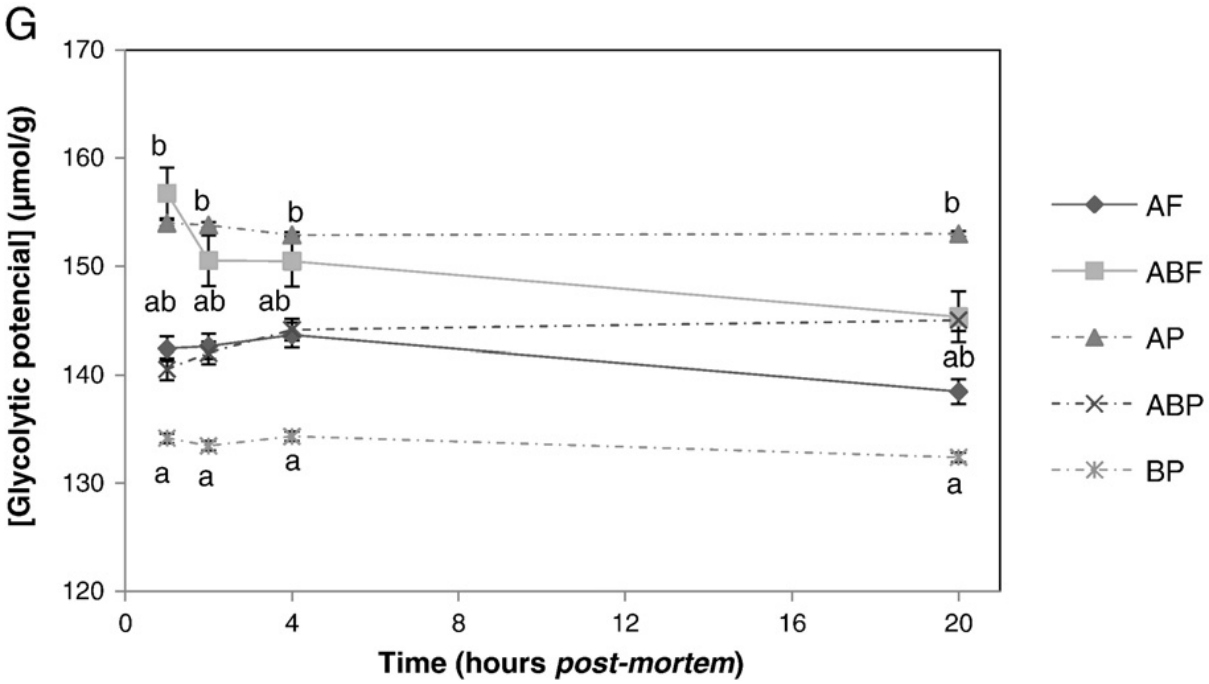


Figure 2.19 Rate of increase/decrease of energy metabolites (A-F) and glycolytic potential (G), over time post-mortem

Key: AF = A age group, grain fed at feedlot; ABF = AB age group, grain fed at feedlot; AP = A age group, pasture finished; ABP = AB age group, pasture finished; BP = B age group, pasture finished (Frylinck et al., 2013)

2.8 Post-mortem proteolysis and proteolytic systems

Proteolysis is the breakdown of proteins into smaller polypeptides or amino acids. It is typically catalysed by cellular enzymes called proteases, which may also occur by intra-molecular digestion. Studies have shown that ES elicits changes (tenderization) in post-mortem muscle through the acceleration of proteolysis which is regarded as a secondary effect that is mediated through time-temperature-pH interaction, which affects enzyme activities and stability (Hwang et al., 2003). There are suggestions that the main determinant of ultimate tenderness is the extent of proteolysis of key target proteins in muscle fibres (Koohmaraie & Geesink, 2006; Taylor, Geesink, Thompson, Koohmaraie & Goll, 1995a). The main proteases that are implicated in the tenderization process are micro-calpain (calpain-1), m-calpain (calpain-2) and calpastatin. There are other groups of enzymes called cathepsins, which consist of exo- and endo-peptidases and are categorized into cysteine, aspartic and serine peptidase families (Sentandreu, Coulis & Ouali, 2002). However, many researchers have discarded the contribution of cathepsins to meat tenderness because of certain observations. The first is that, due to lack of extensive actin and myosin degradation in post-mortem conditioning period considering they are primary substrate for cathepsin (Koohmaraie, Whipple, Kretchmar, Crouse & Mersmann, 1991). Secondly, cathepsins are situated inside lysosomes, and therefore, must be released for them to have access to

myofibrillar proteins to add to meat tenderness (Hopkins & Taylor, 2004). Thirdly, there is little relationship between cathepsins' activities and variation in tenderness in meat samples (Whipple et al., 1990). However, more recently, serine peptidase inhibitor has been found to be a good predictor of meat toughness (Zamora et al., 2005), but calpastatin, which is the calpain proteolytic enzyme inhibitor has been discovered to give a better predictive value of meat quality than enzymes that are directly involved in proteolysis (calpain enzymes) (Kemp, Sensky, Bardsley, Buttery & Parr, 2010). Figure 2.20 shows the muscle myofibrillar proteins in the sarcomere.

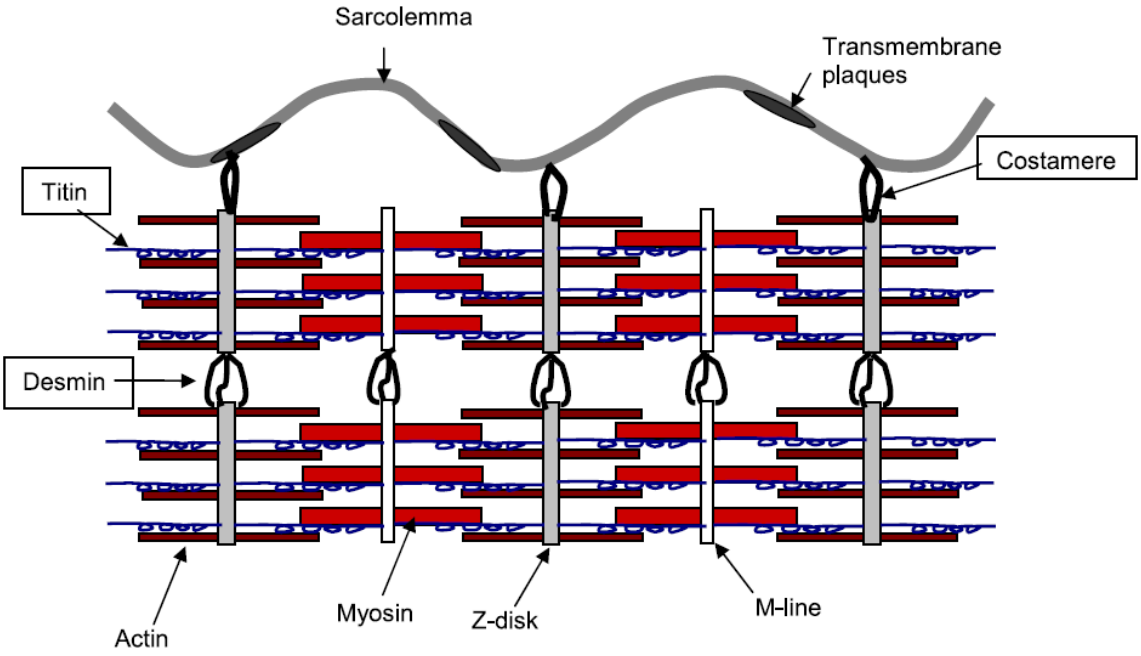


Figure 2.20 Schematic representation of muscle myofibrillar proteins indicating the major component of sarcomere

(Taylor et al., 1995a)

For a protease system to be regarded as being involved in post-mortem proteolysis and tenderization, it must meet certain basic criteria: (i) it must be endogenous to skeletal muscle cells; (ii) it must be able to mimic pm changes in myofibrils in *vitro* under optimum conditions; and (iii) it must have access to myofibrils in tissue (Koohmaraie, 1988). The ultimate tenderization of meat depends on the degree of alteration of muscle structural and related proteins (Hopkins & Taylor, 2004). The myofibril, myofibrillar cytoskeleton and costamere proteins, such as titin, desmin and vinculin respectively, are subjected to cleavage, with cleavage of the main myofibrillar proteins such as actin and myosin (Goll, Thompson, Taylor & Christansen, 1992; Hopkins & Thompson, 2002; Koohmaraie & Geesink, 2006; Taylor, et al., 1995a). There is evidence that ES per se does not lead to increased release of 'free' calcium ions into the cytosol after stimulation (Hopkins & Thompson, 2001b). However, stimulation accelerates pH decline, which causes an increase in

'free' calcium, which also suggest that, at the same temperature, stimulated muscle would be exposed to an increased level of 'free' calcium ion which could cause an increase in proteolysis. Table 2.5 shows the estimate of Ca^{2+} concentration needed for proteolytic enzymes activation.

Table 2.5 Estimated amount of calcium ion concentration required for activation, autolysis and interaction with calpastatin

Calpain property	Autolysed μ-calpain	μ-calpain	Autolysed m-calpain	m-calpain
Proteolytic activity	05-2	3-50	50-150	400-800
Calpastatin binding	0.042	40	25	250-500
Autolysis - phospholipids		50-150		550-800
Autolysis + phospholipids		0.8-50		90-400

(Goll et al., 2003)

2.8.1 The calpain system

Calpains are the most extensively researched enzymes in the protease family and the contribution of calpain activity to meat tenderization is widely accepted (Koohmaraie & Geesink, 2006). Studies have shown that the calpain system is responsible for most of the proteolytic and tenderization processes in the first 72 h pm (Geesink, Kuchay, Chishti & Koohmaraie, 2006; Koohmaraie, 1996). Studies have also shown that in cattle, the identification of a single nucleotide polymorphism (SNP) in the CAPN1 gene, encoding μ- calpain, which is associated with tender meat, strengthened the importance of the role of μ- calpain in tenderization. Calpains are a large family of intracellular cysteine peoteases. So far, 14 members have been identified, which are expressed in a tissue-specific manner (Goll, Thompson, Li, Wei & Cong, 2003). The calpain system in skeletal muscle consists of three proteases that are ubiquitously expressed isoforms of calpain-1, calpain-2, and p94 (calpain-3). Calpains 1 and 2 are calcium activated and requires micro- and millimollar concentrations of Ca^{2+} for activation respectively. Calpain-1 is known to degrade key myofibrillar proteins, including nebulin, titin, troponin-T and desmin (Huff-Lonergan et al., 1996). Calpain-2 is known to persist longer than the calpain-1 in aging muscles (Sensky, Parr, Bardsley, & Buttery, 1996). This suggests that it is not activated early post-mortem and it is also suggested that the Ca^{2+} concentration that exist in muscle post-mortem are less than that required of calpain-2 for activation (Boehm, Kendall, Thompson & Goll, 1998). Studies have suggested that calpain-3 does not appear to play major role in pm proteolysis and the associated development of tenderness (Geesink, Taylor & Koohmaraie, 2005). Calpain-3 is not known to be inhibited by calpastatin (Ono et al., 2004) either. Calpastatin, which is also associated with the proteolytic enzyme family, serves as a calpain-specific endogenous inhibitor (Wendt, Thompson & Goll, 2004).

A number of studies have shown that calpain-1 levels were constant or increased after ES treatment (Ducastaing, Valin, Schollmeyer & Cross, 1985; Uytterhaegan et al., 1992). Ducastaing et al. (1985) revealed that ES resulted in the early depletion in calpain-1 activity which was associated with increased meat tenderness. On the other hand, the combination of high carcass temperature and low pH, results in early exhaustion of calpain-1 activity and as a result, reduced tenderization during extended aging (Dransfield, Etherington & Tayloy, 1992; Simmons, Singh, Dobbie & Devine, 1996). This study is also in accord with the work of Unruh, Kastner, Kropf, Dikeman & Hunt (1986), where stimulated meat was tougher than un-stimulated meat. The autolysis of calpain-1 which reduces the Ca^{2+} requirement for calpain activity (Li, Thompson & Goll, 2004) and also associated with faster pH decline is considered the peak for activation of calpain-1 in pm muscles. Rowe, Maddock, Lonergan & Huff-Lonergan (2004) and Melody et al. (2004) also attested that the accelerated pH decline in *longissimus dorsi* due to ES was linked to earlier autolysis of calpain-1, which resulted in more rapid tenderization. Dransfield (1994) suggested, based on his modelling of pm calpain activity, that calpain activity in rapidly glycolysing muscle would be increased by a factor of 6 compared with muscle with normal glycolytic rate (unstimilated). However, calpain-1 is likely to undergo autolysis under this condition and that is why temperature and free calcium also play important roles.

Hwang and Thompson (2001) showed that acceleration of post-mortem proteolysis in ES meat is due to the increased proteolytic activity of calpain-1 and -2. Electrical stimulation also increases the concentration of intercellular Ca^{2+} (Westerblad & Allen, 1991), which is required by calpains for proteolytic activity. Electrical stimulation accelerates pH decline which also increases calpain proteolytic activity (Dransfield, 1993; Geesink & Koohmaraie, 1999; Hollung et al., 2007; Koohmaraie & Geesink, 2006). Hollung et al. (2007) suggested that ES causes the release of lysosomal proteases such as cathepsin into the cytosol. On their release, these lysosomal proteases can readily hydrolyse several of the myofibrillar and cytoskeletal proteins that are found in muscle thereby accelerating proteolysis (Bechet, Tassa, Taillandier, Combaret & Attaix, 2005). Hwang & Thomson (2001) also showed that calpain-1 and calpastatin activities decreased by 30% and 15% respectively pre- and post-stimulation. The same authors also showed that HVES resulted in significantly lower calpastatin level ($P < 0.05$) and there was tendency for lower levels of calpain-1 after stimulation ($P = 0.11$) than for LVES.

Hwang and Thompson (2001) showed that the time of ES did not affect calpain-1 levels after stimulation. In the same trial, calpastatin levels post-mortem, were affected by the time of stimulation. Electrical stimulation at 3 min pm resulted in a significantly higher level of

calpastatin compared with stimulation at 40 min (P < 0.05) whereas HVES at 40 min & 60 min pm had similar levels of calpain-1 and calpastatin pre- and post-stimulation. In the same trial, at 24 h pm, all stimulation treatments resulted in similar levels of calpastatin, which were significantly lower, compared with the control (NES) (P < 0.05). The control (NES) sides also showed a trend for a higher residual level of calpain-1 compared with LVES at 40 min pm (P < 0.05). Table 2.6 shows the predicted means and *f* ratios for the levels of extractable calpain-1 and calpastatin (unit/ g tissue) pre- and post-stimulation.

Table 2.6 Predicted means and *f* ratio for the levels of extractable calpain-1 and calpastatin (units/gram of tissue) pre- and post- stimulation, and at 24 h pm for comparisons 1, 2, and 3

	Simulation treatment[a]					Average S.E.	d.f.[b]	*F* ratio		
								Type[c]	Time[d]	Type×Time
Comparison 1		HV3	LV3	HV40	LV40					
μ-Calpain	Pre-stim	–	–	1.55	1.64	0.17	1/15	0.11	–	–
	Post-stim	0.85	1.21	0.98	1.18	0.17	1/31	2.6[f]	0.10	0.18
	24 h[e]	0.09a	0.38b	0.29b	0.15b	0.09	1/31	0.7	0.01	5.66*
Calpastatin	Pre-stim	–	–	3.33	3.86	0.23	1/15	2.52	–	–
	Post-stim	3.14	3.92	2.76	3.35	0.18	1/31	14.84***	7.01*	0.28
	24 h	1.26	1.82	1.36	1.43	0.22	1/31	2.07	0.44	1.23
Comparison 2		HV40		HV60						
μ-Calpain	Pre-stim	1.58		1.77		0.24	1/15	–	0.30	–
	Post-stim	0.99		1.00		0.20	1/15	–	0.00	–
	24 h	0.27		0.28		0.09	1/15	–	0.00	–
Calpastatin	Pre-stim	3.28		3.40		0.23	1/15	–	0.15	–
	Post-stim	2.78		3.10		0.24	1/15	–	0.94	–
	24 h	1.38		1.74		0.27	1/15	–	0.89	–
Comparison 3		LV40		Cont						
μ-Calpain	Pre-stim	1.66		–		0.13	–	–	–	–
	Post-stim	1.18		–		0.22	–	–	–	–
	24 h	0.14		0.40		0.09	1/15	–	4.0[g]	–
Calpastatin	Pre-stim	3.87		–		0.23	–	–	–	–
	Post-stim	3.27		–		0.23	–	–	–	–
	24 h	1.39		2.24		0.17	1/15	–	13.34**	–

[a] HV3, HV40 and HV60, high voltage ES applied at 3, 40, and 60 min pm, respectively; LV3 and LV40; LVES applied at 3 and 40 minutes pm respectively; Cont = unstimulated control sides; pre-stim (pre-stimulation); post-stim (post-stimulation).

[b] Degree of freedom (numerator/denominator), [c] Type of stimulation, [d] Time of stimulation,

[e] Means within rows bearing different letters differ significantly ($p < 0.05$), [f] $p = 0.11$, [g] $p = 0.06$,

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (Hwang & Thompson, 2001)

Hwang and Thompson (2001) noted that ES generally decreased the amount of extractable calpain-1 and calpastatin in the muscle at predicted pH post stimulation of *ca* 6.3. This is in

contrast with Dransfield et al. (1992) who showed a threshold effect of pH on protease activity, that at pH greater than 6.1, the activities of μ-calpain and calpastatin were static, while at lower pH, there was an accelerated decrease in both μ-calpain and calpastatin activities. This finding is in contrast with the results of Ducastaing et al. (1985) and Morton et al. (1999) in which the authors suggested that ES induced a temporary increase in calpain-1 and calpastatin activities. The same authors also suggested that the increase in calcium ion concentration owing to ES could have been sufficient to initiate autolysis and / or proteolysis of calpain-1 and calpastatin and as a result, lead to reduced level of extractable calpain-1 and calpastatin post-stimulation. The authors suggested that the application of HVES (800 V) possibly caused greater acceleration of autolysis/proteolytic activity of calpain-1 and calpastatin during stimulation compared with LVES. Regarding calpain-2, the authors suggested that the static level of calpain-2 suggest that the amount of calcium released during stimulation was not sufficient to activate calpain-2. In the same trial, calpastatin levels were higher post stimulation for carcasses stimulated at 3 min pm compared with 40 min pm and this suggests that the extractable level of calpastatin was affected by the time of stimulation, in that ES at 3 min pm resulted in less available substrate (e.g. calpain) for calpastatin to be deactivated (Dransfield, 1993).

Hwang and Thompson (2001) revealed that, at 24 h pm, HVES at 3 min pm resulted in lower amount of calpain-1 compared with other treatments (HVES 40 min, LVES 3 min, LVES 40 min pm). Although, calpastatin levels were similar, the authors reckoned it was due to faster pH decline and higher carcass temperature. During rigor development, the HVES at 3 min pm displayed a marginally faster pH decline and higher carcass temperature compared with LVES at 3 min pm. The authors suggested that the residual calpain-1 levels at 24 h pm might be affected by even small differences in the rate of pH-temperature decline for fast glycolysing muscle. Also, at 24 h pm, HVES at 40 min pm resulted in a significantly faster pH decline compared with HVES at 60 min pm. The authors suggested that there could be a threshold effect, meaning that if ES was not applied very soon after slaughter, leading to a rapid pH decline, there could be little effect on calpain-1 and calpastatin levels. Table 2.7 shows the proteolytic enzyme activity at different time pm for beef and springbok.

Table 2.7 Proteolytic enzyme activity at different time post-mortem for beef and springbok

Beef	Hours post-mortem	
Longissimus	1	24
Calpastatin	2.329[a]	2.022[b]
	(0.047[a])	(0.040[b])
Calpain-I	1.757[a]	1.497[b]
	(0.035[a])	(0.030[b)]
Calpain-II	1.129	1.152
	(0.023)	(0.023)
Calpastatin/Calpain-I	1.373[a]	1.427[b]
Calpastatin/Calpain-I + Calpain-II	0.819[a]	0.764[b]

Springbok	Hours post-mortem		
Longissimus	2	18	30
Calpastatin	1.434[a]	1.365[ab]	1.198[b]
	(0.023[a])	(0.022[ab])	(0.019[b])
Calpain-I	1.116[a]	0.933[b]	0.754[c]
	(0.018[a])	(0.015[b])	(0.012[c])
Calpain-II	0.966	0.977	0.935
	(0.015)	(0.015)	(0.015)
Calpastatin/Calpain-I	1.345[a]	1.727[b]	2.263[c]
Calpastatin/Calpain-I + Calpain-II	0.689[a]	0.726[b]	0.737[b]

[1]One unit of calpastatin activity was defined as the amount that inhibited one unit of m- calpain activity divided by two. [2]One unit of calpain activity is defined as an increase in absorbance of 1.0 at 366nm per hour at 25^0C. [3]Values in brackets denotes specific activities. [a,b,c,d] Means in the same row with different superscripts differ significantly. (Anderson et al., 2015)

2.8.2 Calpastatin and its role in meat toughness

Calpastatin inhibits both calpain-1 and -2 and the process requires ca^{2+} concentration close to or below those that are required to activate calpain (Goll et al., 2003). Calpastatin is also susceptible to proteolysis but the resultant fragment carries inhibitory activity. This was shown by a

chromatography-based observation that identified the nature of the interaction of calpastatin with calpain, which showed a protein inhibitor interacting with proteolytic enzyme (Hanna, Campbell & Davies, 2008). Calpastatin is known to be an unstructured protein but when bound to calpain, it adopts a structure that allows inhibition to take place. It possesses four inhibitory domains, each of which can inhibit calpain activity. When calcium binds to calpain, it causes a change in the calpain molecule, thereby enabling it to become active and simultaneously allowing calpastatin to interact with the enzyme. This experiment also showed that calpastatin requires calcium in order to interact with calpain (Goll et al., 2003).

A number of studies have shown that high levels of calpastatin are related to poor quality meat. This is based on the theory that high levels of calpastatin reduce the activity of calpain, thereby reducing the proteolysis required for tender meat. Studies in ruminant species have shown that there is a relationship between calpastatin activity at 24 h pm and the degree of tenderization achieved after conditioning, with differences in calpastatin activity accounting for 40% of the variation in tenderness (Shackelford et al., 1994). Studies on commercially slaughtered pigs also showed that a high level of calpastatin in the first few hours pm was associated with increased incidence of toughness (Parr et al., 1999b; Sensky et al., 1998). A number of researchers identified calpastatin polymorphisms and have demonstrated that some of these could be used to predict carcass quality in cattle and pigs (Barendse, 2002; Ciobanu et al., 2004). Currently, there are markers within the calpastatin and calpain-1 genes that are able to identify beef cattle with genetic potential to produce tender meat (Casas et al., 2006). Apart from the effect of proteolysis in the mediation of the calpain system, variability in calpastatin and its effect on the calpain system could be mediated by environmental factors such as elevated plasma adrenaline, which increases calpastatin activity and expression in pigs. This implies that the link between stress and meat toughness may be partially mediated through the calpain system (Parr et al., 2000, Sensky et al., 1996). Figure 2.21 shows the relationship between calpastatin activity and shear force in porcine *longissimus dorsi.*

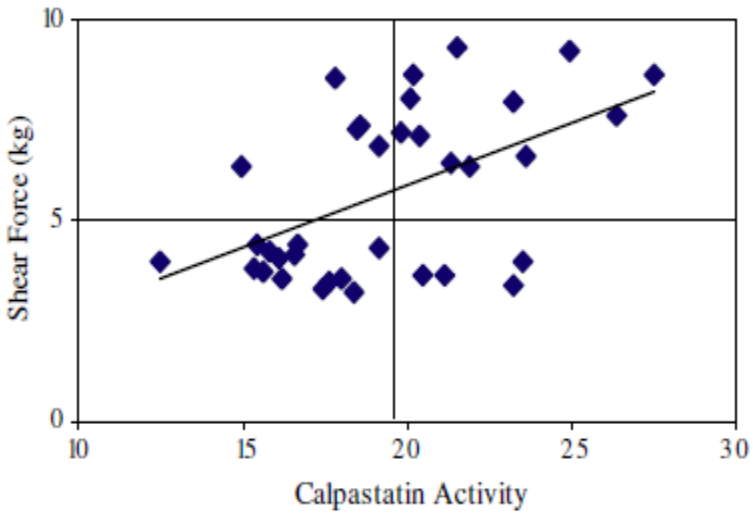


Figure 2.21 Relationship between slaughter (2 hours) calpastatin activity and 8 day shear force in porcine *longissimus dorsi*

(Kemp et al., 2010)

2.8.3 Effect of carcass weight and high rigor temperature on proteolytic activity

One of the adverse effects of high temperature rigor caused by increased glycolysis and faster pH decline at high carcass temperature because of the reduced cooling rate (owing to heavy carcass weight) (Hopkins et al., 2007; Jacob & Hopkins, 2014)), is heat-induced toughening (Rosenvold et al., 2008). The other implication of this phenomenon is reduced ageing potential owing to early exhaustion of proteolytic enzyme activities or elevated protein denaturation, which also results in reduced tenderness. However, there is increased tenderness early pm in high rigor temperature meat due to early activation of the calpain system, but as ageing progressed, there was reduced tenderization (Kim et al., 2014). According to Devine et al. (1999), meat tenderizes better when carcasses enter rigor at 15 °C than at 35-38 °C. It is generally agreed that the early activation of micro-calpain by elevated free calcium ions at high carcass temperature results in faster myofibrillar protein degradation (Hwang et al., 2004). However, there is early exhaustion and autolysis of proteolytic enzymes, which results in reduced proteolysis with extended ageing (Kim et al., 2013; Warner et al., 2014b).

2.9 Zilpaterol hydrochloride and its effects on carcass and meat quality

The use of a class of feed additives called beta-agonists (Beerman, 2004) is a management strategy that was developed recently to increase and improve growth efficiency, and the amount of meat yield and carcass composition. These compounds have been studied intensively and some have been approved for use in meat-producing animals. Beta-agonists are naturally occurring or

synthetic in nature. They share a common base chemical structure with a class of compound called phenethanolamines. The general structure of this compound is shown in the Figure 2.22 and 2.23. Figure 2.22 consists of a phenyl group ring structure attached to the ethanolamine group. The attachment at A, B, C and R positions, denotes or indicate the differences among the family of phenylethanolamine compounds, called beta-agonists (Beermann, 2004). These substituents, which are attached at different positions, influence how long the beta-agonists stay in circulation in the blood, which tissues are affected, and the actions they promote. The base chemical structure of phenethanolamine is provided below.

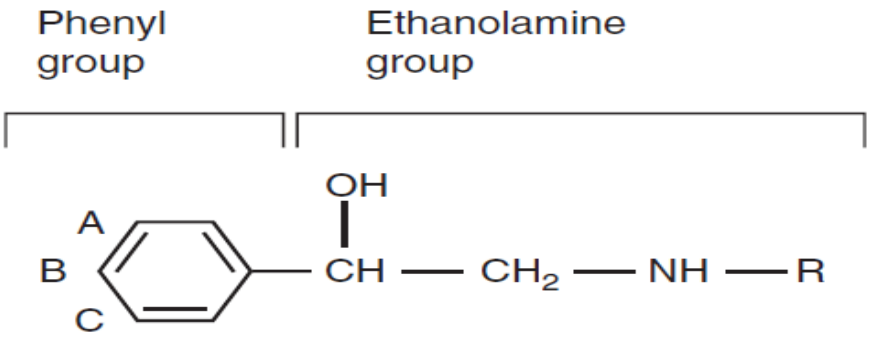


Figure 2.22 Base chemical structure of phenethanolamine

N.B: They all contain the phenyl group coupled with the ethanolamine group, but they differ in the type of substituents that are attached at positions A, B, C and R (Beermann, 2004).

The two known naturally occurring beta-agonists are adrenaline (epinephrine) and noradrenaline (norepinephrine), but benefits animal growth. They are also called catecholamines and play important roles in regulating heart rate, blood flow and other physiological and metabolic functions in humans and animals. Synthetic beta-agonist has also been used in humans and animals for decades. The common ones are ractopamine, climaterol, clenbuterol, L-644,969, salbutanol and zilpaterol hydrochloride (Zilmax). Figure 2.23 shows the chemical structures of common beta agonists that have been studied in meat producing animal species.

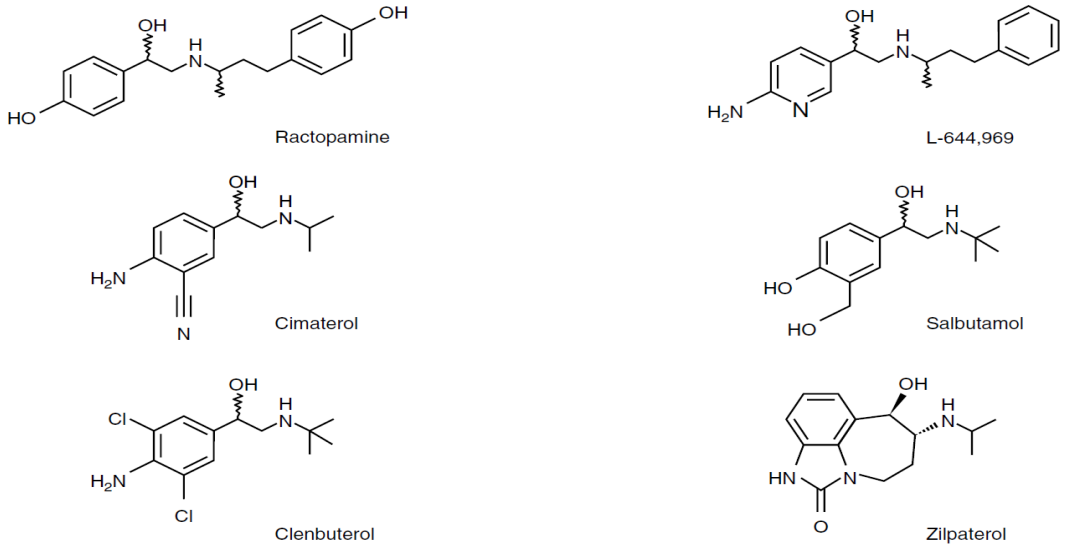


Figure 2.23 Chemical structures of common beta agonists (phenethanolamines) that have been studied in meat animal species (Beermann, 2004)

(Beermann, 2004)

Over the past three decades, these beta-agonists have been extensively evaluated for their potential in enhancing muscle growth and their anti-obesity effects. All were discovered to enhance muscle growth in meat animals, broiler chickens, turkeys, cattle, sheep, goats and laboratory animals, but only ractopamine and zilpaterol were approved for use in meat animals (Beerman, 2004). Ractopamine hydrochloride and zilpaterol hydrochloride are marketed under various names in USA, South Africa, Mexico and many other countries. Zilpaterol, which is manufactured by Hoechst Roussel Vet, is sold commercially as zilmax, and is approved for use in South Africa, Canada, Mexico, Costa Rica, South Korea and Kazakhstan (Codex Alimentarius Commission of Joint FAO/WHO, 2016). None of the beta-agonists is approved for use in the European Union (Dunshea & Gannon, 1995). Zilmax was registered in South Africa by Intervet S.A (Pty Ltd, Reg. No 91/06580/07) in 1997.

Beta-agonists act as repartitioning agent and are administered orally in the feed to increase muscle growth and reduce fat gain (Dunshea & Gannon, 1995). They act directly through beta-adrenergic receptors on adipocytes and influence cellular metabolism through signalling cascades (Dunshea, D'Souza, Pethick, Harper & Warner, 2005). They are usually administered at concentrations of 5-30 ppm (g/tonne of feed) close to the end of the finishing period, between 28 – 42 days prior to harvest. Specific guidelines are provided by the manufacturers for their administration. Beta-agonists are referred to as repartitioning agents because they redirect nutrients used for growth, which increases the rate of muscle protein synthesis (Eisemann, Huntington & Ferrel, 1988; Smith

et al., 1989), leading towards larger muscles and away from deposition of fat in the adipose tissues. That is, they decrease lipogenesis and increase lipolysis (Dunshea, 1993; Liu & Mills, 1989). The magnitude of changes in meat quality is influenced by dose and duration of treatment, type of beta-agonist and species of animals (Beermann, 1993, Mersmann, 1998; Moody, Hanock & Anderson, 2000). They promote muscle leanness and feed efficiency because they are more efficient in growing muscles than fat, thereby reducing feed intake at an equal or greater rate of body weight gain. In summary, beta-agonists are expected to improve higher average daily gain (ADG), feed conversion efficiency, higher dressing percentage, and to lower kidney and channel fat (Dikeman, 2007).

Beta-agonists are known to affect the tenderness of meat and other traits negatively owing to increase in calpastatin activity (Koohmaraie, Shackelford, Muggli-Cockett & Stone, 1991). Beta-agonist such as Zilmax has been linked ro elevated calpastatin activity and mRNA expression, which are known to increase meat toughness (Dunshea et al., 2005). Electrical stimulation is known to improve meat tenderness by early activation of the calpain system (Hwang & Thompson, 2001). However, Hope-Jones, Strydom, Frylinck and Webb, (2010) showed that ES, combined with pm ageing could improve, but not fully overcome the adverse effect of beta-agonists on beef loin tenderness. Other researchers demonstrated the infusion of calcium chloride ($Cacl_2$) in reducing the effects of beta-agonists on tenderness (Goll et al., 1992; Koohmaraie & Shackelford, 1991). Other researchers studied the effect of supplementation of high levels of vitamin D_3 over the final days before slaughter in reducing the induced toughness by beta-agonists (Montgomery et al., 2002; Montgomery et al., 2004). Reports about the duration of supplementation and the magnitude of the effects are not consistent. In one study, dietary zilpaterol (at 6 ppm) did not have any effect on marbling (Plascencia, Torrentera & Zinn, 1999), but dramatically increased instrumental SF (7.7 vs 15.9 kg) in another (Moron-Fuenmayor, Zamorano-Garcia, Ysunza & Gonzalez-Mendez, 2002). The study of Strydom, Osler, Leeuw, & Nel, (1998) also showed that zilpaterol did not have negative effects on meat quality when administered for between 15 and 30 days, whereas, 50 days of administration resulted in lower sensory tenderness and juiciness ratings for the longissimus muscle coupled with a negative effect on SF when fed at 45 mg/day.

Some researchers also demonstrated the effects zilpaterol on beef colour stability. Gunderson et al. (2009a) and VanOverbeke et al. (2009) observed a duration-dependent and packaging specific effect of zilpaterol on beef colour stability. Gunderson et al. (2009) observed that feeding zilpaterol for 20 or 30 days to steers, resulted in steaks with equal or more desirable colour

characteristics than controls when packaged in aerobic or carbon monoxide modified advanced packaging (MAP). VanOverbeke et al. (2009) also examined colour stability of gluteus medius of cattle fed with zilpaterol. They observed that zilpaterol influenced the colour of steaks stored in High Oxygen (HIOX) MAP, but not in aerobic packaging. They reported that after five days of storage, steaks stored under HIOX MAP and fed with zilpaterol for 20 days exhibited greater surface redness compared with animals fed 0, 30, or 40 days. These findings highlight the importance of retail packaging, feeding and animal type when considering the use of zilpaterol as a growth promotant.

Zilmax is usually administered within the last 30 days of fattening and withdrawn at about 48 hours prior to harvest. It is usually administered at the rate of 10-45 ppm and is expected to produce about 15-25% improvement in daily weight gain compared with controls. Carcass yield is also expected to increase by 2-8 kg with less fat and more muscle and protein on a relative basis (Beermann, 2004). A trial by Webb and Morris (2014, unpublished) using Zilmax on feedlot cattle produced 63.1% ($P < 0.001$) dressing percentage compared with 61.9% in the control group. Animals treated with Zilmax gained an average of 10.7 kg more than the controls at slaughter. They also showed improvement in carcass classification. The proportion of A2 category increased to 91% in the Zilmax terated group compared with 76.3% in the control group. The proportion of A3 carcasses also decreased from 22.9% in the control group to 7.6% in the Zilmax treated group. Tenderness was not affected by Zilmax treatment in this trial. Subcutaneous fat was also significantly reduced ($P < 0.025$) in the Zilmax treated group (19.3%) compared with control (21.9%). The authors also showed that the effect of Zilmax on meat tenderness in heavier carcasses was negligible. There was also significant improvement in proportion of primal cuts, namely rumps (11% increase) and sirloins (11% -increase). Carcass compactness was also increased in the Zilmax-treated carcasses compared with controls (Webb & Morris, 2014 unpublished).

Skeletal myocytes express beta-adrenergic receptors that transmits signal from beta-agonists to muscle metabolic enzymes in a dose-dependent manner (Byrem, Beermann & Robinson, 1996). Avendano-Reyes et al. (2006) evaluated the effects of Zilmax on carcass performance traits and meat quality. An amount of 66 mg/steer/day was fed to steers on high grain diet for 33 days before slaughter. Zilmax improved the feed conversion ratio (FCR) ($P < 0.01$) and ADG by 26% compared with control steers. Carcass weight and longissimus areas ($P < 0.05$) were also improved. However, SF was greater ($P < 0.01$) in Zilmax treated meat compared with controls (49.6 N vs 42.6 N). Meat colour was not affected. On the contrary, O'Neill (2001) showed that

there were no differences in tenderness between steers that were treated with Zilmax compared with controls. Table 2.8 shows the least square means and SE for growth performance and carcass characteristics of three β-agonist treatments and the control group.

Table 2.8 Effects of three beta agonists on bovine carcass characteristics

Least square means and standard errors for growth performance and carcass characteristics of the three β-agonist treatments and the control group.

	Control	Zilpaterol	Ractopamine	Clenbuterol	SEM[d]
Starting weight (kg)	504.7	503.2	503.3	506.3	4.06
Final weight (kg)	568.6	575.5	582.7	572.1	6.07
ADG (kg/day)	2.1[a]	2.4[a,b]	2.6[b]	2.2[a]	0.132
FCR (kg/kg – dry matter basis)	6.6[b]	5.4[a]	5.2[a]	5.9[a,b]	0.328
Feed intake (kg DM/day)	13.3[b]	12.5[a,b]	13.2[b]	12.1[a]	0.289
ADG – carcass (kg/day)	1.69[a]	2.19[b]	1.93[a,b]	2.18[b]	0.1033
FCR – carcass (kg/kg)	8.29[c]	5.82[a]	7.01[b]	5.65[a]	0.379
Dry matter digestibility (%)	72.6	74.3	70.9	72.0	1.399
Protein digestibility (%)	71.3	74.0	70.3	73.2	1.374
Final carcass weight (kg)	339.1[a]	353.5[b]	346.1[a,b]	355.0[b]	4.60
Dressing (%)	59.6[a]	61.4[b]	59.4[a]	62.1[b]	0.291
Hide – (%) of carcass weight	12.1[b]	11.5[a,b]	12.5[b]	10.8[a]	0.360
USDA yield grade	3.27[c]	2.52[b]	2.92[b,c]	1.65[a]	0.220
AFT (cm)	1.42[c]	0.91[a,b]	1.27[b,c]	0.74[a]	0.142
REA (cm^2)	75.5[a]	83.9[b]	80.0[a,b]	95.5[c]	2.645
Kidney, pelvic and heart fat (%)	1.38[b]	1.33[b]	1.29[b]	0.83[a]	0.108
Bone maturity score	A[44]	A[50]	A[47]	A[49]	
Marbling scores[e]	220.8[b,c]	196.7[a,b]	234.2[c]	189.2[a]	9.94

ADG, average daily gain; FCR, feed conversion ratio; AFT, adjusted fat thickness; REA, rib eye area.

[a,b,c] Means in the same row without a common superscript differ significantly ($P < 0.05$).

[d] Standard error of means.

[e] Marbling was collected from the 10th rib plane; 100 = Traces[00]; 200 = Slight[00].

(Strydom *et al*., 2009)

CHAPTER 3

MATERIALS AND METHODS

3.1 Location of the trial and environmental conditions

The trial was conducted at Sparta beef abattoir, which is located at Welkom, a major industrial city in the province of Free-State, South Africa. Sparta beef abattoir is regarded as a high throughput abattoir with a slaughter capacity of about a thousand heads of cattle per day. It is regarded as one of the biggest commercial beef abattoirs in South Africa. The abattoir is registered with the Red Meat Abattoir Association (RMAA) of South Africa and International Meat Quality Assurance Services (IMQAS) and is ISO certified (22,000, 14001, 9001 and OHAS 18001). Prior to slaughter, the animals were reared as calves in Marquard cattle farm and later weaned and transferred to a feedlot in the same vicinity as the farm, which also belongs to Sparta Beef. Marquard district is about 125 km from Welkom, where the animals were slaughtered. The trial took place in March 2015, over a period of 5 weeks. The weather conditions of Marquard and Welkom during March is as follows:

Average maximum temperature = 25-29^{0}C; average minimum temperature = 10 - 13^{0}C; average annual precipitation = 77.9 mm; average number of days with precipitation = 10.7; average length of day = 12.6 h; average relative humidity = 64.2%; average wind speed = 7.2 km/h.
The elevation of Marquard is 1514 m above sea level, with GPS coordinates of 28^{0}34’ 54.0^0 S and 27^{0}29’ 11.0^0 E. GPS coordinates of Welkom are 27^{0}59’ 30.0^0 S and 26^{0}46’ 8.0^0 E (AGROMET, 1994).

3.2 Experimental animals

One hundred and forty-four (144), cattle were used for this trial, mostly steers of composite breeds and mostly of Bonsmara type, which are representative of typical South African feedlot cattle. At the feedlot, the animals were fed standard commercial concentrate ration, which provides about

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Part of this chapter has been published in *Food Research International* as Babatunde Agbeniga & Edward C. Webb (2018), Influence of carcass weight on meat quality of commercial feedlot steers with similar feedlot, slaughter and post-mortem management, *Food Research International*, 105 (793 – 800). doi.org/10.1016/j.foodres.2017.10.073; Part of this chapter has also been accepted with minor corrections for publication in *Animal Production Science* as B. Agbeniga & E.C Webb (2018), Effects of timing and duration of low voltage electrical stimulation on selected meat quality characteristics of light and heavy bovine carcasses.

10.5MJ ME Kg^{-1}DM and was available *ad libitum*. Zilpaterol hydrochloride (Zilmax) was fed to the animals as a feed supplement during the last 30 days of the finishing/fattening period. Three additional days were left for the withdrawal of Zilmax. Zilmax was fed at the rate of 162 g/ton of feed (i.e. 162 ppm) at active ingredient, zilpaterol hydrochloride at 4.8%, which was calculated based on current feed with 75% DM content. In terms of liveweight, Zilmax was administered at 0.15 MG/kg live weight. The initial expected daily weight gain was 1.3 kg/day, rising to about 1.8 kg /day towards the finishing time. Standard hormonal growth implants were implanted on the animals according to Sparta feedlot protocol. A product named Ralgro was administered for 45 days, followed by Revalor H for about 60-70 days. The animals received normal medication according to Sparta feedlot protocol and the pre-slaughter conditions were assessed to be satisfactory. Most of the animals were steers in the "A" age group (i.e. no permanent incisors) and a few AB group according to the South African beef classification system (Meat Classification Regulations No 863 in Government Gazzete of September 2006). Heavy carcasses were categorized as ≥ 290 kg, while lighter carcasses were categorized as ≤ 260 kg. Fatness scores (subcutaneous fat thickness) for lighter carcasses, were Class 2 (1 to 3 mm), and a few Class 3 (3 to 5 mm) with a mean value of 3.59 mm (lean to medium). Heavy carcasses were categorized as Class 4 (5 to 7 mm) with a mean subcutaneous fat thickness of 6.5 mm on the $12/13^{th}$ rib.

Cattle were transported from the feedlot to the abattoir for about 2 h at night by trucks. On getting to the abattoir, the animals had about 6 h lairage time, with access to water, but without feed.

3.3 Slaughter process

The slaughter was carried out over five weeks (2nd March – 30th March 2015). Animal live weight was recorded before being presented for slaughter in a slaughter box, with a head restraint and a rear gate to restrain the animals. Pneumatic captive bolt gun was used to stun the animals, to render them unconscious in a humane way (conventional slaughter). Each carcass was suspended by a shackle on one hind leg after the stun, followed by sticking, about 60 s later. Sticking was done with a sharp knife of about 40 cm long. Carcasses that were due to be stimulated before evisceration were stimulated immediately (7 min) after sticking while those that were scheduled for late stimulation were done immediately after grading at 45 min pm.

3.4 Slaughter schedule and post-slaughter processes

Animals were randomly grouped based on live weight by visual examination to estimate carcass weight of small (≤ 260 kg; Code 1) and heavy carcasses (≥ 290 kg; Code 2). For the smaller carcasses, the minimum weight was on average 200 kg, while for the heavy carcass sides, the

maximum carcass weight was on average 350 kg. Carcasses were also grouped based on time of ES, that is, stimulation before evisceration (immediately after sticking – 7 min pm; Code 3) and ES after evisceration (45 min pm; Code 4). Carcasses were also grouped according to the duration of ES, that is, 30 s stimulation (Code 5) and 60 s stimulation (Code 6) as well as non- electrically stimulated groups (NES) (Code 0). These three factors, that is, carcass weight, ES time and ES duration formed the three treatment groups in this trial. The weight of each carcass was measured at the grading point at about 45 min pm by a scale attached to the rail, on which the carcasses were hung. At this point it was possible to group the carcasses according to their weights.

These factors were combined to have 10 scenarios and combinations of treatments, including controls. Table 3.1 shows the experimental layout and the combination of the treatments.

Table 3.1 Tabular layout of the various treatment combinations and controls

	Treatment groups									
TR	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
n	*18*	*14*	*16*	*16*	*14*	*15*	*13*	*11*	*12*	*15*
CW	S	H	S	S	S	S	H	H	H	H
ESd	NES	NES	30 s	60 s	30 s	60 s	30 s	60 s	30 s	60 s
ESt	NES	NES	B	B	A	A	B	B	A	A

Key: TR, treatment; CW, carcass weight; ESd, ES duration; ES, time of stimulation; 30 s, stimulation for 30 seconds; 60s, stimulation for 60 seconds; S, smaller carcasses (≤ 260 kg); H, heavier carcasses (≥ 290 kg); A, stimulation after evisceration (late stimulation- 45 min pm); B, stimulation before evisceration (early stimulation- 7 min pm); NES, non-stimulated controls; *n*, number of sample.

Carcasses were divided into two carcass weights groups (≤ 260 kg (small) and ≥ 290 kg (heavy)). According to the grading of the abattoir, the groupings were T = Tollies (whole carcass under 200 kg); LA = light 'A' (whole carcasses between 200 and 225 kg); MA = medium 'A' (whole carcasses between 226 and 275kg); HA = heavy 'A' (whole carcasses above 275kg). In total, 144 carcass sides (left) were used to evaluate carcass and meat quality attributes in ten treatment groups (Table 3.1). To evaluate the energy metabolites and the enzyme activities in the muscle, an average of six carcass sides were randomly selected from each treatment group. In all, 57 carcass sides were used to determine muscle energy metabolites and enzyme activity.

3.4.1 Slaughter schedule and treatments

Animals were slaughtered over a five-week period because of the hectic nature of the trial at a commercial abattoir. It was difficult to sample all animals in one day because of line stoppages and excessive interference with the slaughter line, and also because of the large number of animals to be sampled. It was also difficult to relocate the position of the electrical stimulator from the slaughter floor, where early stimulation was carried out, to the grading area, where late stimulation was done. However, the research team managed to include each treatment on a weekly basis to have a representation of all the treatment groups per slaughter day. The team also tried to minimize stoppages, which could amount to a significant reduction in the day's production target for the abattoir. An average number of 28 carcasses were sampled per slaughter day over the five-week period.

3.4.2 Electrical stimulation and sampling

Electrical stimulation was done with a portable electrical stimulator (Jarvis Products Corporation R.S.A. (PTY) LTD which was capable of outputs of 110 V and 300 V (low and medium voltage) with a selector switch. The voltage of stimulation was also adjustable with a switch. The settings of the stimulator were: frequency = 17 Hz; pulse = 5 m/s; voltage = 110V and A.C current of 1A. Stimulation was done by hooking the positive electrode on the neck region of each carcass before evisceration (7 min pm) (whole carcass) and after evisceration (45 min pm, on carcass sides). The earth (negative) of the electrode was connected to a metal hook on the rail line. Early stimulation was done at the kill floor, while the carcasses are still bleeding at 7 min pm. ES was done for 30 or 60 seconds (duration). At 45 min pm, after evisceration and immediately after late stimulation, pH and temperature readings were taken on each carcass by inserting pH and temperature probes in the *longissimus dorsi* muscle around the 12th and 13th ribs. Approximately 5g of sample was also taken from the left side of each carcass with a knife on the *longissimus et lumborum* (LL), around the 12^{th} and 13^{th} ribs to analyse energy metabolite and enzyme activity at 1 h pm. Subsequent samples for energy metabolite analysis were taken at 4 and 24 h pm. In a similar way, subsequent samples (5 g) were taken for enzyme activity around the same area (LL) at 24 h pm in the chiller. These samples were frozen immediately in liquid nitrogen, to halt metabolic activities, labelled and stored in dry ice until they are ready to be taken to a freezer where they were stored at -80 °C, until analysis (Dalrymple & Hamm, 1973). Subsequent recordings of pH and temperature were also done at 3, 6, 12 and 24 h pm.

After the collection of the energy metabolite and enzyme activity samples, carcasses were moved to a chiller room of between 0 and 2 °C, 1.5 m/s^{-1}, and *ca* 95% relative humidity. After recording the 24 h carcass pH and temperature and collecting the 24 h pm samples for enzyme activity, large samples of about 1.5 kg were dissected from each left carcass side on the LL between the 2nd last rib and the last lumbar vertebrae. These samples were used to determine meat shear force, meat colour, water holding capacity, drip loss, cooking loss, sarcomere length, myofibrillar length and subcutaneous fat. The large samples were vacuum packed to extend the shelf life. All samples were then transported to Agricultural Research Council, Meat Science Centre, Pretoria, which is about three and a half-hour drive, for further analysis. The samples for energy metabolites and enzyme analysis were stored in a freezer at -80 °C until they were analysed. The big samples were stored at chill temperature at about 4 °C pending analysis, from the following day.

3.5 Methods

3.5.1 pH and temperature measurement

Carcass pH and temperature were recorded by a special portable pH meter (Oakton Instruments, operating under the name Eutech Instruments, RS 232 (2003 model). It is designed for meat pH and has a special glass pH probe and a stainless steel, temperature probe, which are inserted into an incision (*ca* 2cm deep) on the *longissimus dorsi* (around the 12^{th} and 13^{th} ribs) as stated above. pH and temperature readings were taken at 45 min, 3, 6, 12, and 24 h pm and recorded for each carcass. Before use, the pH meter was first calibrated to pH range of 4 -7 to ensure accuracy. Figure 3.1 shows the pH and temperature reading on the carcass at the abattoir.

Figure 3.1 pH and temperature recording on the *longissimus dorsi* at the abattoir

3.5.2 Subcutaneous fat measurement

Subcutaneous fat (Sf)) (mm) was measured on the large *longissimus* samples, over the 13th rib, 50 mm from the medial plane. It was measured directly by placing the jaws of the calliper on the fat layer under the skin. The calliper jaws were inserted to measure the fat thickness (mm) (Swatland, 1984). The measurement was taken at 48 h pm for all treatments.

3.5.3 Percentage cooking loss

Cooking loss (CL) was determined by cutting out about 200 to 400 g of meat from the large samples (LL) that were extracted from the carcasses. These were stored at a temperature of ± 4 °C. Each sample was cut into a rectangular shape of about 10 cm x 8 cm x 6 cm, which was weighed and recorded. The length of each block was cut parallel to the fibre direction. The samples were then placed in thin walled transparent plastic bags and set in continuously boiling water bath, with the opening ends of the bags extending above the water surface on a metal rack. This was done to prevent water from entering the bags while the meat was boiling. The temperature of the water fluctuated between 75 and 78 °C but the meat internal temperature (core temperature) was

maintained at 75 °C (Honikel, 1998; Pearce et al., 2009), monitored by a temperature probe. The boiling was done at about 56 h pm

After boiling for one hour, the bags were removed from the water bath and cooled in ice slurry. They were then stored in chill condition at 4 °C overnight (about 14 h). The following morning (at 72 h pm), the meat samples were taken from the bags, blotted dry and weighed (Offer, 1984; Honikel, 1998). Cooking loss was expressed as a percentage of the initial weight:

$$\% \text{ cooking loss} = \frac{\text{weight loss after cooking}}{\text{initial sample weight}} \times \frac{100}{1}$$

The same procedure was followed to determine the cooking loss at 14 d pm. Duplicate samples were stored at ±4 °C before being cooked, 13 d pm. Percentage cooking loss was determined at the in-vitro lab of the Department of Animal and Wildlife Sciences, University of Pretoria.

3.5.4 Meat shear force

The samples that were used to determine percentage cooking loss were also used to evaluate the meat shear force (SF), 72 h pm. A hollow metal probe (1.27 cm in diameter and 8 cm in length) was used to take out 6 round cores, parallel to the fibre direction of the meat sample (AMSA, 1995; Honikel, 1998). Each core sample was sheared perpendicular to the fibre direction, through the centre. This was done with a Warner Bratzler shear device mounted on a Universal Instron apparatus (Model 4301, Instron Ltd, Buckinghamshire, Englang), with cross head speed of 200 mm/min. The mean value of the six readings in kg was used as the SF value for each sample. Shear force was determined at the Meat Science Centre, Agricultural Research Council, Pretoria. The same procedure was followed to determine the SF values at 14 d pm. Duplicate samples were stored for 13 days at ±4 °C before being analysed for cooking loss and SF. Figures 3.2, 3.3 and 3.4 shows the Warner Bratzler SF device.

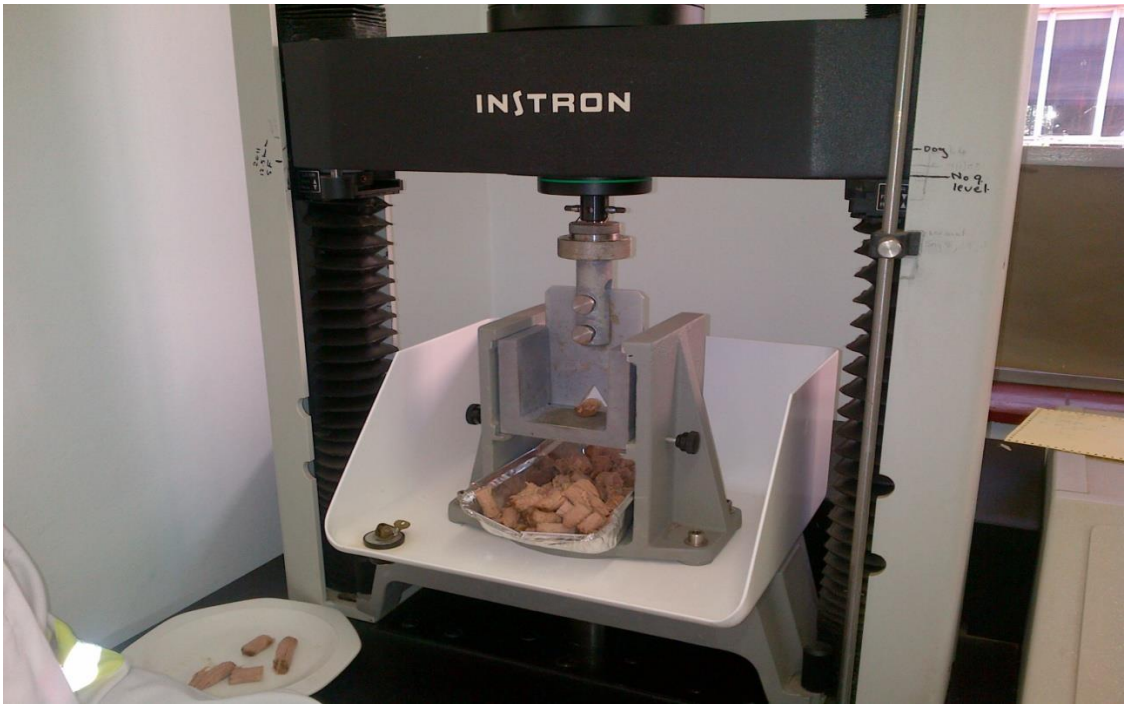

Figure 3.2 Warner Bratzler shear force device mounted on a Universal Instron Machine (Model 4301)

Figure 3.3 Universal Instron apparatus

Figure 3.4 Warner Bratzler shear force device mounted on a Universal Instron, connected to a computer monitoring system which indicates the graphs and individual measurements of each sample

3.5.5 Drip loss

Cubes of meat from the large samples (LL) of about 30 g were cut, 48 h pm and weighed. Prior to this, samples had been stored at ± 4 °C. Each cube (devoid of fat) 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 thin wire through the meat and through the holes to suspend the meat without touching the container. In so doing, the meat was left to release the drip directly to the floor of the container. The plastic bottles were then stored for 24 h at 4 °C. After storage, each sample was taken from the container, gently blotted dry and weighed (Frylinck et al., 2013; Honikel, 1998). Drip loss (DL) was expressed as a percentage of the initial weight:

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

The same procedure was carried out for the measurement of the 14-day DL. Duplicate samples of meat cubes were stored at 48 h pm for the next 13 days at 4 °C before determining DL, following the procedure described above. Figure 3.5 shows the meat cubes in plastic bottles during storage.

Figure 3.5 Cubes of meat suspended in bottles to measure of drip loss

3.5.6 Water holding capacity

About 0.5 g of meat from the large samples (LL), which were stored at ± 4 °C was pressed on a filter paper (Whatman 4), while sandwiched between two Perspex plates at 3 d pm. Each sample was pressed at a constant pressure and force of about 5N for 5 min (Grau & Hamm, 1953). The fluid and the meat area on the filter paper were then measured by a video image analysis (VIA) (Soft Imaging System, Olympus, Japan) as described by Irie, Izumo & Mohri (1996). The instrument was calibrated prior to use. The program detects the larger area and small area of the fluid. Water holding capacity (WHC) was calculated as the ratio of the area of meat to the liquid area on the filter paper after pressing. Figures 3.6 to 3.8 shows the samples and the pressing machine.

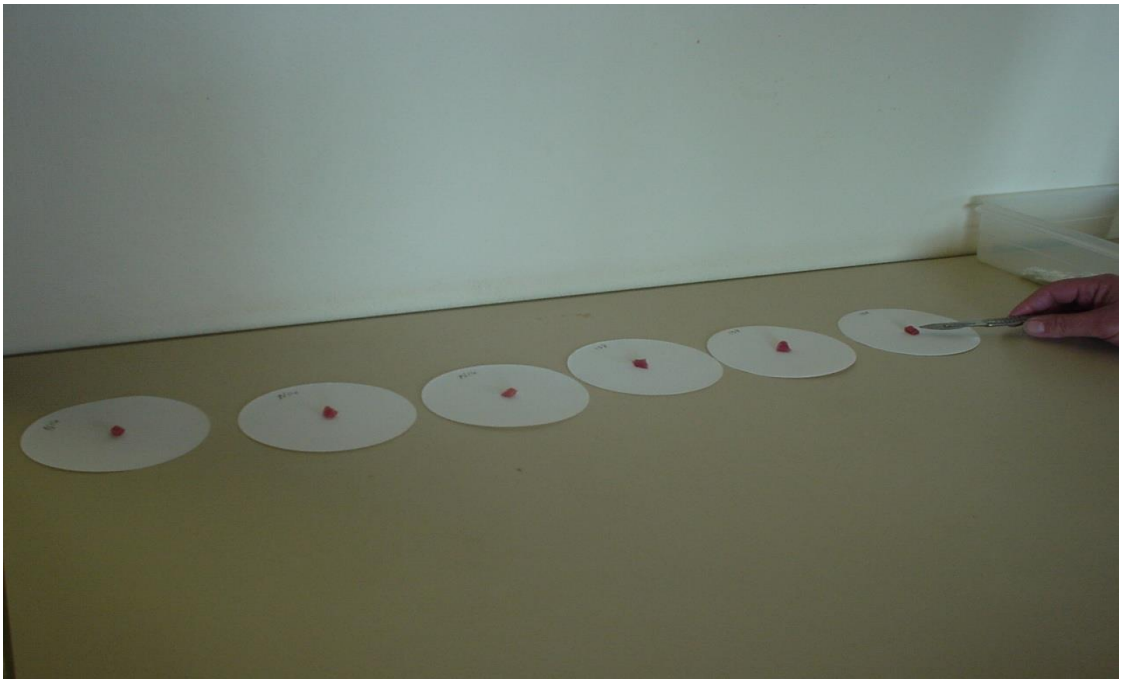

Figure 3.6 Small pieces of meat on the filter paper before pressing

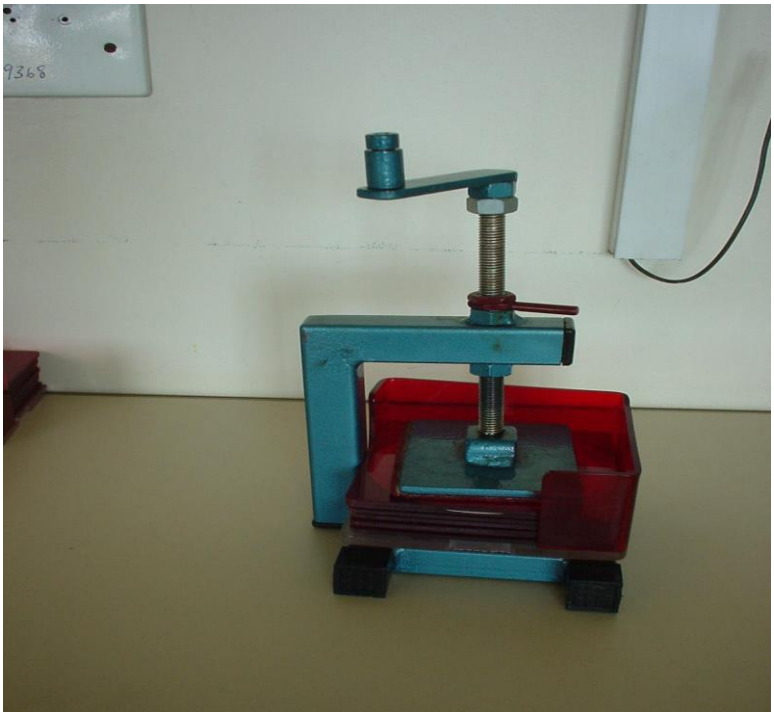

Figure 3.7 Meat pressing machine

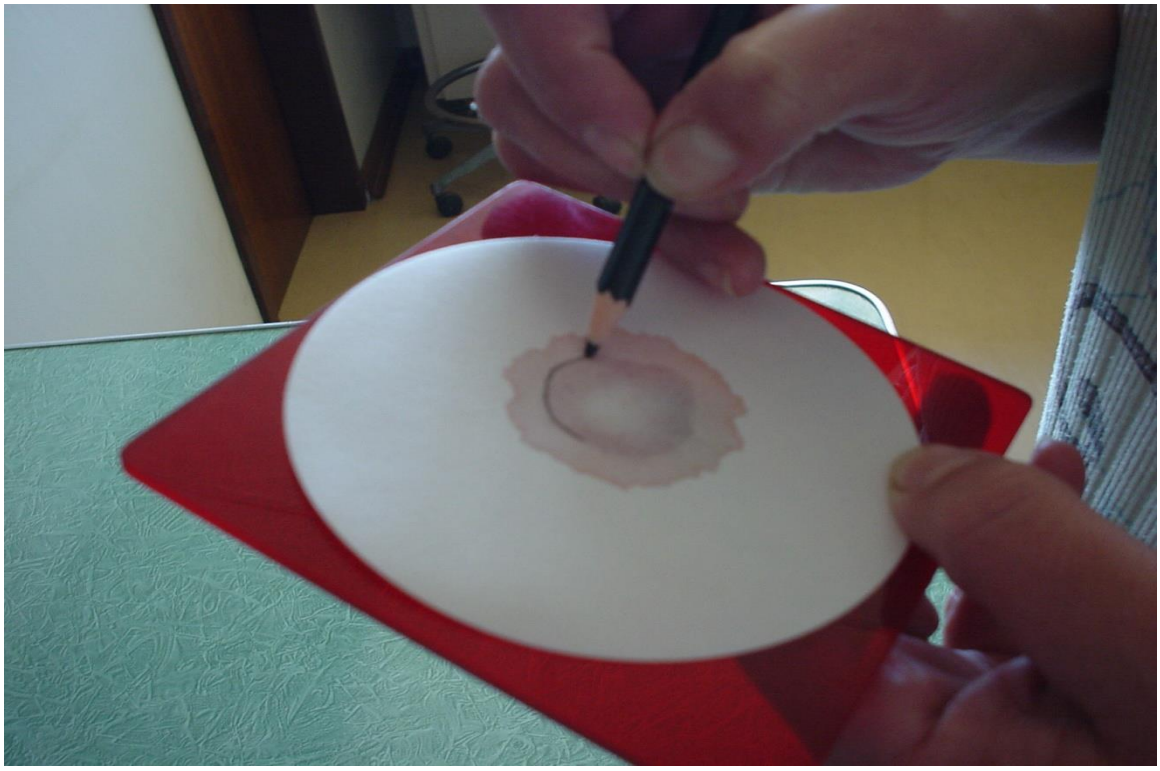

Figure 3.8 Meat and the fluid area on the filter paper

The same procedure was repeated for the 14-day measurement of the WHC. Duplicate samples were stored at 4 °C before pressing at 14 d pm.

3.5.7 Sarcomere length

Sarcomere length (SL) (μm) was measured on day 3 pm. Two grams of meat from each sample were homogenised according to (Hegarty & Naude, 1970). A small drop of the homogenised meat sample was put on slide. Sarcomere length was measured with a VIA system, using an Olympus B340 microscope at a magnification of 31000. The system was equipped with a CC12 video camera (Olympus, Tokyo, Japan). The processing and the quantification of measurements were done with AnalySIS Life Science software package (Soft Imaging System Gmbh, Munster, Germany). Five sarcomeres were measured and divided by five to obtain the size of each sarcomere because of their small size (Frylinck et al, 2013). Figure 3.9 shows the measurement of the SL. The green and the yellow lines were drawn over five sarcomeres.

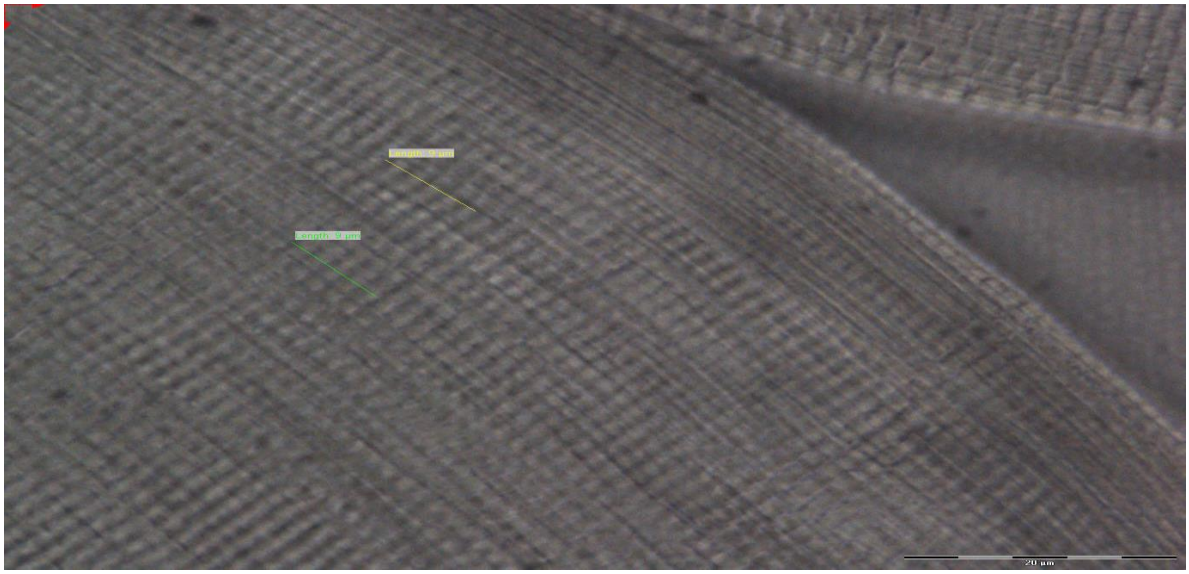

Figure 3.9 Sarcomere length measurement

N.B: The green and yellow lines cover five sarcomeres

3.5.8 Myofibril fragment length

Myofibril fragment length (MFL) was measured with a VIA (Soft Imaging System, Olympus, Japan) at 3 and 14 days pm on the samples extracted from the LL stored at 4 °C. The myofibrils were extracted according to Culler, Parrish, Smith & Cross (1978) and as modified by Heinze and Bruggemann (1994). Hundreds of fragments per sample were examined and measured with an Olympus BX41 system microscope at a magnification of 400X. Figure 3.10 and 3.11 shows MFL measurements at 3 and 14 d pm.

Figure 3.10 Myofibrillar fragment length at 3-days post-mortem

N.B: The red line indicates the MFL

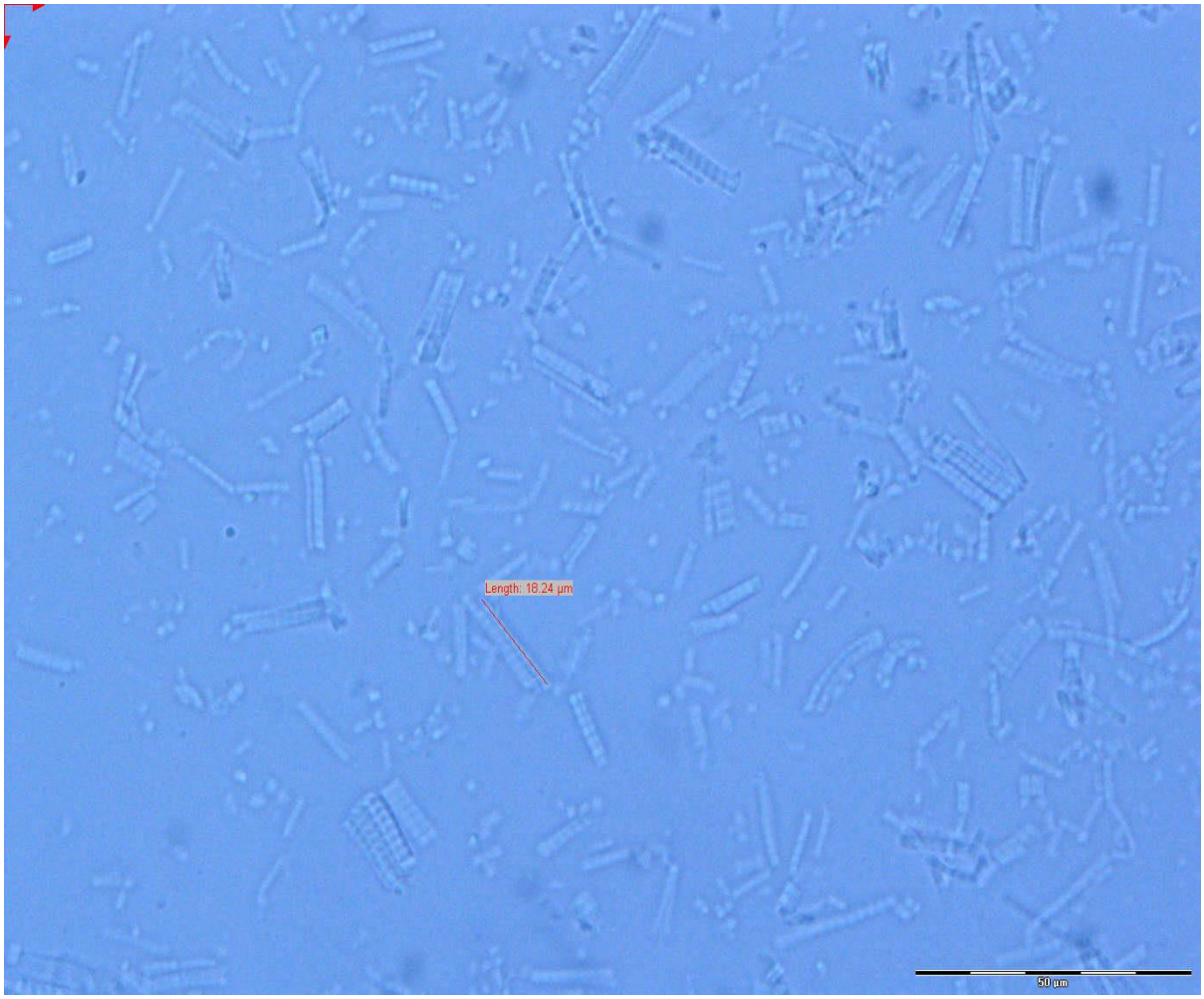

Figure 3.11 Myofibrillar fragment length at 14-days post-mortem

N.B: The red line indicates the MFL

3.5.9 Meat colour

Meat colour attributes were measured with a Minolta meter (Model CR200, Osaka, Japan) on fresh samples at 48 h pm and 14 d pm. Cut steaks of about 1.5 cm thick from the large samples (LL) were left to bloom for 1 h at a temperature of 4 °C before readings were taken. The colour attributes were lightness, L^* value (dark to light), a^* value (redness) and b^* value (yellowness). The physiological attribute of chroma (C*), which is the intensity of the red colour (saturation index) was calculated as: $(a^{*2} + b^{*2})^{1/2}$ (MacDougall, 1982) and the hue angle (H) (discoloration), was calculated as $\tan^{-1}$ (b^*/a^*) (Young, Priolo, Simmons & West, 1999). The values of the attributes were recorded with spectral component excluded (SCE) and spectral component included (SCI) but SCE was used, for the purpose of this trial and analysis. Two lamps were also

used, namely D65 lamp and luminant A lamp, but the D65 was used for the current analysis. Figure 3.12 shows the recording of the colour attributes with a colorimeter.

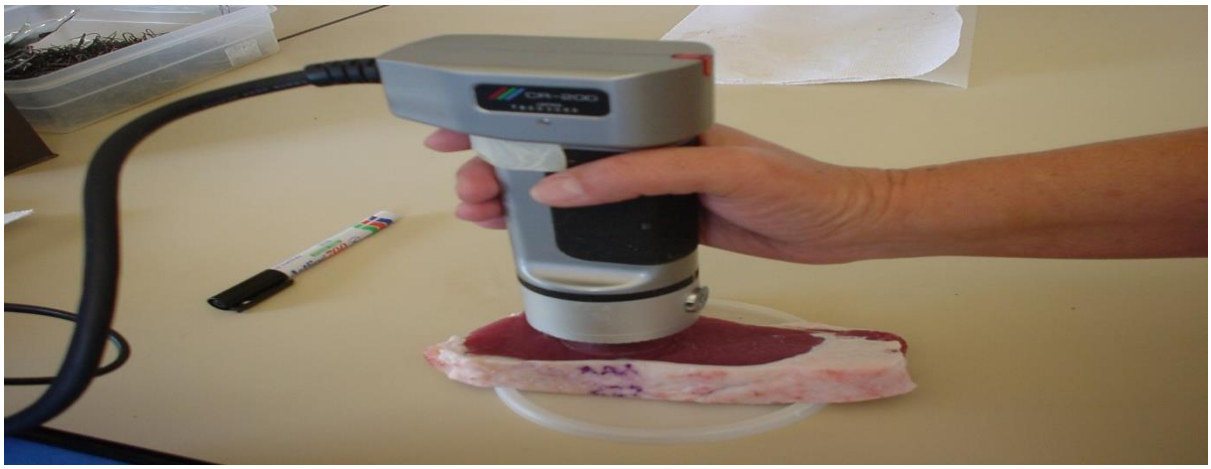

Figure 3.12 Measurement of the meat colour attributes

3.5.10 Energy metabolites determination (µmol/g)

At 1, 2 and 24 h pm, about 3 g of samples were taken between the 12th and 13th rib, on the LL muscle. The samples were taken with a small sharp knife, snap frozen in liquid nitrogen to stop all metabolic activities and stored in dry ice before being transported to the Agricultural Research Council (ARC), Meat Science Centre, Pretoria, where they were stored at -80 °C till the time of analysis. Samples were about two and a half months after slaughter at the ARC, Meat Science Centre. Samples were analysed for lactate, glucose, glycogen, creatine phosphate, ATP and glucose-6-phosphate (G-6-P). Lactic acid concentration, glucose, glycogen, glucose-6-phosphate (G-6-P), ATP, and creatine phosphate (CP) concentrations were determined using the modified method of Dalrymple and Hamm (1973). For lactic acid concentration, the method was further amended by Gutmann & Wahlefeld (1974). For glucose and glycogen concentration, the method was revised by Keppler & Decker (1974) while for G-6-P, ATP and CP determination, the method was modified by Bernt, Bergmeyer and Mollering (1974) and Lamprecht, Stein, Heinz and Weisser (1974).

These buffers were prepared before the start of the analyses; *perchloric acid* (0.6, $HCLO_4$, used for extraction) 60% (64.8 ml to 1 l water) or 70% (55.9 ml to 1l water), 200 ml per 8 samples were kept in the fridge and the remainder at room temperature; *lactate buffer* (hydrazine/glycine) (I lactate, pH 9, 1 l, stored at room temperature) 83.33m l hydrazine hydrate + 38g glycine + 800 ml water; *acetate buffer* (glycogen, pH 4.8, 0.2M, 1 l, stored at room temperature) 4.8 ml (96%) acetic acid + 9.75 g sodium acetate + 800 ml water; *1M KOH* (glycogen, stored at room

temperature) 28.05 g + 500 ml water; *Trieth (glycol) 0.3M buffer* (1l, 0.3M; pH 7.5; glycogen, stored at room temperature) 56 g triethanolamine hydrochloride + 1 g $MgSO_4$. $7H^2O$ + 120 ml 1M KOH + 800 ml water. Adjust pH to make up; *5.4M KOH* (glycogen, neutralize samples, stored at room temperature) 151.47 g KOH + 500 ml water (it gets hot); 1M NaOH (for creatine phosphate analysis, store at room temperature) 2 g + 50 ml water; *Trieth (CP, G-6-P)0.05M buffer* (1l, pH 7.5- 7.6, 50 mM, for CP, G-6-P, ATP, stored at room temperature) 9.3 g triethanolamine hydrochloride + 22 ml 1M NaOH + 800ml water; *$Mgcl_2$* (for creatine, stored at room temperature) 2.033 g $Mgcl_2.6H^2O$ + 100 ml water; *Glucose* (for creatine phophate analysis, stored at 4 °C) 2.48 g + 25 ml water or 1.5 g to 15 g to 15 ml H^2O; Methyl orange (indicator) (for sample extraction, stored at room temperature) 1 g + 25 ml water.

These solutions were also prepared and used weekly for analyses (for 16 samples): *AGS* (for extraction) 2.2 ml AGS + 17.8 ml acetate buffer (16 ml); *NAD* (for lactate) 0.150 g NAD + 5 ml water (3.5 ml); *glycogen buffer* 0.018 g ATP + 0.024 g NADP + 30 ml Trie (gly) buffer + 150 µl GP suspension (32 ml); NADP (for CP, ATP, G-6-P) 0.01 g + 2 ml water (2 ml), ADP 0.012 g + 1 ml water (0.5 ml), creatinee phosphate 0.005g + 1 ml Triethol (CP, G-6-P) buffer (0.5 ml). They were stored in the fridge at -8 °C. The chemicals were purchased from Merck, Roche and Separations chemical companies, SA.

Synopsis of analysis

The samples were first extracted. The steps for the extraction were as follows:

A water bath was heated to 40 °C.

1. Eight sets 16 mm glass tubes were set aside; to each tube were added 100 µl 0.6M perchloric acid buffer + 50 µl 1M KOH + 1 ml AGS solution (kept in frigde).
2. Three sets of 30ml plastic tubes were also set aside + an extraction form. The first set of tubes (1-8) were filled with 10 ml 0.6 M perchloric acid + 2 g of frozen sample (-80 °C). The sample was homogenized in the tube for 15 s by a homogenizer at 8000 rpm till mixed. The weights of each tube + the mixture was recorded on the extraction form.
3. Each homogenised mixture in tube was then centrifuged for 15 min at 10,000 rpm (cold). After this, the aliquot was poured into the next set of 30 ml plastic tube (1-8).
4. A 100 µl aliquot sample was taken from each plastic tube and placed in the glass tube that had been set aside in Step 1 above. The tubes were placed in the water bath at 40 °C for 2 h. After 2 h in the water bath, the samples were taken out and 1 ml 0.6 M perchloric acid buffer was

added to each glass tube to stop the reaction. These clear samples were stored in the fridge till needed to determine glycogen concentration.

5. For the remaining homogenized samples in the 30 ml plastic tubes, a few drops of methyl orange indicator were added to each tube with 5.4 M KOH slowly added to neutralize the pH. The colour turned from pink to yellow. These samples were placed in the fridge to precipitate out for 20 min. These were then filtered into measuring glass tubes with filter paper, a pump and clamps. The volume of each filtrate was recorded on the extraction form. The filtrate (yellow samples) was then poured into the last set of 30ml plastic tubes and stored in the fridge till analysed.

Spectrophotometer analyses

The Spectrophotometer machine (Agilent 8453E UV-Visible Spectroscopy System, Agilent Technologies Deutschland GmbH. Hewlett-Packard Strasse 8, 76337 Waldbronn, Germany) was switched on until a temperature of 39 °C was attained on the temperature monitor. Wavelength was set at 340 nm, while both lamps were on.

To determine the lactate concentration (µmol/g), 2.5ml lactate buffer + 200µl NAD solution + 20µl sample (yellow sample) were placed in each 1cm cuvette. Special mathematical software then calculated the concentration of each sample. Values increased from 1-24 h for samples from each cuvette.

To determine glycogen concentration (µmol/g), the same set-up was used. Glycogen buffer was heated up to 37 °C, clear and yellow samples were added to the cuvettes, namely 1 ml glycogen buffer + 50 µl sample + 5 µl hexokinase and read. The software automatically calculated the glycogen content which decreases from 1 to 24 h pm.

To determine the CP, ATP, glucose, and G-6-P concentrations (µmol/g), yellow samples were used; 2.5 Trieth (CP, G-6-P) buffer + 100 µl NADP + 100 µl $Mgcl_2$ + 20 µl ADP + 50µl sample (yellow). These were mixed and left to stand for 5 min and read. To determine G-6-P concentration, 5 ml G-6-P suspension was added and read. To determine glucose, 100 µl glucose was added and read. To determine ATP concentration, 5 ml hexokinase was added and read. To determine CP, 20 µl craetine kinase was added and read. All these values were calculated by the software on the computer attached to the spectrophotometer machine. Figures 3.13 and 3.14 show the spectrophotometer and the attached computer system for metabolite analyses.

Figure 3.13 Computer attached to spectrophotometer showing readings and graphs for each sample

Figure 3.14 Spectrophotometer analysis of energy metabolites

3.5.11 Proteolytic enzymes determination

About 5 g muscle samples were taken from *M.longissimus et lumborum* on the 12th and 13th ribs of each carcass at 1 and 24 h pm. Samples (devoid of fat) were taken with a small sharp knife on each carcass. The samples were snap frozen in liquid nitrogen and stored in dry ice pending transportation to ARC, Meat Science Centre, after 24 h pm. The samples were stored at -80 °C till they were analysed about two and a half months later.

Enzyme determination was done according to Geesink and Koohmaraie (1999) and Koohmaraie (1990), modified by Anderson, Frylinck, Strydom and Heinz (2015). Extract was prepared from samples in 15 ml extraction buffer. Samples were loaded in a 20 ml DEAE Sepharose (GE Healthcare Bio Sciences AB, Upsala, Sweden) packed column. Calpastatin was extracted from sample with 50 ml 0.04 M Tris HCL pH 7.5; 5 mM EDTA, and 0.025% MCE elution buffer containing 0.15M Nacl. Calpain-2 (m-calpain) was collected through a fraction collector after elution with 100 ml buffer containing 0.35 M Nacl. The column resin was washed with 50 ml 1.0 M Nacl and regenerated with 100 ml elution buffer containing no Nacl. All the test tubes containing sample fractions were tested for calpain-2 (m-calpain) activity with the azo-casein assay and all the fractions with absorbance of ≥ 0.100 than the blank at 366 nm were collected and used to determine the activity of calpastatin and indirectly calpain-1.

Separation and calculation of calpain*-1, calpain-2*, and calpastatin in muscle

Extraction of the samples was carried out according to the method of Dransfield (1996), in which about 3 g frozen muscle was homogenized in 15 ml extraction buffer (75 mM Tris HCL, 10 mM EDTA, pH 7.8, 0.05% (vol/vol) 2-mercaptoethanol (MCE), 2 mM phenylmethylsulfonyl fluoride (PMSF), 1 µl/L pepstatin A, at 4 °C and centrifuged at 10000 x for 30 min, filtered through vitrace, pH adjusted to 7.5 and volume made up to 20 ml with extraction buffer.

Separation of calpain-1, calpain-2, and calpastatin was based on the three-step gradient ion exchange chromatography-method of Geesink and Koohmaraie (1999). The Gilson LPLG system (pump, DEAE Sepharose column, valve mate, fraction collector, from Gilson Medical Electronics, Inc. 3000W Beltline Hwy, Middletown, USA) with the automated software program (Unipoint ™ LC version 5.1) was applied to separate the calpastatin and calpain-1 from the calpain-2 with Nacl-Tris/HCL buffers at pH 7.5. After loading the 20 ml extract, the column was eluted with 0.0 M Nacl (to partly separate calpastatin), 0.175 M Nacl (to collect the remainder of calpain-1 and calpastatin, 0.35 M Nacl (to collect calpain II) and also 1.0 M (to regenerate column resin). Calpastatin activity was determined in each of the 0.0 M and 0.175 M Nacl pooled elutes.

Calpain-1 activity was determined indirectly by the estimated calpastatin activity before and after the heat treatment of the 0.175 M salt fraction. A 5 ml aliquot from the pooled 0.175 M Nacl eluent containing both calpastatin and calpain 1 was heated for 15 min at a temperature of 95 °C, cooled on ice and centrifuged at 4000x for 15 min to remove the precipitate to eliminate calpain activity. Calpain-2 was determined in the pooled fractions of 0.35 M Nacl elute.

The activity of calpain was determined using the azo-casein assay as described by Dransfield (1996). Azo-casein removes the background absorbance of non-specific proteins in the extracts. Chemical reaction was started by the addition 400 µl sample (described below) to 400 µl assay buffer and incubated at 25 °C. This reaction was stopped after 1 h with 400 µl of 10% trichloroacetic acid (TCA) and the reaction mixture was centrifuged at 4000x. The absorbance of the supernatant was determined at 366 nm (spectrophotometer: Beckam Coulter DU 730 Life Science UV/Vis spec).

A triplicate of each of the following was prepared:

- Blank: replacing sample with 400 µl extraction buffer;
- Stock calpain-2, 100 µl + extraction buffer: 300 µl;
- 0.0 M Nacl fraction (for calpastatin only): 100 µl + stock calpain-2, 100 µl + extraction buffer, 200 µl.
- 0.175 M Nacl fraction (calpastatin plus calpain-1): 100 µl + stock calpain-2, 100 µl + extraction buffer, 200 (before heat).
- 0.175 M Nacl fraction (calpain-1 + calpastatin): 100 µl + stock calpain-2; 100 µl + extraction buffer (after heat).
- 0.35 M Nacl fraction (calpain Π): 400 µl

The calpain-1 activity was estimated by subtracting calpastatin activity in the 0.175 M Nacl fraction before and after heat treatment and could be determined within a 10% margin of error (Geesink & Koohmaraie, 1999). One unit of calpain activity is defined as an increase in absorbance of 1.0 at 366 nm per hour at 25 °C.

Calpastatin activity was determined by adding the same quantity of extracted calpain-2 stock to each assay (component) against a blank prepared from assay buffer which was prepared in triplicate. One unit of calpastatin was defined as the amount that inhibited one unit of calpain-2 (calpain-2) activity divided by two. The values were divided because of the nature of the activity/reaction of the molecules when combined with calpain (1 and 2) molecules (Hanna, Campbell & Davies, 2008). Data was expressed as units per gram of muscle or units/mg of

extractable protein (specific activity). The calpain and calpastatin determination described above were considered estimates and not the exact values because they were influenced by a number of factors such as protein extractability, inseparability of calpastatin and calpain-1 and enzyme activity. Enzyme activities were measured in unit/g of meat.

3.6 Statistical analysis

The general linear model procedure (GLM) (SAS 9.3) was used to compare the variations between the mean values of the treatment groups. The effects of the main treatments (carcass weight, ES time of application and ES duration) and their interactions was evaluated on selected/important meat quality attributes (SF, WHC, DL, L*, C* and H*). General statistics to determine the degree of freedom, sum of squares, F-values (F) and probability value (P) were also done using GLM. Pairwise comparison was used to compare and rank (in order of magnitude) the means of the treatment groups to determine significant differences. For all attributes with repeated measures, analysis of variance (ANOVA) was used to determine the variance of contrast variables at different times of measurement. Means for the interactions between treatments for all the dependent variables were separated and compared using Fisher's protected least significant difference (LSD) at 5% level of probability (Snedecor & Cochran, 1980).

Multiple analysis of variance (MANOVA) was used as test criteria to test the F-statistics and *P*-values for the hypothesis of stimulation time effects, using Wilk's-Lambda, Pillai's trace, Hotelling-Lawley's trace and Roy's greatest root. For all the attributes with repeated measures, univariate test was done by repeated measures ANOVA to test the hypothesis for within subject effects, that is, time, and time × treatment interactions. Partial correlation coefficient and the level of significance were also determined at the various time levels for all attributes with repeated measures. Correlations were done using the CORR procedure to determine the correlation coefficients among all variables to ascertain those with strong and significant relationships.

Regression analysis was done using the REG procedure to determine the level of influence of the strongly correlated independent and dependent variables on key quality attributes (SF, DL and meat colour) at the abattoir /butchery level and the retail/supermarket/display level. This was to enable the researchers to predict the quality of the carcass and meat products based on their treatments at the various levels of processing, for quality assurance. The general regression equation was:

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + \beta_9 X_9 + \beta_{10} X_{10}$$

Where,

y = the independent variable, e.g. shear force day 3

β_0 = intercept (overall mean value of y)

X_1 to X_9 = dummy variables associated with the ten treatments.

CHAPTER 4

RESULTS

The main aim of this study was to optimise the conversion of muscle to meat using low voltage electrical stimulation (ES) (110 V) in light and heavy beef carcasses from Zilmax-treated cattle. The three main effects or factors (independent variables) that were evaluated were, time of stimulation (before evisceration/early stimulation, 7 min pm (B) or after evisceration/late stimulation 45 min pm (A)); duration of stimulation (30 s or 60 s); and carcass weight ($\leq$ 260 kg (Small (S) or $\geq$ 290 kg (Heavy (H)). The initial number of animals used was 161, but after discovering some outliers probably due to the hectic nature of conducting the research at a commercial abattoir, numbers of carcasses were trimmed down by removing some carcasses with extreme/erratic values. At the end, 144 carcasses were used, with mostly normally distributed values of carcass and meat quality attributes that were measured. However, some of the values of these quality attributes were still extreme (outliers) and those particular values were removed, instead of condemning the whole carcass. These values were regarded as missing values. To dtermine energy metabolites and enzyme activity in muscles, a total of 57 carcass sides were used after random selection of about six carcass sides from each treatment group as indicated in Chapter 3. This was because the reagents for analysing these attributes were expensive.

4.1 pH

One hundred and forty-four (144) carcass sides were evaluated for carcass pH. Table 4.1 shows the mean values and standard deviations of pH measurement for each treatment group at 45 min pm, and 3, 6, 12 and 24 h pm.

Part of this chapter has been published in *Food Research International* as Babatunde Agbeniga & Edward C. Webb (2018), Influence of carcass weight on meat quality of commercial feedlot steers with similar feedlot, slaughter and post-mortem management, *Food Research International*, 105 (793 – 800). doi.org/10.1016/j.foodres.2017.10.073; Part of this chapter has also been accepted with minor corrections for publication in *Animal Production Science* as B. Agbeniga & E.C. Webb (2018), Effects of timing and duration of low voltage electrical stimulation on selected meat quality characteristics of light and heavy bovine carcasses.

Table 4.1 ANOVA for the effects of treatments on carcass pH mean values and standard deviation at different times of measurements

Treatments	N	pH 45 min Mean ± SD	pH 3 h Mean ± SD	pH 6 h Mean ± SD	pH 12 h Mean ± SD	pH 24 h Mean ± SD
(1) S NES	18	6.51±0.23^{d}	6.35±0.29^{b}	6.13±0.22^{a}	5.81±0.16^{ab}	5.56±0.10^{bc}
(2) H NES	14	6.55±0.24^{cd}	6.40±0.28^{ab}	6.13±0.23^{a}	5.73±0.22^{b}	5.48±0.09^{cd}
(3) S 30s B	16	6.15±0.29^{e}	5.81±0.25^{c}	5.66±0.27^{b}	5.59±0.20^{c}	5.49±0.15^{cd}
(4) S 60s B	16	5.94±0.25^{f}	5.70±0.18^{c}	5.64±0.25^{b}	5.51±0.09^{c}	5.47±0.09^{d}
(5) S 30s A	14	6.84±0.17^{ab}	6.37±0.28^{b}	6.08±0.30^{a}	5.91±0.21^{a}	5.65±0.09^{a}
(6) S 60s A	15	6.89±0.18^{a}	6.57±0.28^{a}	6.08±0.16^{a}	5.91±0.16^{a}	5.64±0.10^{ab}
(7) H 30s B	13	6.06±0.20^{ef}	5.69±0.20^{c}	5.58±0.20^{b}	5.48±0.11^{c}	5.46±0.12^{d}
(8) H 60s B	11	5.94±0.16^{f}	5.65±0.23^{c}	5.56±0.11^{b}	5.51±0.07^{c}	5.46±0.09^{d}
(9) H 30s A	12	6.84±0.20^{ab}	6.31±0.26^{b}	5.97±0.30^{a}	5.81±0.19^{ab}	5.71±0.14^{a}
(10) H 60s A	15	6.69±0.24^{bc}	6.31±0.27^{b}	6.03±0.26^{a}	5.77±0.15^{b}	5.64±0.16^{ab}

Key: N, number of carcasses; S, carcass side less than or equals 130 kg; H, carcass side more than or equal 145 kg; 30s, 30 seconds stimulation; 60s, 60 seconds stimulation; B, stimulation before evisceration (early stimulation); A, stimulation after evisceration (late stimulation); SD, standard deviation

^{a,b,c,d} Means in the same column without a common superscript letters differ ($P < 0.05$).

The GLM repeated measures showed a declining trend in variation between groups in terms of pH decline from 45 min to 24 h pm in this manner: At 45 min pm, there was a significant variation (73%), ($R^2 = 0.73$; $P < 0.0001$); at 3 h pm, there was a significant variation (64%), ($R^2 = 0.64$; $P < 0.0001$); at 6 h pm, there was a significant variation (50%), ($R^2 = 0.51$; $P < 0.0001$); at 12 h pm, there was a significant variation (49%), ($R^2 = 0.49$; $P < 0.0001$) while at 24 h pm, there was a lower but significant variation among the treatment groups (37%), ($R^2 = 0.37$; $P < 0.0001$). Figure 4.1 shows the plot of pH decline for the treatment groups over time.

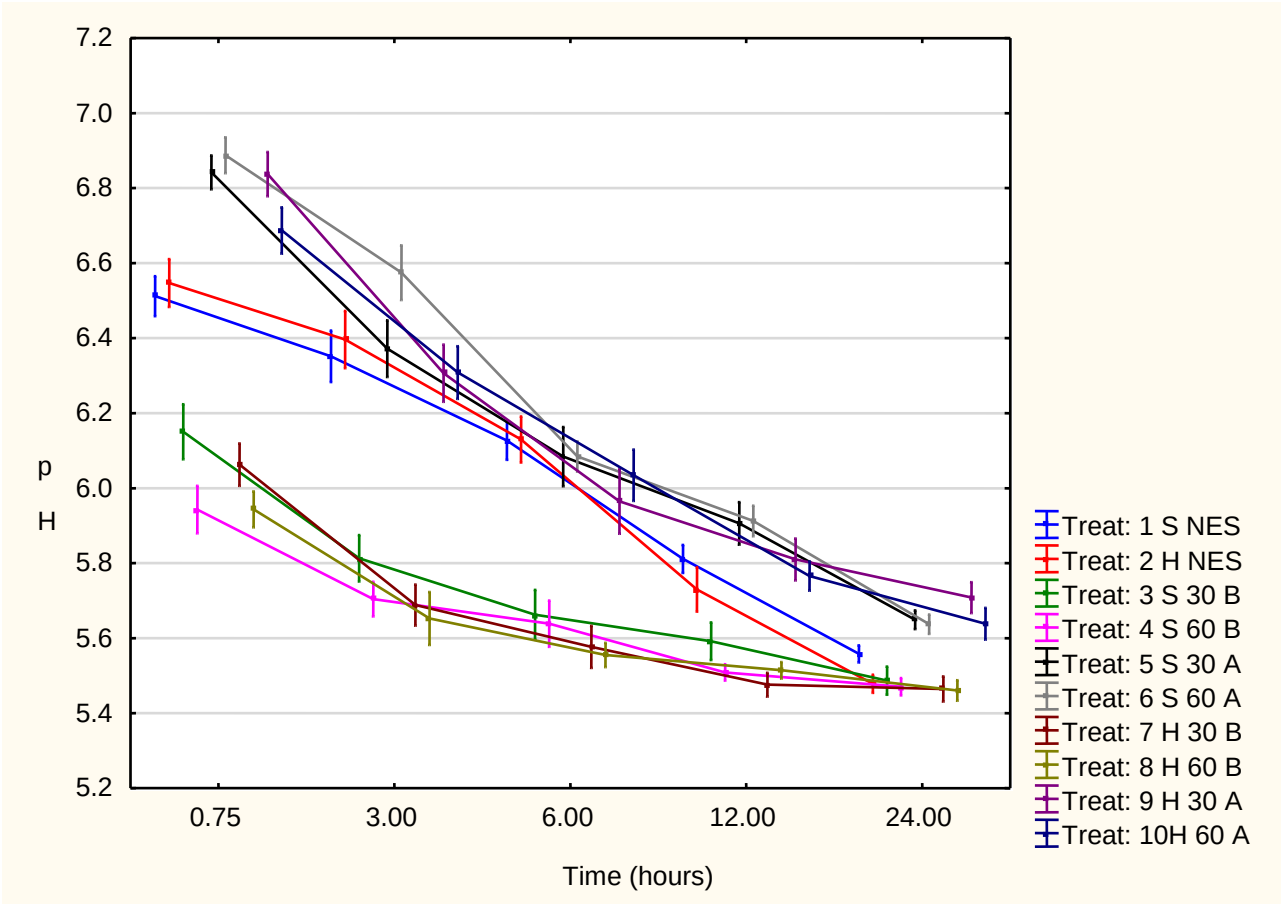

Figure 4.1 Time plot profile of pH decline for the treatment groups

Key: S, small carcasses; H, heavy carcasses; A, stimulation after evisceration/late stimulation; B, stimulation before evisceration/early stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

The rate of pH decline was ranked among the treatment groups at various times of measurement, in descending order (i.e. from highest to lowest value) thus:

pH 45 min pm: 6 (S 60 A) > **5** (S 30 A) > **9** (H 30 A) > **10** (H 60 A) > **2** (H NES) > **1** (H NES) > **3** (S 30 B) > **7** (H 30 B) > **8** (H 60 B) >**4** (S 60 B)

pH 3 h pm: 6 (S 60 A) > **2** (H NES) > **5** (S 30 A) > **1** (S NES) > **10** (S 60 A) > **9** (H 30 A) > **3** (S 30 B) > **4** (S 60 B) > **7** (H 30 B) > **8** (H 60 B)

pH 6 h pm: 2 (H NES) > **1** (S NES) > **6** (S 60 A) > **5** (S 30 A) > **10** (H 60 A) > **9** (H 30 A) > **3** (S 30 B) > **4** (S 60 B) > **7** (H 30 B) > **8** (H 60 B)

pH 12 h pm: 6 (S 60 A) > **5** (S 30 A) > **1** (S NES) > **9** (H 30 A) > **10** (H 60 A) > **2** (H NES) > **3** (S 30 B) > **8** (H 60 B) > **4** (S 60 B) > **7** (H 30 B)

pH 24 h pm: 9 (H 30 A) > **5** (S 30 A) > **10** (H 60 A) > **6** (S 60 A) > **1** (S NES) > **3** (S 30 B) > **2** (H NES) > **4** (S 60 B) > **7** (H 30 B) > **8** (H 60 B)

Treatments 4, 7 and 8 had the fastest pH decline, and the lowest readings at all time points. These carcasses were all stimulated before evisceration (B) (7 min pm). This was followed by Treatment 3, which was also stimulated early post-mortem (pm). On the other hand, all carcasses simulated late/ after evisceration (A) and the control (NES) had the slowest pH decline rate, irrespective of the carcass weight. This shows that LVES (110 V) immediately after slaughter, before evisceration brought about faster pH decline throughout the time profile, compared with stimulation after evisceration (45 min pm). Heavy carcasses with early ES (Treatments 7 and 8) had the numerically lowest pH 3 h pm, lowest rigor pH (pH 6 h pm) and the lowest ultimate pH, compared with the small/light carcasses throughout the pH profile. There were mixed reactions to the duration of ES (30 and 60 s) and there was no definite pattern of reaction among the treatment groups. MANOVA and exact F statistics were carried out to test for the hypothesis of number of time effect and the interaction between time and treatments, using Wilks' lambda, Pillai's trace, Hotelling-Lawley Trace, and Roy's greatest root. They were all significant ($P < 0.0001$), which shows that there were significant differences between the times of measurement, and likewise, in the interaction between time and treatments.

At 45 min pm, all carcasses stimulated early pm (Treatments 3, 4, 7 and 8) had significantly ($P < 0.05$) lower pH value compared with the late stimulated carcasses (5, 6, 9 and 10) and the controls (1 and 2) Table 4.1. The late stimulated carcasses also had slightly higher pH at 45 min compared with the NES controls. A similar pattern of reaction to pH 45 min pm was observed at 3 h pm among the carcass groups. Heavy carcasses that were stimulated early (7 and 8) had the lowest pH values but were not significantly different from the other early stimulated carcasses (3 and 4). At 6 h pm (rigor pH), a similar pattern of reaction was observed, but the NES carcasses had the highest pH values, followed by the late stimulated carcasses. The heavy carcasses stimulated early (7 and 8), retained the lowest values, but were not significantly different from the other early stimulated lighter carcasses (3 and 4). At 12 h pm a similar pattern of reaction was observed for all treatment groups, but the numerical gaps among groups were reduced. All early stimulated carcasses had significantly ($P < 0.05$) lower pH at 12 h compared with the late stimulated carcasses and controls. At 24 h pm, a much lower numerical gap was observed among all treatment groups. Early stimulated carcasses had significantly lower pH_u (ultimate pH) compared with the late stimulated carcasses as shown in Table 4.1.

Partial correlation was also carried out using the GLM repeated measure procedure of ANOVA, within the time levels of pH measurement. There were significant ($P < 0.01$) positive correlations between the time levels of measurements for all the treatment groups, as expected, with pH3 and pH6 h pm having the highest correlation ($r = 0.67$, $P < 0.0001$).

4.2 Temperature

One hundred and forty-four carcass sides were evaluated for carcass temperature decline. Table 4.2 shows the mean values and standard deviations of temperature readings for each treatment group at 45 min, and 3, 6, 12 and 24 h pm.

Table 4.2 Mean values, standard deviation and P- values for the ten treatment groups and at different times of temperature measurements

Treatments	**N**	**Temp 45 min Mean ± SD**	**Temp 3 h Mean ± SD**	**Temp 6 h Mean ± SD**	**Temp 12 h Mean ± SD**	**Temp 24 h Mean ± SD**
(1) S NES	17	35.55 ± 1.62^{e}	25.50 ± 2.15^{cd}	17.42 ± 1.75^{b}	6.70 ± 1.28^{f}	3.16 ± 0.30^{e}
(2) H NES	14	35.16 ± 1.97^{e}	26.44 ± 2.97^{c}	19.50 ± 2.78^{a}	7.83 ± 1.68^{cde}	3.43 ± 0.44^{de}
(3) S 30s B	16	38.78 ± 0.88^{cd}	23.84 ± 2.34^{de}	14.60 ± 1.81^{c}	7.33 ± 0.96^{def}	3.10 ± 0.41^{e}
(4) S 60s B	16	38.93 ± 1.15^{c}	24.83 ± 2.12^{cd}	15.23 ± 1.08^{c}	8.31 ± 0.85^{cd}	3.38 ± 0.29^{de}
(5) S 30s A	14	37.96 ± 1.55^{d}	26.67 ± 2.78^{c}	14.61 ± 2.72^{c}	6.99 ± 1.35^{ef}	3.25 ± 0.38^{e}
(6) S 60s A	14	38.93 ± 0.57^{cd}	31.05 ± 2.76^{b}	15.76 ± 1.99^{c}	8.64 ± 0.70^{bc}	3.81 ± 0.36^{cd}
(7) H 30s B	13	39.81 ± 0.58^{ab}	22.46 ± 2.64^{e}	18.45 ± 2.01^{ab}	9.74 ± 1.12^{a}	3.98 ± 0.50^{bc}
(8) H 60s B	10	40.50 ± 0.57^{a}	26.66 ± 2.99^{c}	19.03 ± 3.69^{ab}	9.56 ± 1.35^{ab}	3.74 ± 0.74^{cd}
(9) H 30s A	14	39.56 ± 0.55^{bc}	34.04 ± 3.77^{a}	17.54 ± 1.83^{b}	9.78 ± 1.85^{a}	4.60 ± 1.11^{a}
(10)H60s A	15	39.17 ± 0.94^{bc}	34.42 ± 3.76^{a}	17.55 ± 2.08^{b}	9.56 ± 2.33^{ab}	4.26 ± 0.88^{ab}

Key: N, number of carcasses; S, small carcass side (≤ 130 kg); H, heavy carcass side (≥ 145 kg); 30s, 30 seconds stimulation; 60s, 60 seconds stimulation; B, stimulation before evisceration (early stimulation); A, stimulation after evisceration (late stimulation); SD, standard deviation.

^{a,b,c,d} Means in the same column without a common superscript letters differ ($P < 0.05$).

The GLM for temperature repeated measures indicated a declining trend in the variations among the treatment groups from 45 min to 24 h pm. At 45 min pm, the variation between the treatment groups was 68% ($R^2 = 0.68$; $P < 0.0001$); at 3 h pm, the variation was 67% ($R^2 = 0.67$; $P < 0.0001$); at 6 h pm, the variation was 39% ($R^2 = 0.39$; $P < 0.0001$); at 12 hr pm, the variation was 41% ($R^2 = 0.41$; $P < 0.0001$) and at 24 h pm, the variation was 42% ($R^2 = 0.42$; $P < 0.0001$).

Figure 4.2 shows the plot of temperature decline over time for the ten treatment groups.

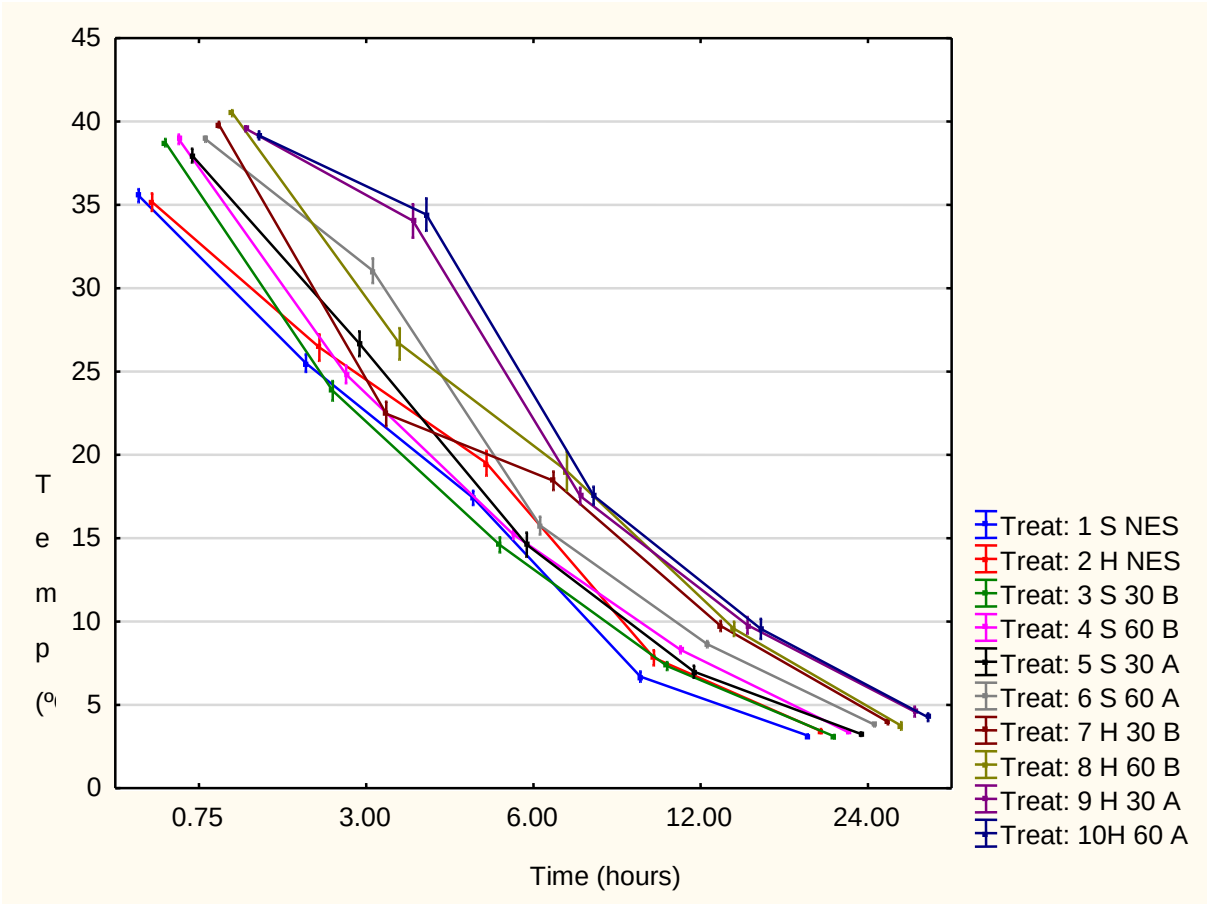

Figure 4.2 Time plot profile of temperature decline for treatment groups

Key: S, small carcasses; H, heavy carcasses; A, stimulation after evisceration/late stimulation; B, stimulation before evisceration/ early stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

The rate of temperature decline was ranked among the treatment groups at different times of measurement, in descending order:

Temperature 45 min pm: 8 (H 60 A) > **7** (H 30 B) > **9** (H 30 A) > **10** (H 60 A) > **6** (S 60 A) > **4** (S 60 B) > **3** (S 30 B) > **5** (S 30 A) > **1** (S NES) > **2** (H NES)

Temperature 3 h pm: 10 (H 60 A) > **9** (H 30 A) > **6** (S 60 A) > **5** (S 30 A) > **8** (H 60 B) > **2** (H NES) > **1** (S NES) > **4** (S 60 B) > **3** (S 30 B) > **7** (H 30 B)

Temperature 6 h pm: 2 (H NES) > **8** (H 60 B) > **7** (H 30 B) > **10** (H 60 A) > **9** (H 30 A) > **1** (S NES) > **6** (S 60 A) > **4** (S 60 B) > **5** (S 30 A) > **3** (S 30 B)

Temperature 12 h pm: 9 (H 30 A) > **7** (H 30 B) > **8** (H 60 B) > **10** (H 60 A) > **6** (S 60 A) > **4** (S 60 B) > **2** (H NES) > **3** (S 30 B) > **5** (S 30 A) > **1** (S NES)

Temperature 24 h pm: 9 (H 30 A) > **10** (H 60 A) > **7** (H 30 B) > **6** (S 60 A) > **8** (H 60 B) > **2** (H NES) > **4** (S 60 B) > **5** (S 30 A) > **1** (S NES) > **3** (S 30 B)

As expected, the temperature of the smaller/lighter carcasses (Treatments 1, 3, 4, 5 and 6) declined faster across the board from 45 min to 24 h pm (see Table 4.2 and the illustration above). On the other hand, Treatments 7, 8, 9 and 10 (heavy carcasses, except Treatment 7 and 8, at 3 h pm) had the slowest temperature decline from 45 min to 24 h pm. Aside controls, all heavy carcasses had significantly higher ($P < 0.05$) rigor temperature (pH_6). The graph follows a more linear pattern for all the treatment groups compared with the pH profile graph. Regarding the effect of time of ES on temperature decline, at 45 min pm, there were mixed reactions (i.e. no definite pattern) among the treatment groups. At 3 h pm, the late stimulated carcasses (A) (5, 6, 9 and 10) recorded the slowest temperature decline followed by the NES groups and the groups stimulated before evisceration (B), respectively. From 6 h to 24 h pm, there were mixed reactions from all the groups in terms of time of ES. Regarding the duration of stimulation, there were mixed responses from the treatment groups. This implies little or no influence of duration of stimulation on carcass temperature decline. MANOVA was carried out to test the variation among time levels (i.e. times of measurement), using Wilks lambda, Pillai's trace, Hotelling-Lawley trace, and Roy's greatest root. All were significant ($P < 0.0001$). This was affirmed by the test of hypothesis for between -subject effects, using repeated measures ANOVA, for all the treatment groups. They were all significantly different ($P < 0.0001$) from each other. MANOVA and F-value approximation for the hypothesis of interaction of times by treatment effect were also carried out using Wilks' Lambda, Pillai's test, Hotelling-Lawley Trace, and Roy's Greatest Root. The interactions were all found to be significant ($P < 0.0001$).

Partial correlation was carried out using the GLM procedure repeated measures ANOVA, within the time levels of temperature measurement. The initial temperature readings showed a weak positive correlation with the final temperature readings but from 3 to 12 h, there were strong positive and significant ($P < 0.01$) correlations. Temperature readings at 6 and 12 h pm showed the strongest correlation ($r = 0.52$, $P < 0.0001$).

4.3 pH and temperature relationship

Figure 4.3 shows the relationship between pH and temperature decline. It also indicates treatment groups that were probably cold or hot/heat shortened owing to the prevailing temperature in relation to the various treatments.

Cold shortening occurs when the pH is greater than 6.0, while ATP is still available for muscle contraction and muscle temperature has dropped to less than 10 °C. On the other hand, Thompson (2002) observed that heat/hot shortening occurs when the combined effect of high temperature and low pH in the muscle exhausts proteolytic activity owing to accelerated glycolysis and protein denaturation. The window set to avoid this phenomenon is when pH is lower than 6 when muscle temperature is greater than 35 °C. Figure 4.3 shows a plot of pH and temperature showing the cold and hot/heat shortening windows.

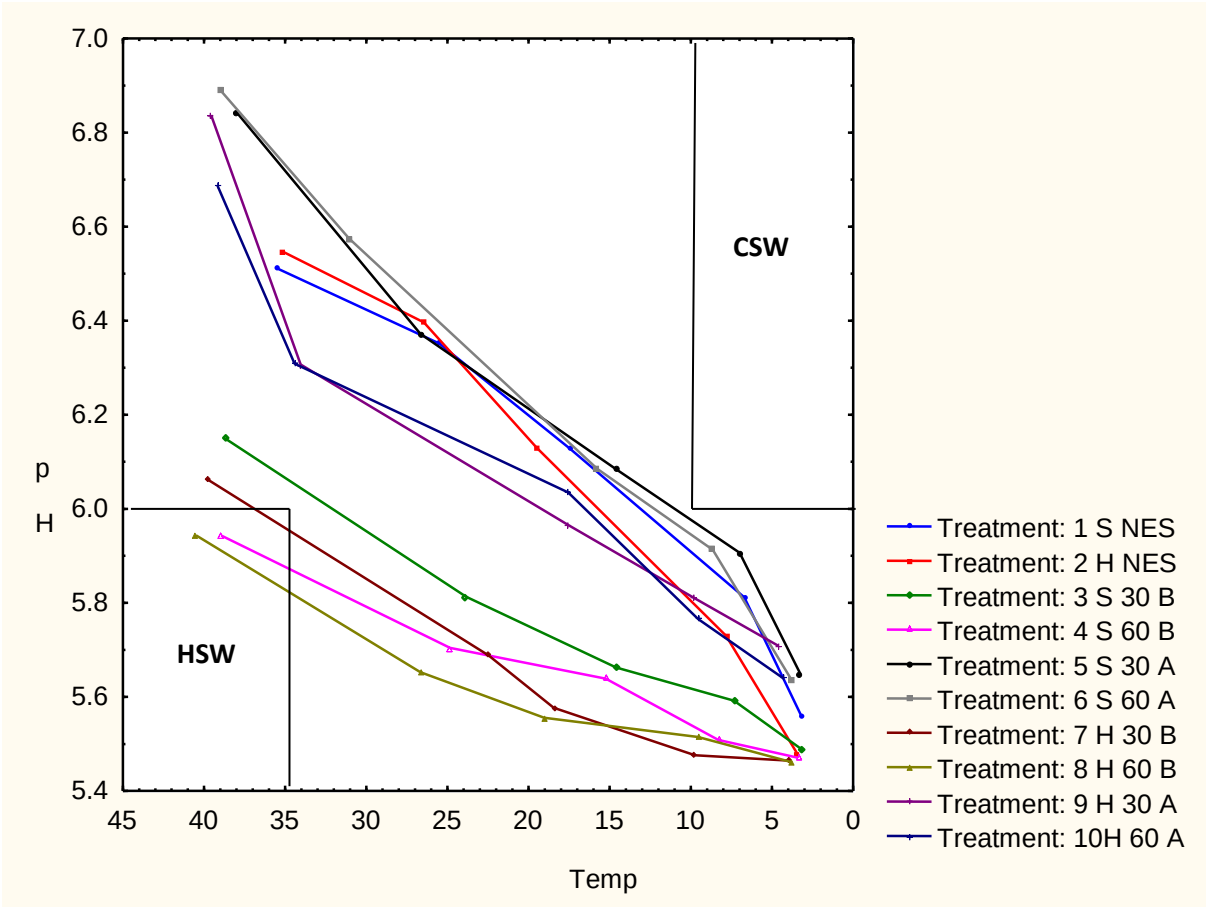

Figure 4.3 Cold and hot/heat shortening windows for pH and temperature interaction for the treatments

Key: CSW, Cold shortening window; HSW, hot/heat shortening window; S, small carcasses; H, heavy carcasses; B, stimulation before evisceration (early stimulation); A, stimulation after evisceration (late stimulation)

None of the carcasses and treatment groups fell within the cold shortening window (CSW) (Figure 4.3). However, Groups 4, 7 and 8 fell within the hot/heat shortening window (HSW) at the initial chilling period, between 45 min and 3 h pm.

4.4 Subcutaneous fat

One hundred and forty-nine carcasses were evaluated for subcutaneous fat (mm). General linear model ANOVA revealed significant difference ($P < 0.0001$) between the 10 treatment groups. The variation R^2 was 46% ($R^2 = 0.46$; $P < 0.0001$) among the groups. T-test (LSD) was carried out using pairwise comparison at 5% level of significance to check the differences between means of the treatments. Table 4.4 shows the differences between the mean values and their standard deviations in terms of subcutaneous fat thickness (mm) among treatment groups.

Table 4.3 ANOVA results for subcutaneous fat for treatment groups

Treatment	**N**	**Subcutaneous fat (mm)** **Mean ± SD**
(1) S NES	20	3.90 ±1.48^{cd}
(2) H NES	15	8.27 ±3.25^{a}
(3) S 30s B	16	3.72 ±1.99^{cd}
(4) S 60s B	16	3.34 ±1.56^{d}
(5) S 30s A	14	3.36 ±1.12^{d}
(6) S 60s A	15	3.63 ±0.92^{cd}
(7) H 30s B	13	6.27 ±1.83^{b}
(8) H 60s B	11	6.50 ±1.89^{b}
(9) H 30s A	14	6.57 ±2.39^{b}
(10) H 60s A	15	4.90 ±0.93^{c}

Key: N, number of carcasses; S, small carcasses; H, heavy carcasses; 30s, 30 seconds stimulation; 60s, 60 seconds stimulation; B, stimulation before evisceration (early stimulation); A, stimulation after evisceration (late stimulation); SD, standard deviation.

^{a,b,c,d} Means in the same column without a common superscript letters differ ($P < 0.05$).

The treatment groups were ranked in descending order according to the thickness of their subcutaneous fat:

Subcutaneous fat: 2 (H NES) > **9** (H 30 A) > **8** (H 60 B) > **7** (H 30 B) > **10** (H 60 A) > **1** (S NES) > **3** (S 30 B) > **6** (S 60 A) > **5** (S 30 A) > **4** (S 60 B)

There was a clear pattern in the ranking, as expected. The groups with heavy carcasses (i.e. 2, 9, 8, 7 and 10) had the thickest subcutaneous fat cover, while the groups with smaller/lighter carcasses (i.e. 1, 3, 6, 5 and 4) had the thinnest subcutaneous fat cover. Figure 4.4 shows the distribution of mean subcutaneous fat cover (mm) for the treatment groups.

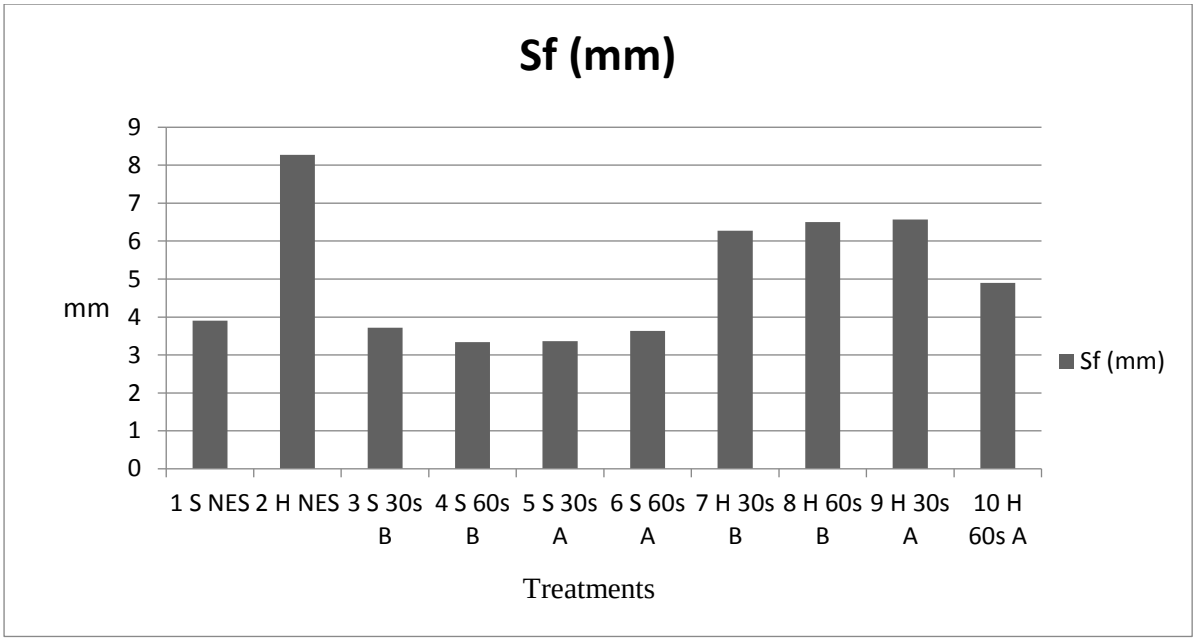

Figure 4.4 Bar chart of subcutaneous fat cover (mm) for treatment groups

Key: Sf (mm), subcutaneous fat; H, heavy carcasses; S, smaller carcasses; B, early stimulation; A, late stimulation; 30s, 30 seconds stimulation; 60s, 60 seconds stimulation; NES, non-stimulated.

There were no correlations between time of stimulation and duration of stimulation and subcutaneous fat. There were no significant differences ($P > 0.05$) between Groups 7, 8, and 9 (heavy carcasses), while the heavy control carcasses (2) had the highest ($P < 0.05$) subcutaneous fat cover. There was no significant difference ($P > 0.05$) between groups 1, 3, 4, 5 and 6 (smaller/lighter carcasses). Treatment 2 shows a significant difference ($P < 0.05$) from all the other groups. The thickness of the subcutaneous fat cover was reflected in the temperature decline rate. Treatment groups with thickest fat (heavy carcasses) (7, 8, 9 and 10) had the slowest temperature decline, while the lighter carcasses had the fastest temperature decline rate.

4.5 Shear force

Meat samples from 144 carcasses were evaluated for meat shear force (SF). From the analysis, MANOVA and partial correlation coefficients showed a significant ($P < 0.0001$) difference between the two times of SF measurements. Table 4.4 shows the GLM t-test (LSD) and SD for SF 3 and 14 d pm measurement. Partial correlation between the two times of measurements showed there was moderate and significant correlation ($r = 0.47$; $p < 0.0001$).

Table 4.4 Mean shear force values for treatment groups 3 and 14-days post-mortem

Treatment	N	SF (Kg) 3 dpm Mean ± SD	SF (Kg) 14 dpm Mean ± SD	%Δ SF
1) S NES	**20**	10.73 ± 1.62^{a}	8.16 ± 1.11^{a}	**23.95**
2) H NES	**15**	9.44 ± 1.32^{b}	7.59 ± 1.76^{ab}	**19.60**
3) S 30 B	**16**	9.09 ± 1.20^{bc}	6.13 ± 0.91^{cde}	**32.56**
4) S 60 B	**16**	8.14 ± 1.11^{cd}	6.19 ± 1.04^{cde}	**23.96**
5) S 30 A	**14**	9.59 ± 1.69^{b}	6.66 ± 1.46^{cd}	**30.55**
6) S 60 A	**15**	8.91 ± 0.98^{bcd}	6.44 ± 1.31^{cde}	**27.72**
7) H 30 B	**13**	7.99 ± 1.08^{d}	5.60 ± 0.75^{e}	**29.91**
8) H 60 B	**11**	8.39 ± 1.10^{cd}	5.90 ± 1.29^{de}	**29.68**
9) H 30 A	**14**	9.07 ± 1.49^{bc}	6.57 ± 1.48^{cd}	**27.56**
10) H 60 A	**14**	9.48 ± 1.08^{b}	6.82 ± 0.98^{bc}	**28.06**

Key: S, small carcass sides; H, heavy carcass sides; NES, non-electrically stimulated carcasses; A, after evisceration (late stimulation); B, before evisceration (early stimulation); N, number of carcasses; SF 3d, SF at 3 days pm; SF 14d, SF at 14 days pm; SD, standard deviation. ^{a,b,c,d} Means in the same column without a common superscript letters differ ($P < 0.05$).

Figure 4.5 shows the graphical distribution of mean shear force values at 3 and 14 days pm for treatment groups.

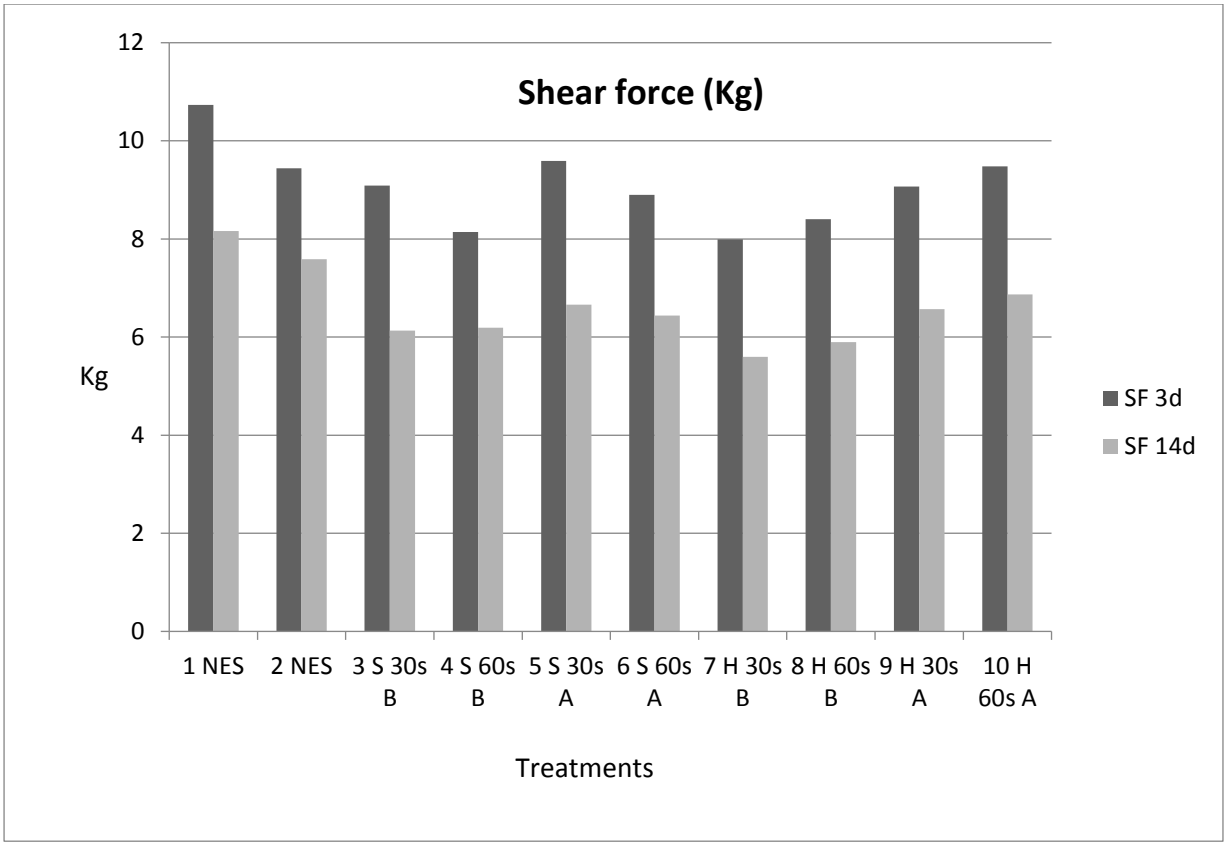

Figure 4.5 Shear force at 3 and 14 days pm by treatment groups

Key: S, small carcasses; H, heavy carcasses; NES, non-stimulated (control); B, early stimulation; A, late stimulation; 30s, 30 seconds stimulation; 60s, 60 seconds stimulation.

From the GLM ANOVA, there was low but significant variation of 29% for 3-day SF ($R^2 = 0.29$; $P < 0.0001$) & 29.2% for 14-day SF ($R^2 = 0.29$; $P < 0.0001$) within the treatment groups. For shear force at 3 and 14 dpm, the following illustration shows the ranking of the magnitude of SF for treatment groups in descending order:

Shear force 3 day pm: 1 (S NES) > **5** (S 30 A) > **10** (H 60 A) > **2** (H NES) > **3** (S 30 B) > **9** (H 30 A) > **6** (S 60 A) > **8** (H 60 B) > **4** (S 60 B) > **7** (H 30 B)

Shear force 14 day pm: 1(S NES) > **2** (H NES) > **10** (H 60 A) > **5** (S 30 A) > **9** (H 30 A) > **6** (S 60 A) > **4** (S 60 B) > **3** (S 30 B) > **8** (H 60 B) > **7** (H 30 B)

At 3 dpm there were mixed reactions regarding the effects of carcass weight on shear force (from the ranking above and Table 4.4). At 14 dpm, early stimulated carcasses (Treatments 3, 4, 7 and 8) had the lowest shear force values and out these early stimulated carcasses, Treatments 7 and 8 (heavy carcasses), had the numerically lowest shear force while the controls and the late stimulated carcasses had the highest SF values respectively. In terms of the duration of stimulation, at 3 and 14 dpm, there were mixed reactions among the treatment groups. The NES

group (1) had the highest SF at 3 dpm while at 14 dpm, both NES groups (1 & 2) had the highest SF (Table 4.4).

Table 4.5 shows the mean values of the main effects (carcass weight, ES time and ES duration) on shear force values.

Table 4.5 Mean values and standard deviation of main effects on shear force at 3 and 14-days post-mortem

Main effects		**N**	**SF(N) day 3 pm Mean ± SD**	**SF(N) day 14 pm Mean ± SD**
CW	**S**	70	89.63 ± 14.72	65.02 ± 13.08
	H	55	86.73 ± 12.35	63.73 ± 13.11
ES t	**0 (NES)**	23	97.75 ± 13.63	78.22 ± 12.47
	B	52	82.67 ± 11.90	59.24 ± 9.75
	A	50	89.95 ± 13.02	63.53 ± 12.00
ES d	**0 (NES)**	23	97.75 ± 13.63	78.22 ± 12.47
	30 s	54	87.47 ± 14.11	60.41 ± 11.14
	60 s	48	84.85 ± 11.44	62.39 ± 11.01

Key: CW, carcass weight; S, small carcasses; H, heavy carcasses; SF, shear force; ES t, electrical stimulation time; ES d, electrical stimulation duration; 0 (NES), non-stimulated carcasses; B, stimulation before evisceration (early stimulation); A, stimulation after evisceration (late stimulation); 30 s, 30 seconds stimulation; 60 s, 60 seconds stimulation; SD, standard deviation.

Table 4.6 Effects of carcass weight, electrical stimulation time, electrical stimulation duration and their interactions on meat shear force at 3 and 14-days post-mortem

Effects	***P* value**	**F ratio**	**SEM**	***P* value**	**F ratio**	**SEM**
Main effects	**SF day 3**			**SF day 14**		
CW	0.116	2.507	1.580	0.522	0.411	1.450
ES time (ES t)	0.004	8.797	2.034	0.060	3.606	1.860
ES dura (ES d)	0.436	0.611	2.033	0.351	0.878	1.860
Interactions	**SF day 3**			**SF day 14**		
CW x ES t	0.519	0.418	2.869	0.516	0.426	2.628

CW x ES d	0.005	8.340	2.870	0.104	2.678	2.628
ES t x ES d	0.992	0.000	2.501	0.661	0.194	2.290
CW x ESt x ES d	0.759	0.095	3.527	0.546	0.366	3.230

Key: CW, carcass weight; ES t, ES time; ES d, ES duration; *P* = 0.05; SEM, standard error of means.

Analysis revealed that carcass weight and ES duration did not produce significant effects on shear force. However, heavy carcasses produced numerically lower SF at 3 and 14 dpm (Table 4.5 and 4.6). Table 4.6 showed that, 60 seconds ES produced numerically lower SF at 3 dpm, while 30 s ES produced slightly lower SF at 14 dpm. Early stimulation produced significantly lower SF at 3 dpm and a high tendency to be significant (P = 0.060) at 14 dpm (Table 4.6). Additionally, the interaction of heavy carcass weight and shorter duration of stimulation produced a significantly (P < 0.05) lower SF at 3 dpm (Table 4.6). Early stimulated carcasses had marginally higher average decline (combined) in SF (29%) compared with late stimulated carcasses (28%) and the controls (22%) (Table 4.4).

4.6 Cooking loss

Samples from 144 carcasses were evaluated for cooking loss. At 3 dpm, there was 18% (R^2 = 0.18; *P* < 0.0001) variation among treatment groups while at 14 dpm, there was low variation, 6% (R^2 = 0.06; *P* < 0.4445). MANOVA test, the GLM repeated measure ANOVA revealed significant difference (*P* < 0.0001) between the two times of measurement for all the carcasses. MANOVA test also reveals significant difference (*P* < 0.0384) for the interaction of time and treatments for all the carcasses in terms of cooking loss. Partial correlation revealed significant difference (r = 0.27; *P* < 0.0012) between the two times of measurement. Table 4.7 shows the t-test (LSD) between the mean values of each treatment group.

Table 4.7 Table of mean percentage cooking loss, standard deviation and *P*- values for treatment groups

Treatments	**N**	**% CL 3 day pm Mean ± SD**	**% CL 14 day pm Mean ± SD**
1) S NES	**19**	31.49 ± 2.00^{ab}	30.35 ± 2.16^{ab}
2) H NES	**15**	29.65 ± 2.76^{c}	29.68 ± 2.83^{ab}
3) S 30 B	**16**	32.79 ± 2.28^{a}	30.60 ± 2.15^{ab}
4) S 60 B	**15**	32.85 ± 2.08^{a}	29.53 ± 2.25^{ab}
5) S 30 A	**14**	32.29 ± 2.26^{ab}	29.36 ± 2.58^{ab}

6)	**S 60 A**	**15**	31.82 ± 2.69^{ab}	30.89 ± 2.03^{a}
7)	**H 30 B**	**12**	30.97 ± 1.44^{bc}	29.19 ± 2.37^{b}
8)	**H 60 B**	**10**	32.23 ± 1.83^{ab}	30.31 ± 1.59^{ab}
9)	**H 30 A**	**14**	31.72 ± 1.39^{ab}	29.81 ± 2.21^{ab}
10)	**H 60 A**	**15**	30.74 ± 1.39^{bc}	29.35 ± 1.97^{ab}

Key: N, Number of carcasses; S, small carcasses; H, heavy carcasses; SD, standard deviation; B, early stimulation; A, late stimulation

^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.6 shows the distribution of the mean values for percentage cooking loss at 3 and 14 dpm

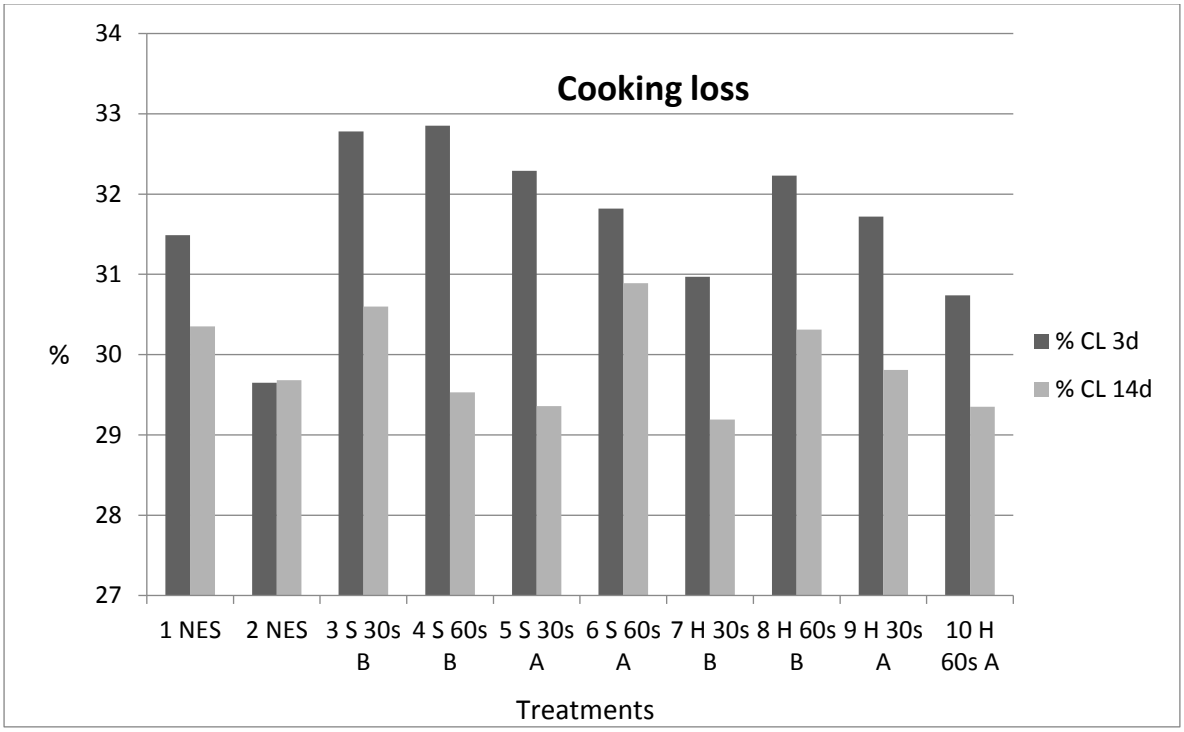

Figure 4.6 Distribution of percentage cooking loss at 3 and 14-days post-mortem among treatments

Key: S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30s, 30 seconds stimulation; 60s, 60 seconds stimulation.

From Table 4.7 and Figure 4.6, the following illustration shows the ranking of the percentage CL at 3 and 14 dpm in descending order:

Percentage CL 3dpm: **4** (S 60 B) > **3** (S 30 B) > **5** (S 30 A) > **8** (H 60 B) > **6** (S 60 B) > **9** (H 30 A) > **1** (S NES) > **7** (H 30 B) > **10** (H 60 A) > **2** (H NES)

Percentage CL 14d pm: **6** (S 60 A) > **3** (S 30 B) > **1** (S NES) > **8** (H 60 B) > **9** (H 30 A) > **2** (H NES) > **4** (S 60 B) > 5 (S 30 A) > **10** (H 60 A) > **7** (H 30 B)

At 3 dpm, the illustration reveals that smaller carcasses (Treatments 4, 3, 5) had the highest percentage cooking loss while the heavier carcasses (2, 7, 9 and 10) had the lowest percentage cooking losses. At 14 dpm, most of the smaller carcasses (Treatments 1, 3, 6) had the highest cooking loss while a few of the heavy carcasses (7 and 10) had the lowest cooking loss in terms of carcass weights. Generally, there were mixed reactions and low variations regarding carcass weight. In terms of time and duration of ES, there were mixed reactions and no definite patterns from the treatment groups (Figure 4.6). From the ANOVA (Table 4.7), there were no significant differences ($P > 0.05$) among most of the treatment groups at 3 dpm save Treatments 2, 3 and 4. Also, at 14 dpm, there were no significant differences ($P > 0.05$) among the groups save Treatments 6 and 7 (Table 4.7). As indicated earlier by the R^2 values, there was very little variation between the groups at 3 and 14 dpm. Numerical differences were low among treatments.

4.7 Drip loss

One hundred and forty-four carcasses were evaluated for percentage drip loss but 141 carcasses were used owing to outliers. At 3 dpm, the variation among the treatment groups was 37% ($R^2 = 0.37$; $P < 0.0001$). At 14 dpm, a similar variation pattern was observed among the treatment groups at 36.9% ($R^2 = 0.37$; $P < 0.0001$). The GLM procedure showed significant differences ($P < 0.0001$). Partial correlation between the two times of measurement (3 and 14 dpm) ($r = 0.67$) showed a significant difference ($P < 0.0001$). GLM, repeated measures analysis of variance using MANOVA revealed a significant difference at 10% level ($P < 0.0931$) in the interaction of time and treatment among the groups. Table 4.8 shows the t- test (LSD) between the mean values of the treatment groups.

Table 4.8 Mean percentage drip loss and and standard deviation for treatment groups

Treatments	N	% DL 3d Mean ± SD	% DL 14d Mean ± SD
1) H NES	17	1.47 ± 0.45^{d}	1.76 ± 0.50^{c}
2) H NES	12	1.61 ± 0.43^{d}	2.22 ± 1.01^{c}
3) S 30 B	16	3.10 ± 2.08^{b}	4.26 ± 2.51^{b}
4) S 60 B	16	2.95 ± 1.09^{bc}	4.20 ± 1.92^{b}
5) S 30 A	14	1.99 ± 0.72^{d}	2.82 ± 1.44^{c}
6) S 60 A	14	2.03 ± 0.69^{d}	2.85 ± 1.36^{c}
7) H 30 B	13	3.41 ± 1.39^{b}	4.87 ± 2.09^{ab}
8) H 60 B	11	4.59 ± 1.98^{a}	5.75 ± 1.79^{a}
9) H 30 A	14	2.10 ± 0.78^{cd}	2.57 ± 1.39^{c}
10) H 60 A	14	2.22 ± 0.61^{cd}	2.53 ± 1.21^{c}

Key: N, Number of carcasses; S, smaller carcasses; H, heavier carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation; SD, Standard Deviation.

^{a,b,c,d} Means in the same column without a common superscript letter differs ($P < 0.05$).

Figure 4.7 shows the distribution of the mean values of percentage DL at 3 and 14 dpm

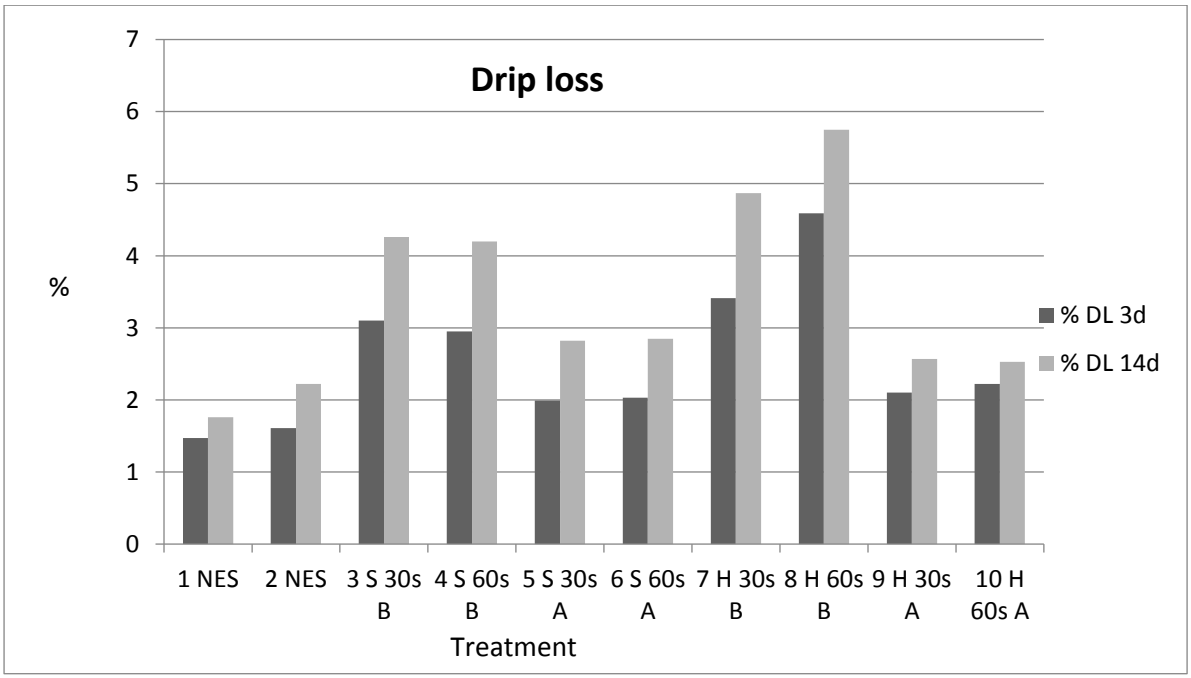

Figure 4.7 Distribution of mean values of percentage drip loss at 3 and 14-days post-mortem

Key: S = smaller carcasses; H = heavier carcasses; B = early stimulation; A = late stimulation; 30 = 30 s stimulation; 60 = 60 s stimulation

The following illustration shows the ranking of percentage drip loss at 3 and 14 dpm in descending order, according to the treatments.

Percentage drip loss 3 dpm: 8 (H 60 B) > **7** (H 30 B) > **3** (S 30 B) > **4** (S 60 B) > **10** (H 60 A) > **9** (H 30 A) > **6** (S 60 A) > **5** (S 30 A) > **2** (H NES) > **1** (S NES)

Percentage drip loss 14 dpm: 8 (H 60 B) > **7** (H 30 B) > **3** (S 30 B) > **4** (S 60 B) > **6** (S 60 A) > **5** (S 30 A) > **9** (H 30 A) > **10** (H 60 A) > **2** (H NES) > **1**(S NES)

There were clear patterns of reaction among the treatment groups in terms of time of ES at 3 and 14 dpm. The bar chart (Figure 4.7) and the illustration above reveals that carcasses stimulated before evisceration (B) (Treatments 8, 7, 4 and 3) had the highest DL followed by the late stimulated carcasses (A) (10, 9, 6 and 5) and the controls. Regarding the duration of stimulation, there was no clear pattern. That is, there was mixed reactions from the treatment groups. Heavy and early stimulated carcasses, Treatment 8 (60 s), had the highest drip loss followed by Treatment 7 (30 s) but, further down the scoring line, the reaction was mixed. There was a similar pattern of reaction for percentage DL at 14 dpm. Regarding the effects of carcass weight on drip loss, heavy carcass sides (Group 7 and 8) had the highest ($P < 0.05$) drip loss at 3 dpm. A similar pattern of reaction was observed for 14-day percentage DL. At 3 dpm, there were no significant differences between the controls and late stimulated smaller carcasses, but the late stimulated carcasses had numerically higher DL (Table 4.8). Early ES carcasses produced the highest ($P < 0.05$) amount of DL at both times of measurement. Overall, the non-stimulated controls had the lowest percentage DL.

Tables 4.9 and 4.10 shows the mean values of carcass weight, ES time and ES duration, and the significant differences in the main effects and their interactions.

Table 4.9 Mean values and standard deviation of main effects on drip loss at 3 and 14-days post-mortem

Main effects		N	% DL day 3 pm Mean ± SD	% DL day 14 pm Mean ± SD
CW	**S**	70	2.35 ± 1.34	3.30 ± 1.95
	H	55	2.77 ± 1.58	3.56 ± 2.15
ES t	**0 (NES)**	23	1.54 ± 0.42	1.97 ± 0.80
	B	52	3.42 ± 1.78	4.74 ± 2.18
	A	50	2.06 ± 0.69	2.69 ± 1.37
ES d	**0 (NES)**	23	1.54 ± 0.42	1.97 ± 0.80
	30s	54	2.70 ± 1.51	3.70 ± 2.14
	60s	48	2.81 ± 1.54	3.78 ± 2.06

Key: CW, carcass weight; S, small carcasses; H, heavy carcasses; ES t, ES time; ES d, ES duration; 0 (NES), non-stimulated carcasses; B, ES before evisceration (early ES); A, ES after evisceration (late ES); 30s, 30 seconds stimulation; 60s, 60 seconds stimulation; SD, standard deviation.

Table 4.10 Effects of carcass weight, electrical stimulation time and electrical stimulation duration and their interactions for percentage drip loss at 3 and 14-days post-mortem

Effects	***P* value**	**F ratio**	**SEM**	***P* value**	**F ratio**	**SEM**
Main effects	**%DL day 3**			**% DL day 14**		
CW	0.037	4.441	0.160	0.204	1.633	0.216
ES time	0.000	37.490	0.200	0.000	42.214	0.278
ES dura	0.361	0.842	0.199	0.588	0.296	0.277
Interactions	**% DL day 3**			**% DL day 14**		
CW x ES t	0.077	3.189	0.282	0.023	5.289	0.392
CW x ES d	0.178	1.840	0.282	0.742	0.109	0.392
ES t x ES d	0.261	1.274	0.246	0.458	0.555	0.342
CW x ESt x ES d	0.193	1.714	0.346	0.428	0.633	0.482

Key: CW, carcass weight; ES t, Electrical stimulation time; ES d, electrical stimulation duration; *P* = 0.05; SEM, standard error of means.

Heavy carcasses produced significantly (P < 0.05) higher DL at 3 dpm but at 14 dpm, the difference was not significant (Tables 4.9 and 4.10). Early stimulation produced significantly (P < 0.000) DL at 3 and 14 dpm, followed by late stimulated carcasses and the NES. Duration of ES did not influence DL *per se* but longer stimulation (60 s) produced slightly higher DL compared with 30 s ES at 3 and 14 dpm. The interaction of heavy carcass weight and early ES produced a significantly (P < 0.05) higher drip loss at 14 dpm and a high tendency to be significant (P = 0.077) at 3 dpm. Other interactions were not significant.

4.8 Water Holding Capacity

One hundred and forty-four carcasses were evaluated for WHC at 3 and 14 dpm. The GLM procedure revealed significant difference ($P < 0.0001$) among treatment groups at 3 and 14 dpm. At 3 dpm, there was 29% variation among the treatment groups ($R^2 = 0.29$; $P < 0.0001$). Similar variation (29%; $R^2 = 0.29$) was observed at 14 dpm. Partial correlation was done to relate the two times of measurement. The correlation was weak (r = 0.033) and not significant ($P = 0.7001$). The GLM MANOVA test revealed no significant difference ($P > 0.05$) in time/treatment effect using Wilks' lambda, Pillai's test, Hotelling-Lawley trace and Roy's greatest root. Table 4.11 shows the GLM t- test (LSD) and SD for WHC at 3 and 14 dpm

Table 4.11 Mean values and standard deviation for water holding capacity at 3 and 14-days post-mortem

Treatments	**N**	**WHC 3 dpm Mean ± SD**	**WHC 14 dpm Mean ± SD**
1) S NES	20	0.45 ± 0.04^{a}	0.44 ± 0.04^{ab}
2) H NES	15	0.41 ± 0.03^{bc}	0.44 ± 0.04^{ab}
3) S 30 B	16	0.44 ± 0.05^{ab}	0.41 ± 0.05^{cd}
4) S 60 B	16	0.40 ± 0.03^{cd}	0.39 ± 0.03^{d}
5) S 30 A	14	0.45 ± 0.04^{a}	0.45 ± 0.03^{ab}
6) S 60 A	15	0.46 ± 0.04^{a}	0.44 ± 0.05^{ab}
7) H 30 B	13	0.42 ± 0.04^{bc}	0.39 ± 0.04^{d}
8) H 60 B	11	0.38 ± 0.03^{d}	0.40 ± 0.04^{cd}
9) H 30 A	14	0.47 ± 0.04^{a}	0.47 ± 0.03^{a}
10) H 60 A	14	0.45 ± 0.04^{a}	0.43 ± 0.03^{bc}

Key: N, Number of carcasses; NES, non-electrically stimulated carcasses; S, smaller carcasses; H, heavier carcasses; SD, Standard Deviation; 30, 30 seconds stimulation; 60, 60 seconds stimulation; B, early stimulation; A, late stimulation.

[a,b,c,d] Means in the same column without a common superscript letter differs ($P < 0.05$).

Figure 4.8 shows a graphical distribution of WHC at 3 and 14 dpm

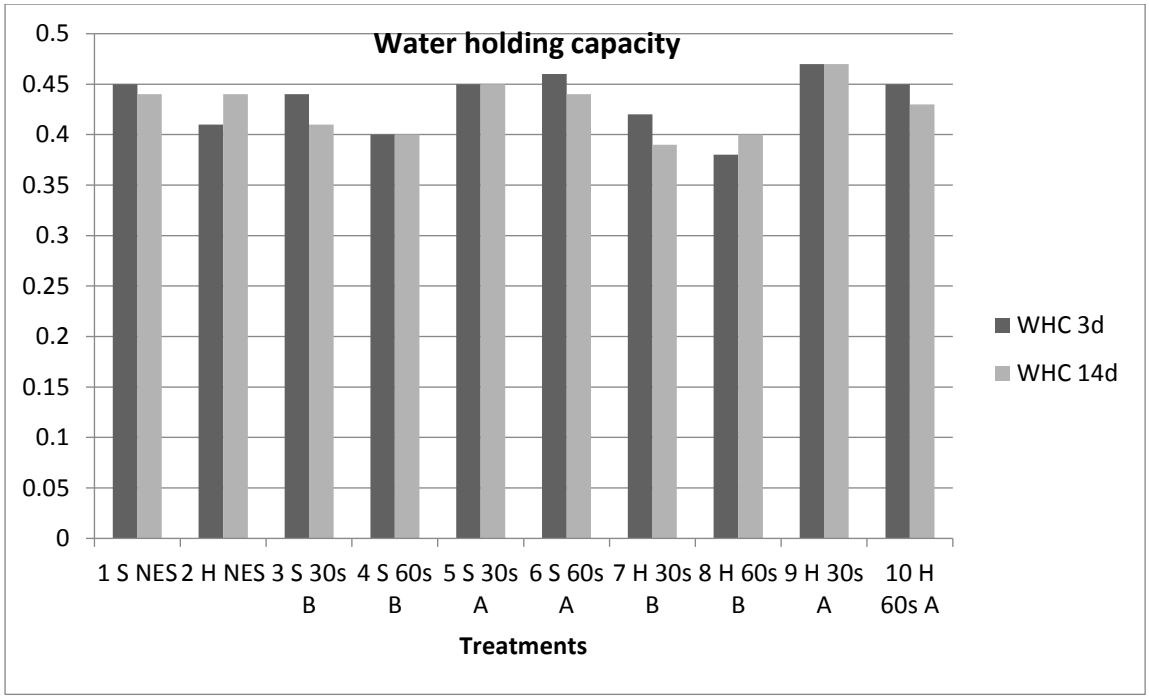

Figure 4.8 Distribution of mean values of water holding capacity at 3 and 14-days post-mortem according to treatment groups

Key: S**,** small carcasses; H, heavier carcasses; 30, 30 seconds ES; 60, 60 seconds ES; B, early stimulation; A, late stimulation; NES, non-electrically stimulated carcasses.

The illustration below shows the ranking of WHC at 3 and 14 dpm in descending order.

Water hoding capacity 3 dpm: **9** (H 30 A) > **6** (S 60 A) > **10** (H 60 A) > **1** (S NES) > **5** (S 30 A) > **3** (S 30 B) > **7** (H 30 B) > **2** (H NES) > **4** (S 60 B) > **8** (H 60 B)

Water holding capacity 14 dpm: 9 (H 30 A) > **5** (S 30 A) > **2** (H NES) > **6** (S 60 A) > **1** (S NES) > **10** (H 60 A) > **3** (S 30 B) > **8** (H 60 B) > **4** (S 60 B) > **7** (H 30 B)

Small numerical differences were observed between the 3 and 14 -day WHC for the groups (Table 4.11 and Figure 4.8). This was reflected in the partial correlation between the times, which showed a very weak and insignificant correlation (r = 0.033). According to the scoring (illustration above) and regarding the time of stimulation, there was a clear pattern of reaction.

Carcasses that were stimulated after evisceration and the controls (Treatments1, 5, 6, 9 and 10) had the highest water holding capacity, while carcasses that were stimulated before evisceration (3, 7, 4 and 8) had the lowest WHC at 3 and 14 dpm. Regarding the duration of stimulation, there was no definite pattern of reaction at both times of measurement. Also, regarding the influence of carcass weight on WHC, there was no clear pattern of reactions from the treatment groups at both times of measurement. At 3 dpm, the t- test revealed that Treatment 9, 6, 10 and 5 (late stimulated) were not significantly different ($P > 0.05$) from one another but differed from the other groups. Early stimulated carcasses (except Treatment 3) were not significantly different from one another and the other groups ($P < 0.05$) at 3 dpm. At 14 dpm, late stimulated carcasses and controls (Treatments 1, 2, 5, 6, 9 and 10) were not significantly different from one another but were significantly different from the other groups. Table 4.12 and 4.13 shows the mean values of the main effects and the significance of main effects and their interactions on WHC.

Table 4.12 Mean values and standard deviation of main effects on water holding capacity at 3 and 14-days post-mortem

Main effects		**N**	**WHC day 3 pm Mean ± SD**	**WHC day 14 pm Mean ± SD**
CW	**S**	70	0.44 ± 0.5	0.43 ± 0.5
	H	55	0.43 ± 0.5	0.43 ± 0.5
ES t	**0 (NES)**	23	0.44 ± 0.4	0.45 ± 0.4
	B	52	0.41 ± 0.5	0.40 ± 0.4
	A	50	0.46 ± 0.4	0.45 ± 0.4
ES d	**0 (NES)**	23	0.44 ± 0.4	0.45 ± 0.4
	30s	54	0.44 ± 0.5	0.43 ± 0.5
	60s	48	0.43 ± 0.5	0.42 ± 0.5

Key: CW, carcass weight; S, small carcasses; H, heavy carcasses; ES t, ES time; ES d, ES duration; 0 (NES), non-stimulated carcasses; B, ES before evisceration (early ES); A, ES after evisceration (late ES); 30s, 30 seconds stimulation; 60s, 60 seconds stimulation; SD, standard deviation.

Table 4.13 Significance carcass weight, electrical stimulation time and electrical stimulation duration on water holding capacity at 3 and 14-days post-mortem

Effects	***P* value**	**F ratio**	**SEM**	***P* value**	**F ratio**	**SEM**
Main effects	**WHC day 3**			**WHC day 14**		
CW	0.029	4.884	0.007	0.970	0.001	0.007
ES time (ES t)	0.000	36.873	0.006	0.000	39.900	0.006
ES dura (ES d)	0.010	6.839	0.008	0.052	3.851	0.010
Interactions	**WHC day 3**			**WHC day 14**		
CW x ES t	0.077	3.185	0.007	0.831	0.046	0.009
CW x ES d	0.346	0.896	0.008	0.323	0.986	0.009
ES t x ES d	0.061	3.570	0.008	0.299	1.087	0.008
CW x ESt x ES d	0.463	0.543	0.011	0.148	2.125	0.010

Key: CW, carcass weight; ES t, Electrical stimulation time; ES d, electrical stimulation duration; *P* = 0.05; SEM, standard error of means.

Heavier carcasses had slightly lower (numerically) but significant ($P < 0.05$) WHC compared with the smaller carcasses at 3 dpm, but at 14 dpm, there were no significant differences between carcass weights in terms of WHC (Tables 4.12 and 4.13). In terms of the influence of time of stimulation on WHC, early stimulation produced significantly lower WHC ($P < 0.000$) compared with the late stimulated carcasses and controls at 3 and 14 dpm. Longer duration of stimulation (60 s) produced slightly lower but significant ($P < 0.05$) WHC compared with shorter duration (30 s) ES and NES carcasses at 3 dpm and a high tendency to be significant ($P = 0.052$) at 14 dpm. The interactions of heavy carcasses and early stimulation also showed a high tendency to produce significantly ($P = 0.077$) lower WHC at 3 dpm, likewise the interaction of early stimulation and longer duration ($P = 0.061$) at 3 dpm.

4.9 Sarcomere length

One hundred and forty-eight carcasses were observed for SL at 3 dpm. GLM procedure revealed low variation ($R^2 = 0.066616$) between the treatment groups. The differences were also found insignificant ($P = 0.3709$). The mean values and standard deviation of SL for each treatment group and how they differed from one another are provided in Table 4.14.

Table 4.14 Mean values and SD of sarcomere length for each treatment groups

Treatments	N	SL (µm) Mean ± SD
1) S NES	**20**	1.94 ± 0.10^{ab}
2) H NES	**14**	1.91 ± 0.07^{b}
3) S 30 B	**16**	1.95 ± 0.06^{ab}
4) S 60 B	**16**	1.95 ± 0.08^{ab}
5) S 30 A	**14**	1.94 ± 0.08^{ab}
6) S 60 A	**15**	1.94 ± 0.05^{ab}
7) H 30 B	**13**	1.99 ± 0.09^{a}
8) H 60 B	**11**	1.96 ± 0.05^{a}
9) H 30 A	**14**	1.95 ± 0.04^{ab}
10) H 60 A	**15**	1.95 ± 0.06^{ab}

Key: N, Number of carcasses; SD, Standard Deviation; S, smaller carcasses; H, heavier carcasses; NES, non-ES carcasses; B, early stimulated carcasses; A, late stimulated carcasses; 30, 30 seconds ES; 60, 60 seconds ES

[a,b,c,d] Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.9 shows the distribution of sarcomere length according to the treatment groups.

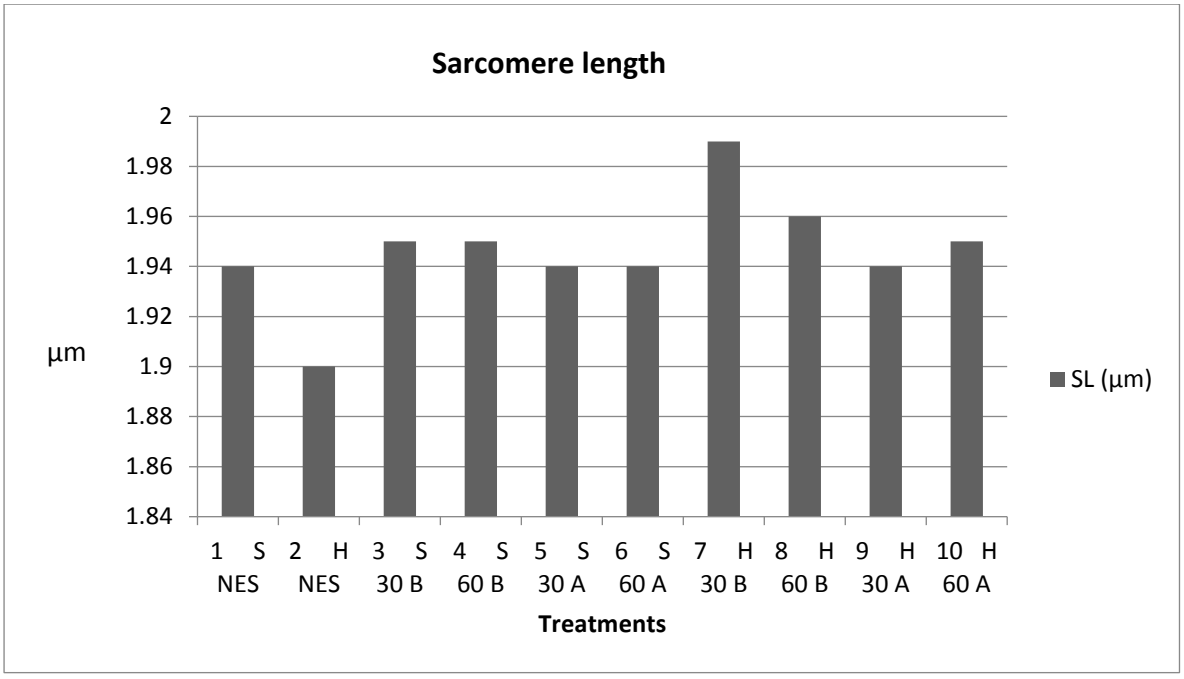

Figure 4.9 Distribution of sarcomere length according to treatment groups

Key: S, small carcasses; H, heavy carcasses; NES, non-electrically stimulated carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

This illustration shows the ranking of SL among treatments in descending order:

Sarcomere length (μm): **7** (H 30 B) > **8** (H 60 B) > **4** (S 60 B) > **10** (H 60 A) > **3** (S 30 B) > **9** (H 30 A) > **5** (S 30 A) > **6** (S 60 A) > **1** (S NES) > **2** (H NES)

There were no significant differences among treatment groups regarding the time of stimulation (Figure 4.9 and Table 4.14). In terms of the duration of stimulation, there was no significant difference among treatment groups. Analyses revealed a mixed reaction from the groups regarding carcass weight and there were no significant differences among treatments. There was no definite pattern of reaction from the two carcass weights, however, heavy early stimulated carcasses (7 and 8) had the longest SL, but not significantly different. Numerical differences between groups were low and negligible, and there was no sign of cold shortening in any of the carcasses.

4.10 Myofibril fragment length

One hundred and forty-nine carcasses were evaluated for myofibril fragment length (MFL) analysis at 3 and 14 dpm. GLM reveals very low variation among the treatment groups at 3 dpm (R^2 = 0.06; P = 0.4801) and 14 dpm (R^2 = 0.11; P = 0.0495). However, there were statistically significant differences (P < 0.05) between some of the groups at 3 and 14 dpm. Using the repeated measures ANOVA, partial correlation between the times of measurements showed moderate correlation (r = 0.54; P < 0.0001) between the 3 and 14-days measurements. MANOVA using Wilks' Lambda; Pillai's Trace; Hotelling-Lawley trace; and Roy's greatest root showed no significant difference (P = 0.2822) in the interaction of time and treatments for the groups. Table 4.15 shows the mean and standard deviations with significant differences between treatments.

Table 4.15 Mean and standard deviation for myofibril fragment length according to treatments at 3 and 14-days post-mortem

Treatments	**N**	**MFL 3 dpm Mean ± SD**	**MFL 14 dpm Mean ± SD**	**%Δ MFL**
1) S NES	**20**	49.73 ± 7.30^{ab}	34.76 ± 6.62^{ab}	30.10
2) H NES	**15**	48.23 ± 4.71^{ab}	33.73 ± 4.88^{b}	30.06
3) S 30 B	**16**	48.64 ± 10.44^{ab}	35.17 ± 7.60^{ab}	27.69
4) S 60 B	**16**	49.27 ± 7.48^{ab}	39.97 ± 9.19^{a}	19.67

5) S 30 A	**14**	46.08 ± 7.21[b]	32.11 ± 8.03[b]	30.32
6) S 60 A	**15**	48.42 ± 6.75[ab]	33.55 ± 9.72[b]	30.71
7) H 30 B	**13**	52.17 ± 5.94[a]	36.67 ± 13.05[ab]	29.71
8) H 60 B	**11**	52.52 ± 7.73[a]	40.77 ± 5.64[a]	22.37
9) H 30 A	**14**	48.48 ± 5.93[ab]	31.40 ± 9.20[b]	35.23
10) H 60 A	**15**	47.84 ± 5.94[ab]	32.55 ± 8.03[b]	31.44

Key: N, Number of carcasses; SD, Standard Deviation; S, small carcasses; H, heavy carcasses; 30, 30 seconds stimulation; 60, 60 seconds stimulation; A, late stimulation; B, early stimulation.

[a,b,c,d] Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.10 shows the distribution of MFL at 3 and 14 dpm for the treatment groups.

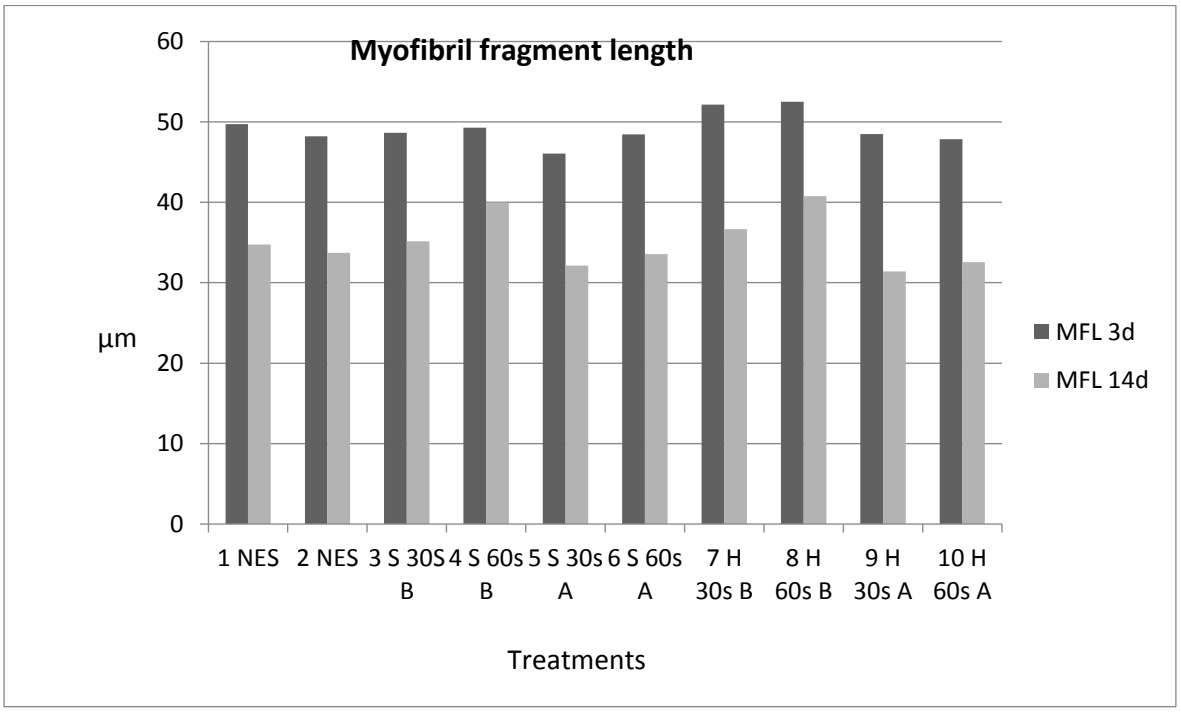

Figure 4.10 Distribution of mean values of myofibril fragment length in treatment groups at 3 and 14 dpm

Key: S, small carcass; H, heavy carcass; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

The following illustration shows the scoring of MFL in descending order, 3 and 14 dpm.

Myofribril fragment length 3 dpm: 8 (H 60 B) > **7** (H 30 B) > **1** (S NES) > **4** (S 60 B) > **3** (S 30 B) > **9** (H 30 A) > **6** (S 60 A) > **2** (H NES) > **10** (H 60 A) > **5** (S 30 A)

Myofibril fragment length 14 dpm: 8 (H 60 B) > **4** (S 60 B) > **7** (H 30 B) > **3** (S 30 B) > **1** (S NES) > **2** (H NES) > **6** (S 60 A) > **10** (H 60 A) > **5** (S 30 A) > **9** (H 30 A)

The early stimulated heavy carcasses (7 and 8) showed the longest MFL at 3 dpm (Table 4.15 and illustration above). However, there were no significant differences among groups. All other groups had numerically close values (except Group 5) and were not significantly ($P > 0.05$) different from one another. At 14 dpm, early stimulated carcasses (3, 4, 7 and 8) had the longest MFL. Of these groups, Treatments 4 and 8 (60 s ES) displayed the longest ($P < 0.05$) MFL compared with the late stimulated carcasses. Carcasses stimulated late (5, 6, 9 and 10) and the controls had the shortest MFL. Of these groups, treatments 5 and 9 (30 s ES), displayed the shortest MFL, regardless of the carcass weight. At 14 dpm, the early stimulated carcasses had the lowest mean percentage MFL degradation (24%), while the late stimulated carcasses had an average of 32% and controls, 30% degradation. The result suggests that late (45 min pm) and shorter (30 s) ES favoured MFL degradation but did not result into lower SF at 3 and 14 dpm, compared with carcasses stimulated early.

4.11 Meat Colour- Lightness

One hundred and forty-four carcasses were evaluated for meat lightness (L^*) at 3 and 14 dpm. GLM procedure revealed a moderate and significant variation ($R^2 = 0.47$, $P = 0.0001$ at 3 dpm; $R^2 = 0.40$ at 14 dpm, $P = 0.0001$) among the treatment groups. Partial correlation between the two times of measurement revealed a significant difference ($r = 0.76$; $P = 0.0001$). MANOVA test also revealed significant difference ($P < 0.05$) among the treatments at both times of measurement. Using GLM univariate test, the interaction of time and treatment was not significant ($P = 0.5704$). Table 4.16 shows the mean values and SD for meat lightness of treatment groups at 2 and 14 dpm.

Table 4.16 Mean and standard for meat lightness values of treatments at 2 and 14-days post mortem

Treatments	**N**	**L^* 2 day pm Mean ± SD**	**L^* 14 day pm Mean ± SD**
1) S NES	**20**	31.11 ± 1.51^{d}	33.66 ± 2.32^{d}
2) H NES	**13**	31.07 ± 1.40^{d}	33.69 ± 2.22^{d}
3) S 30 B	**15**	33.10 ± 2.35^{c}	34.96 ± 3.18^{cd}
4) S 60 B	**16**	35.70 ± 2.84^{ab}	37.63 ± 2.96^{b}
5) S 30 A	**14**	31.53 ± 2.08^{cd}	32.99 ± 2.97^{d}
6) S 60 A	**14**	30.93 ± 1.92^{d}	33.12 ± 2.16^{d}
7) H 30 B	**12**	35.06 ± 2.36^{b}	36.82 ± 2.43^{bc}

8) H 60 B	**11**	37.37 ± 4.03[a]	40.45 ± 3.15[a]
9) H 30 A	**14**	31.24 ± 1.47[d]	33.41 ± 2.64[d]
10) H 60 A	**14**	31.56 ± 3.10[cd]	33.58 ± 3.83[d]

Key: N, Number of carcasses; SD, standard deviation; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation; NES, non-electrically stimulated.

[a,b,c,d] Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.11 shows the distribution of mean L^* values at 2 and 14 dpm

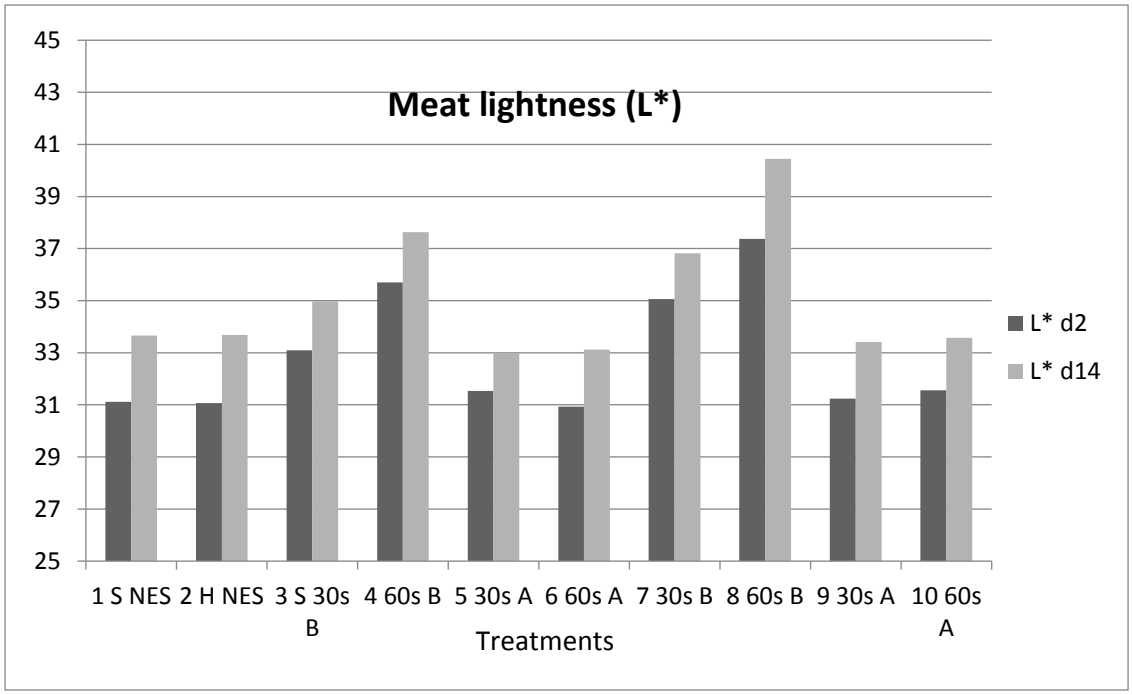

Figure 4.11 Distribution of mean values of meat lightness at 2 and 14-days post-mortem

Key: S, small carcass; H, heavy carcass; B, early stimulation; A, late stimulation; 30s, 30 seconds stimulation; 60s, 60 seconds stimulation.

The illustration below shows the ranking of meat lightness at 2 and 14 dpm, in descending order among the treatment groups.

Meat lightness (*L) 2 dpm: 8** (H 60 B) > **4** (S 60 B) > **7** (H 30 B) > **3** (S 30 B) > **10** (H 60 A) > **5** (S 30 A) > **9** (H 30 A) > **1** (S NES) > **2** (H NES) > **6** (S 60 A)

Meat lightness (*L) 14 dpm: 8** (H 60 B) > **4** (S 60 B) > **7** (H 30 B) > **3** (S 30 B) > **2** (H NES) > **1** (S NES) > **10** (H 60 A) > **9** (H 30 A) > **6** (S 60 A) > **5** (S 30 A)

There was a clear pattern of reaction from the treatment groups regarding the time of ES (Table 4.16 and the illustration above). Carcass groups (8, 7, 4 and 3) stimulated before evisceration/early stimulation (B) had the highest L* values ($P < 0.05$) followed by the groups stimulated after evisceration (A) and the controls. Regarding duration of stimulation, there was no definite pattern of reaction among the treatment groups. However, the 60 seconds stimulated carcasses (8 and 4) produced the lightest ($P < 0.05$) meat among the early stimulated carcasses at 3 and 14 dpm. There was no definite pattern of reaction in terms of carcass weight among the groups. This implies a weak influence of carcass weight on meat lightness. A similar trend was noted for the 14-days pm measurement of meat lightness, but at this time, the control groups were lighter than the groups stimulated after evisceration. All carcasses generally had increased *L** values from 2 to 14 dpm. The early and late stimulated sides had numerically close mean percentage increase (5.6 and 5.9% respectively), while the control sides had the highest percentage increase (7.7%) in *L** value. Table 4.17 and 4.18 shows the mean values of the main effects (ES time, duration and carcass weight) and their significance on L* values at 2 and 14 dpm.

Table 4.17 Mean values and standard deviations of main effects on meat lightness values at 2 and 14-days post-mortem

Main effects		***L*** day 2 pm* **Mean ± SD**	***L*** day 14 pm* **Mean ± SD**
CW	**S**	32.45 ± 2.78	34.55 ± 3.22
	H	33.05 ± 3.14	35.16 ± 3.95
ES t	**0 (NES)**	31.09 ± 1.44	33.59 ± 2.78
	B	35.18 ± 3.20	37.12 ± 3.62
	A	31.32 ± 2.18	33.37 ± 2.95
ES d	**0 (NES)**	31.09 ± 1.44	33.59 ± 2.78
	30s	32.66 ± 2.51	34.37 ± 3.19
	60s	33.77 ± 3.94	36.06 ± 4.15

Key: CW, carcass weight; S, small carcasses; H, heavy carcasses; ES t, electrical stimulation time; ES d, electrical stimulation duration; 0 (NES), non-electrically stimulated carcasses; B, stimulation before evisceration (early ES); A, stimulation after evisceration (late ES); 30s, 30 seconds stimulation; 60s, 60 seconds stimulation; SD, standard deviation.

Table 4.18 Significance of carcass weight, electrical stimulation time, electrical stimulation duration and their interactions on meat lightness values at 2 and 14-days post-mortem

Effects	***P* value**	**F ratio**	**SEM**	***P* value**	**F ratio**	**SEM**
Main effects	**L* day 2**			**L* day 14**		
CW	0.107	2.633	0.419	0.059	3.638	0.499
ES time (ES t)	0.000	75.265	0.331	0.000	57.118	0.395
ES dura (ES d)	0.022	5.371	0.326	0.005	8.321	0.388
Interactions	**L* day 2**			**L* day 14**		
CW x ES t	0.122	2.430	0.463	0.120	2.452	0.553
CW x ES d	0.599	0.278	0.493	0.567	0.329	0.588
ES t x ES d	0.004	8.562	0.482	0.007	7.551	0.574
CW x ES t x ES d	0.399	0.715	0.667	0.770	0.085	0.796

Key: CW, carcass weight; ES t, ES time; ES d, ES duration; *P* = 0.05; SEM, standard error of means.

Heavier carcasses had slightly higher *L** values at 2 and 14 dpm, and had a high tendency to be significant (P = 0.059) at 14 dpm (Tables 4.17 and 4.18). The time of stimulation (ES t) had a profound and significant effect (P < 0.000) at 2 and 14 dpm on *L** values. Early stimulated carcasses had the highest *L** values relative to late stimulated carcasses and controls at 2 and 14 dpm. Longer duration of stimulation (60 s) also produced significant differences (P < 0.05) on *L** values at 2 and 14 dpm compared 30 seconds stimulation and NES. The interaction of early stimulation and longer duration of stimulation also produced significantly (P < 0.05) higher *L** values at 2 and 14 dpm as shown in Table 4.18.

4.12 Meat Redness

One hundred and forty-four carcasses were evaluated for meat redness values (a* value) at 2 and 14 dpm. GLM reveals low but significant variations at both times of measurements between treatment groups. At 2 dpm, the variation was low, but significant at 22% (R^2 = 0.22; P = 0.0001); likewise, at 14 dpm, the variation was low but significant at 19% (R^2 = 0.19; *P* = 0.0007). Partial correlation between the two times of measurement revealed a small but significant relationship (r = 0.26; P < 0.05) between the 2 and 14 dpm measurements. Mean values and SD for the treatment groups (pair-wise comparison) are shown in Table 4.19.

Table 4.19 Mean and standard deviation of meat redness values at 2 and 14-days post-mortem

Treatments	N	a^* 2 dpm Mean ± SD	a^* 14 dpm Mean ± SD
1) S NES	18	9.87 ± 1.15^{bcd}	10.54 ± 1.39^{cd}
2) H NES	13	10.61 ± 0.93^{bc}	11.08 ± 1.44^{bc}
3) S 30 B	16	10.99 ± 2.01^{ab}	11.73 ± 2.15^{b}
4) S 60 B	16	10.65 ± 1.96^{bc}	11.60 ± 0.99^{b}
5) S 30 A	14	9.67 ± 1.49^{cd}	11.74 ± 1.96^{b}
6) S 60 A	13	10.59 ± 0.84^{bc}	13.16 ± 1.69^{a}
7) H 30 B	13	11.95 ± 2.33^{a}	11.41 ± 1.94^{b}
8) H 60 B	11	10.98 ± 1.83^{ab}	9.94 ± 2.06^{c}
9) H 30 A	14	8.92 ± 1.25^{d}	10.72 ± 1.36^{bc}
10) H 60 A	15	9.68 ± 1.14^{cd}	11.62 ± 1.71^{b}

Key: N, Number of carcasses; SD, standard deviation; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.12 shows the distribution of a^* values at 2 and 14 dpm for the treatment groups.

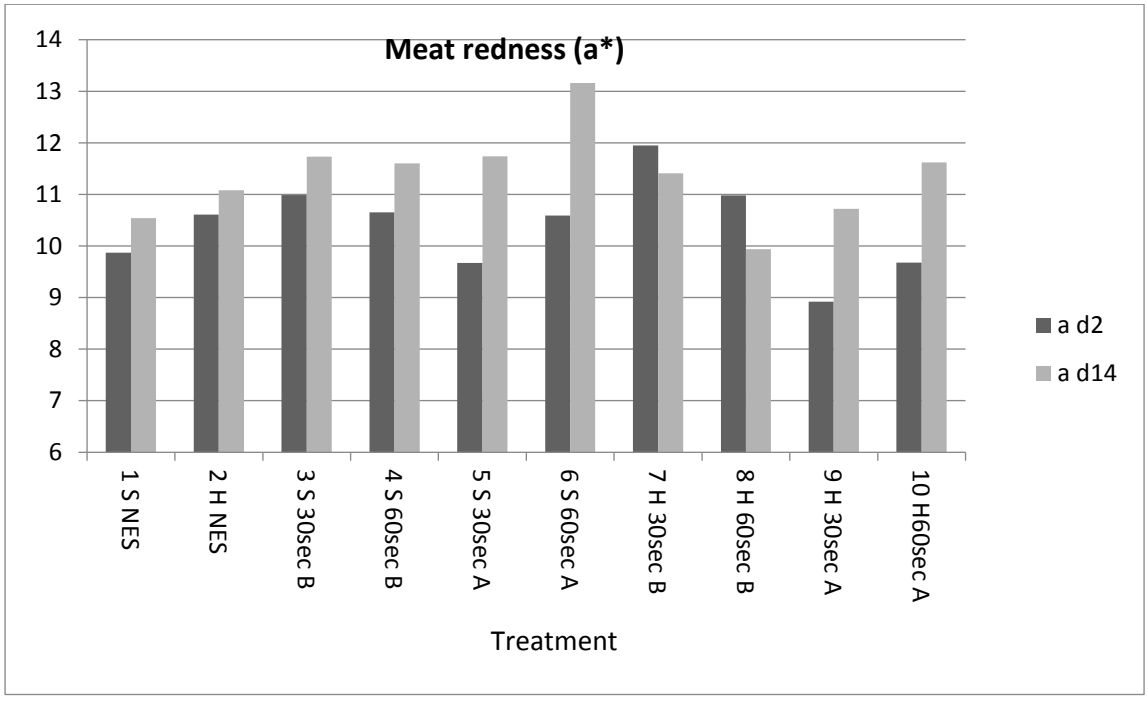

Figure 4.12 Graphical distribution of meat redness values at 2 and 14-days post-mortem

Key: S, small carcass; H, heavy carcass; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

The following illustration shows the reaction pattern and ranking of meat redness values according to treatment groups at 2 and 14 dpm in descending order.

Meat redness 2 dpm: 7 (H 30 B) > **3** (S 30 B) > **8** (H 60 B) > **4** (S 60 B) > **2** (H NES) > **6** (S 60 A) > **1** (S NES) > **10** (H 60 A) > **5** (S 30 A) > **9** (H 30 A)

Meat redness 14 dpm: 6 (S 60 A) > **5** (S 30 A) > **3** (S 30 B) > **10** (H 60 A) > **4** (S 60 B) > **7** (H 30 B) > **2** (H NES) > **9** (H 30 A) > **1** (S NES) > **8** (H 60 B)

At day 2 pm, there was a clear pattern of reaction among the treatment groups in relation to meat redness (a*) (See illustrations above and Table 4.19). Carcasses stimulated before evisceration (B) produced numerically higher a* compared with the ones stimulated after evisceration (A) and controls. However, the numerical differences were low between groups, as stated earlier. In terms of duration of stimulation, there were mixed reactions from the treatment groups. In terms of carcass weight there were mixed reactions and no clear pattern. This implied that carcass weight did not influence meat redness at 2 dpm.

At 14 dpm, there were mixed reactions from the treatment groups and no clear pattern of reaction in terms of time of stimulation. Regarding the duration of stimulation, there was no clear pattern of reaction from the treatment groups. That is, there were mixed reactions from the treatment groups. This suggested that meat redness was not influenced by duration of ES at 14 dpm. Regarding carcass weight, there were mixed reactions and there were no significant differences among the groups, except Treatment 6 (with the highest a*) and Treatment 8 (with the lowest a* value). Apart from Treatments 6 and 8, all other carcass groups had numerically close values. All carcass groups had marginal increases in a* values from 2 to 14 dpm except Treatments 8 and 7 (early stimulated, heavy carcasses) which recorded marginally lower a* value. This showed that LVES could improve a* values, even after 14 days of ageing.

4.13 Meat yellowness

One hundred and forty-four carcasses were evaluated for meat yellowness (b^* value) at 2 and 14 dpm. At 2 dpm there was moderately low but significant ($P < 0.0001$) variation among the groups (31%; $R^2 = 0.31$), while at 14 dpm, there was much lower but significant ($P = 0.0349$) variation (12%; $R^2 = 0.12$). Partial correlation between the times of measurements (2 and 14 dpm) showed a weak correlation ($r = 0.17$; $p = 0.049$). GLM ANOVA also showed significant differences

between the groups at 2 and 14 dpm (P = 0.0005). Table 4.20 shows the pairwise comparison of b^* values, mean values, and SD for the treatment groups at 2 and 14 dpm.

Table 4.20 Mean values and and standard deviation for meat yellowness values at 2 and 14-days post-mortem

Treatments	**N**	***b**** **2 dpm** **Mean ± SD**	***b**** **14 dpm** **Mean ± SD**
1) S NES	17	11.57 ± 0.74^{d}	12.54 ± 1.18^{bc}
2) H NES	15	11.87 ± 1.48^{cd}	12.84 ± 1.67^{abc}
3) S 30 B	16	12.89 ± 1.98^{bc}	13.18 ± 1.43^{abc}
4) S 60 B	16	13.31 ± 2.17^{ab}	13.75 ± 0.66^{a}
5) S 30 A	14	11.24 ± 0.97^{d}	12.95 ± 1.48^{abc}
6) S 60 A	15	11.47 ± 0.51^{d}	13.75 ± 1.49^{a}
7) H 30 B	13	13.75 ± 2.31^{ab}	13.43 ± 0.87^{ab}
8) H 60 B	11	14.22 ± 2.29^{a}	13.24 ± 0.89^{abc}
9) H 30 A	14	10.98 ± 0.89^{d}	12.35 ± 1.27^{c}
10) H 60 A	15	11.72 ± 1.68^{cd}	13.17 ± 1.10^{abc}

Key: N, Number of carcasses; SD, standard eviation; S, small carcasses; H, heavy carcasses; B, early ES; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation; NES, non-stimulated (control).
^{a,b,c,d} Means in the same column without a common superscript letters differ ($p < 0.05$).

Figure 4.13 shows the distribution of b^* values at 2 and 14 dpm for the treatment groups.

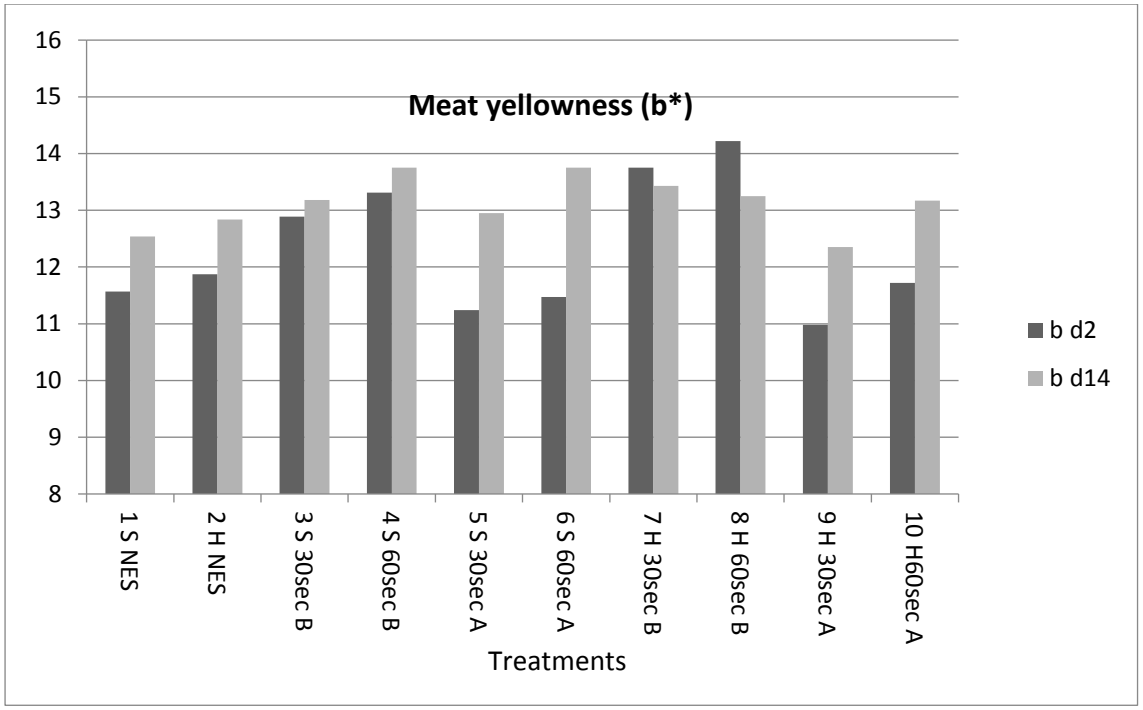

Figure 4.13 Distribution of meat yellowness values at 2 and 14-days post-mortem for the treatment groups

The following illustration shows the reaction pattern and ranking of b^* values according to treatment groups at 2 and 14 dpm, in descending order.

Meat yellowness 2 dpm: 8 (H 60 B) > **7** (H 30 B) > **4** (S 60 B) > **3** (S 30 B) > **2** (H NES) > **10** (H 60 A) > **1** (S NES) > **6** (S 60 A) > **5** (S 30 A) > **9** (H 30 A)

Meat yellowness 14 dpm: 6 (S 60 A) > **4** (S 60 B) > **7** (H 30 B) > **8** (H 60 B) > **3** (S 30 B) > **10** (H 60 A) > **5** (S 30 A) > **2** (H NES) > **1** (S NES) > **9** (H 30 A)

At 2 dpm, there was a clear pattern of reaction from treatments regarding the time of stimulation (see illustration (above) and Table 4.20). Carcasses that were stimulated before evisceration (B) had the highest ($P < 0.05$) meat yellowness values followed by the controls and the late stimulated carcasses (A). Regarding the effect of duration of stimulation on meat yellowness, there were mixed reactions from the treatment groups. However, 60 s ES produced the highest *b** values at 2 and 14 dpm. Concerning the effect of carcass weight on b^* value at 2 dpm, analysis revealed no clear pattern of reaction although, two heavy and early stimulated carcass groups (Treatments 8 and 7) had the highest ($P < 0.05$) values followed by Groups 4 and 3 (early stimulated). None of the other treatment groups showed definite patterns of reaction.

At 14 dpm, there were low numerical differences among treatment groups. However, there was a clear pattern of reaction in the time of stimulation. There were mixed reactions from the groups

and no significant differences in duration of stimulation, which indicatd a weak influence of duration of stimulation on meat yellowness. Regarding carcass weight influence on meat yellowness, there were also mixed reactions. However, two lighter carcass weight groups, stimulated for 60 seconds (Treatments 6 and 4), had the highest *b** values. Similar to a* values, Groups 7 and 8 (heavy, early stimulated carcasses) had marginal decreases in *b** values from 2 to 14 dpm while all other treatment groups including controls, had marginal increases (Table 4.20).

4.14 Meat Chroma

Meat chroma (C*) is derived from the intensity of the red colour or redness of meat (saturation index) (MacDougal, 1977). It is calculated as: $(a^{*\ 2} + b^{*2})^{1/2}$. A total of 144 carcasses were evaluated at 2 and 14 dpm. At 2 dpm, there was low but significant variation (28%), ($R^2 = 0.28$, $P < 0.0001$) among the treatment groups and at 14 dpm, there was even lower but also significant variation (15%), ($R^2 = 0.15$, $P = 0.0082$). Partial correlation between the two times of measurement shows a weak and insignificant correlation ($r = 0.1114$; $P = 0.2016$) between the two. GLM MANOVA showed significant difference ($P = 0.0003$) between the treatment groups in terms of C* at both times of measurements. Table 4.21 shows the mean values and SD for the treatment groups at 2 and 14 dpm for C*.

Table 4.21 Mean values and standard deviation for meat chroma at 2 and 14-days post-mortem

Treatments	**N**	**C* 2 dpm Mean ± SD**	**C* 14 dpm Mean ± SD**
1) S NES	17	15.25 ± 1.20^{cd}	16.36 ± 1.75^{c}
2) H NES	13	15.90 ± 1.49^{bc}	17.04 ± 2.07^{bc}
3) S 30 B	16	16.97 ± 2.62^{ab}	17.67 ± 2.40^{abc}
4) S 60 B	16	17.06 ± 2.84^{ab}	17.99 ± 1.00^{ab}
5) S 30 A	14	14.85 ± 1.58^{cd}	17.50 ± 2.24^{bc}
6) S 60 A	13	15.68 ± 0.71^{bcd}	18.97 ± 2.14^{a}
7) H 30 B	13	18.24 ± 3.14^{a}	17.66 ± 1.73^{abc}
8) H 60 B	11	17.99 ± 2.79^{a}	16.61 ± 1.82^{bc}
9) H 30 A	14	14.15 ± 1.42^{d}	16.37 ± 1.73^{c}
10) H 60 A	15	15.22 ± 1.81^{cd}	17.62 ± 1.49^{abc}

Key: N, Number of carcasses; SD, standard deviation; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.14 shows the distribution of meat chroma at 2 and 14 dpm for treatment groups.

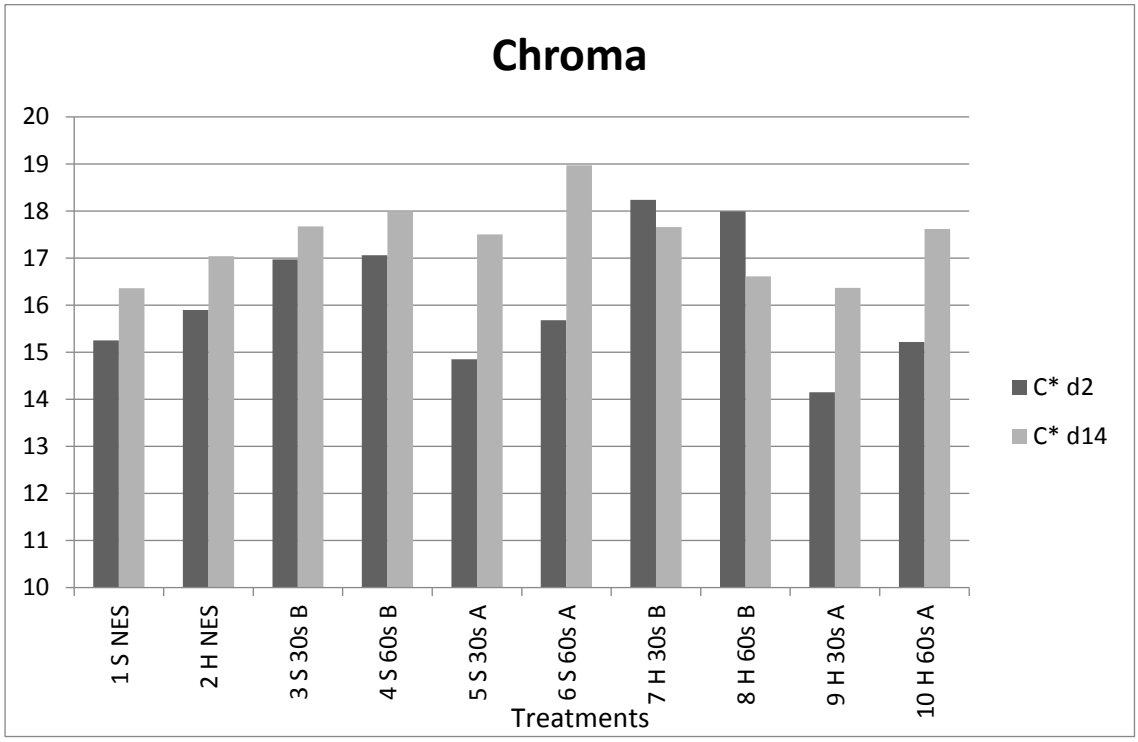

Figure 4.14 Distribution of meat chroma at 2 and 14 dpm according to treatment groups

Key: S, small carcass; H, heavy carcass; B, early stimulation; A, late stimulation; 30s, 30 seconds stimulation; 60s = 60 seconds stimulation.

The following illustration shows the reaction and ranking of meat chroma among treatment groups at 2 and 14 dpm in descending order.

Meat chroma 2 dpm: 7 (H 30 B) > **8** (H 60 B) > **4** (S 60 B) > **3** (S 30 B) > **2** (H NES) > **6** (S 60 A) > **1** (S NES) > **10** (H 60 A) > **5** (S 30 A) > **9** (H 30 A)

Meat chroma 14 dpm: 6 (S 60 A) > **4** (S 60 B) > **3** (S 30 B) > **7** (H 30 B) > **10** (H 60 A) > **5** (S 30 A) > **2** (H NES) > **8** (H 60 B) > **9** (H 30 A) > **1** (S NES)

At 2 dpm, there was a clear pattern of reaction from the treatment groups regarding the time of stimulation (Table 4.21 and illustration above). Carcasses that were stimulated before evisceration (B) (7, 8, 3 and 4) had the highest chroma values but the early stimulated heavy carcasses (7 and 8) were significantly higher ($P < 0.05$), followed by the controls and carcasses stimulated after evisceration (A). This trend is similar to meat redness and yellowness at 2 dpm and it's not surprising because chroma is a derivative of meat redness and yellowness. Regarding the effect of duration of stimulation on chroma, there was no clear pattern of reaction and no significant differences between carcasses and treatments. Regarding the effect of carcass weight on chroma

(C*), there were mixed reactions. However, heavy carcass groups (Treatments 7 and 8) had the highest chroma values at 2 dpm. This trend was also seen in meat yellowness. Treatments 7 and 8 (heavy, early stimulated carcasses) had significantly ($P < 0.05$) higher chroma values than all other treatment groups.

At 14 dpm, there were marginal increases in all treatment groups including the controls. However, marginal decreases were observed in Treatment 7 and 8. There was also low variation and low numerical differences in the chroma values of the groups. Similar trends were also observed in a* and b* profile. Regarding the effects of stimulation time on chroma, there were mixed reactions, but most of the carcasses that were stimulated before evisceration (B) (3, 4, and 7), had the higher chroma values but unexpectedly, Treatment 6 had the highest chroma at 14 dpm. Regarding the duration of stimulation, there were mixed reactions among the treatment groups. In terms of the effects of carcass weight on chroma, there were also mixed reactions but lighter carcasses (3, 4, 5 and 6) had the highest C* values. Late stimulated carcasses had the highest increase in chroma (average 17.4%) from 2 to 14 dpm compared with the early stimulated carcasses (4.8% for small carcasses and -5.5% for heavy carcasses) and controls (average 7.3%). This implied that late application of LVES favoured higher chroma values after ageing for 14 days. Table 4.22 and 4.23 shows the mean values of the main effects and their significance on chroma values.

Table 4.22 Mean values of main effects on meat chroma at 2 and 14-days post-mortem

Main effects		**C* day 2 pm Mean ± SD**	**C* day 14 pm Mean ± SD**
CW	**S**	15.99 ± 2.15	17.64 ± 2.08
	H	16.18 ± 2.66	17.08 ± 1.79
ES t	**0 (NES)**	15.53 ± 1.35	16.66 ± 1.89
	B	17.49 ± 2.82	17.55 ± 1.83
	A	14.97 ± 1.52	17.59 ± 2.07
ES d	**0 (NES)**	15.53 ± 1.35	16.66 ± 1.89
	30 s	16.04 ± 2.76	17.31 ± 2.08
	60 s	16.42 ± 2.41	17.85 ± 1.77

Key: CW, carcass weight; S, small carcasses; H, heavy carcasses; ES t, stimulation time; ES d, stimulation duration; 0 (NES), non-stimulated; B, stimulation before evisceration (early stimulation); A, stimulation after evisceration (late stimulation); 30 s, 30 s stimulation; 60 s, 60 seconds stimulation; SD, standard deviation.

Table 4.23 Significance of carcass weight, stimulation time and duration and their interactions on meat chroma 2 and 14-days post-mortem

Effects	*P* value	F ratio	SEM	*P* value	F ratio	SEM
Main effects	**C* day 2 pm**			**C* day 14 pm**		
CW	0.219	1.524	0.355	0.157	2.027	0.318
ES time (ES t)	0.000	42.988	0.281	0.591	0.290	0.252
ES dura (ES d)	0.293	1.115	0.276	0.117	2.485	0.247
Interactions	**C* day 2 pm**			**C* day 14 pm**		
CW x ES t	0.012	6.426	0.393	0.585	0.301	0.352
CW x ES d	0.618	0.250	0.418	0.368	0.816	0.374
ES t x ES d	0.296	1.103	0.409	0.030	4.817	0.366
CW x ES t x ES d	0.524	0.408	0.567	0.607	0.266	0.507

Key: CW, carcass weight; ES t, stimulation time; ES d, stimulation duration; *P* = 0.05; SEM, standard error of means.

Early stimulated carcasses produced a significantly higher ($P < 0.05$) chroma value at 2 dpm (Tables 4.22 and 4.23). At 14 dpm, there were no significant differences between the carcasses in terms of stimulation time. Carcass weight and duration of stimulation did not produce any significant difference in C* value. However, the interaction of heavy carcasses and early stimulation produced significantly higher chroma values at 2 dpm. Similarly, the interaction of longer duration of stimulation (60 s) and early stimulation (7 min pm) produced significantly higher chroma values at 14 dpm.

4.15 Hue angle

Hue angle (H*) which is a measure of discoloration is calculated as $\tan^{-1}$ (b^*/a^*) (Young, Priolo, Simmons and West, 1999). A total of 149 carcasses were evaluated. At 2 dpm, analysis revealed a low but significant variation (18.9%) ($R^2 = 0.189$; $P = 0.0008$) among the groups. Also, at 14 dpm, there was a significant but low variation (22%) ($R^2 = 0.22$; $P < 0.0001$) among the groups. Partial correlation between the two times of measurement reveals a high and significant correlation ($r = 0.70$; $P < 0.0001$). MANOVA results also revealed a significant difference ($P = 0.0122$) between the two times of measurements. Table 4.24 shows the mean values and SD for the treatment groups at 2 and 14 dpm.

Table 4.24 Mean values and standard deviation of Hue angle at 2 and 14-days post-mortem for treatment groups

Treatments	N	H^* 2 dpm Mean ± SD	H^* 14 dpm Mean ± SD
1 S NES	17	49.51 ± 2.64^{bcd}	50.16 ± 2.11^{b}
2 H NES	13	48.00 ± 2.68^{d}	49.35 ± 2.51^{b}
3 S 30 B	16	49.66 ± 3.62^{bcd}	48.63 ± 3.17^{b}
4 S 60 B	16	51.44 ± 2.44^{ab}	49.91 ± 2.08^{b}
5 S 30 A	14	49.48 ± 3.21^{bcd}	47.98 ± 3.36^{bc}
6 S 60 A	13	47.50 ± 2.28^{d}	46.07 ± 2.25^{c}
7 H 30 B	13	49.11 ± 3.09^{cd}	49.92 ± 4.00^{b}
8 H 60 B	11	52.33 ± 3.04^{a}	53.47 ± 4.52^{a}
9 H 30 A	14	51.06 ± 2.40^{abc}	49.13 ± 2.43^{b}
10 H 60 A	15	50.35 ± 3.32^{abc}	48.73 ± 4.64^{b}

Key: N, Number of carcasses; SD, standard deviation; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation

[a,b,c,d] Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.15 shows the graphical distribution of Hue angle at 2 and 14 dpm for the treatment groups.

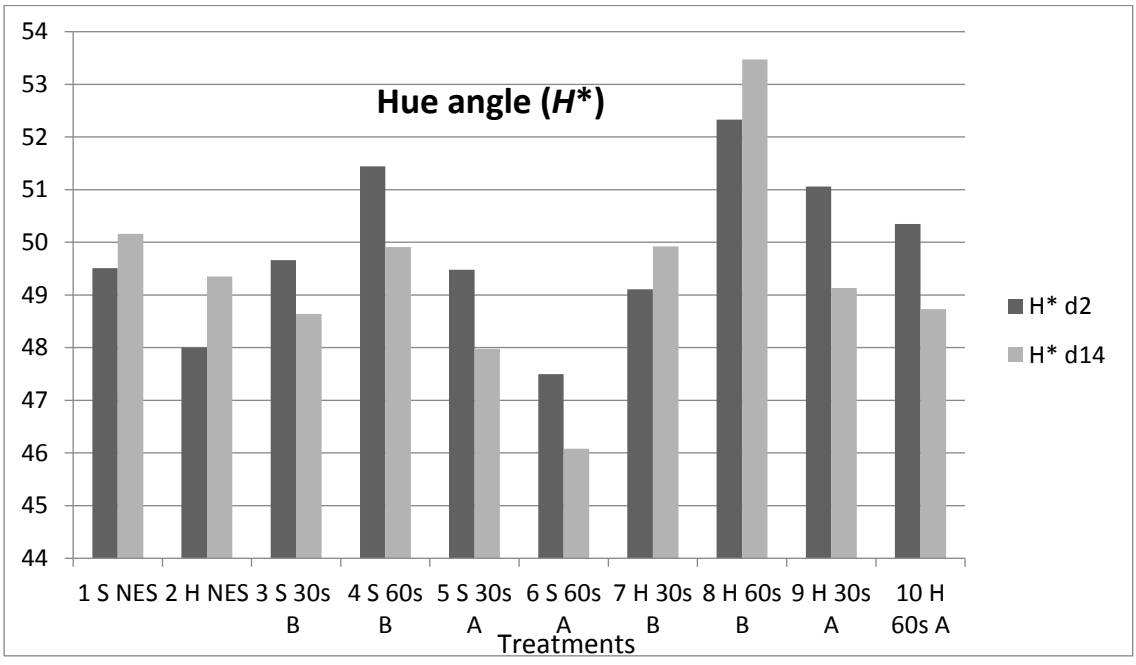

Figure 4.15 Bar chart of Hue angle at 2 and 14 dpm for the treatment groups

Key: S, small carcass; H, heavy carcass; B, early stimulation; A, late stimulation; 30s, 30 seconds stimulation; 60s, 60 seconds stimulation.

The following illustration shows the reaction pattern and ranking of Hue angle according to treatment groups at 2 and 14 dpm in descending order.

Hue angle 2 dpm: 8 (H 60 B) > **4** (S 60 B) > **9** (H 30 A) > **10** (H 60 A) > **3** (S 30 B) > **1** (S NES) > **5** (S 30 A) > **7** (H 30 B) > **2** (H NES) > **6** (S 60 A)

Hue angle 14 dpm: 8 (H 60 B) > **1** (S NES) > **7** (H 30 B) > **4** (S 60 B) > **2** (H NES) > **9** (H 30 A) > **10** (H 60 A) > **3** (S 30 B) > **5** (S 30 A) > **6** (S 60 A)

From the ranking above, at 2 dpm, regarding the time of stimulation, there were mixed reactions from the treatment groups. However, two of the carcass groups stimulated before evisceration (B) (4 and 8) for 60 seconds, had numerically highest but not significantly different hue angle. Regarding the effects of carcass weight on hue angle, there were mixed reactions from the groups, which suggested weak or no influence of carcass weight on H*.

At 14 dpm, regarding the time of stimulation, there was a clear pattern of reaction from the treatment groups. There were no significant differences from most of the treatment groups except Treatments 5 and 6 (smaller carcasses stimulated after evisceration) (Table 4.24), which had the lowest ($P < 0.05$) H*. Treatment 8 (heavy carcasses stimulated early for 60 s) had the highest ($P < 0.05$) H* values. Table 4.25 and 4.26 shows the mean values of the main effects (carcass weight, ES time and ES duration) and the significance of the main effects on H*, respectively.

Table 4.25 Mean values and standard deviation of main effects on hue angle at 2 and 14-days post-mortem

Main effects		**H* day 2 pm Mean ± SD**	**H* day 14 pm Mean ± SD**
CW	**S**	49.60 ± 3.06	41.31 ± 2.94
	H	50.13 ± 3.19	40.04 ± 3.96
ES t	**0 (NES)**	48.86 ± 2.72	40.19 ± 2.29
	B	50.57 ± 3.25	39.75 ± 3.75
	A	49.65 ± 3.08	41.97 ± 3.46
ES d	**0 (NES)**	48.86 ± 2.72	40.19 ± 2.29
	30s	49.83 ± 3.13	41.11 ± 3.26

60s	50.39 ± 3.24	40.61 ± 4.23

Key: CW, carcass weight; S, small carcasses; H, heavy carcasses; ES t, stimulation time; ES d, stimulation duration; 0 (NES), non-stimulated; B, early stimulation; A, late stimulation; 30s, 30 seconds stimulation; 60s, 60 seconds stimulation; SD, standard deviation.

Table 4.26 Significance of carcass weight, electrical stimulation time and duration and their interactions on hue angle at 2 and 14-days post-mortem

Effects	***P* value**	**F ratio**	**SEM**	***P* value**	**F ratio**	**SEM**
Main effects	**H* day 2 pm**			**H* day 14 pm**		
CW	0.363	0.834	0.492	0.018	5.774	0.519
ES time (ES t)	0.030	4.793	0.390	0.000	23.710	0.411
ES dura (ES d)	0.456	0.559	0.383	0.602	0.273	0.404
Interactions	**H* day 2 pm**			**H* day 14 pm**		
CW x ES t	0.154	2.061	0.545	0.293	1.117	0.575
CW x ES d	0.418	0.659	0.580	0.311	1.037	0.612
ES t x ES d	0.001	12.051	0.566	0.002	9.740	0.598
CW x ES t x ES d	0.956	0.003	0.785	0.728	0.121	0.828

Key: CW, carcass weight; ES t, stimulatin time; ES d, stimulation duration; *P* = 0.05; SEM, standard error of means.

Early stimulation brought about a significantly higher ($P < 0.05$) H* at 2 dpm (Tables 4.25 and 4.26), but at 14 dpm, the late stimulated carcasses produced the highest ($P < 0.000$) H* (Table 4.26). Heavier carcasses produced significantly lower ($P < 0.05$) H* although the numerical difference was low between the lighter and heavier carcasses was low. The interaction of early stimulation and longer duration also produced the highest H* value at 2 and 14 dpm (Table 4.26). At 14 dpm, the non-stimulated controls (1 and 2) and the early stimulated heavy carcasses (7 and 8) had marginal increases in H* values while all other carcass groups had marginal decreases in H* values.

4.16 Energy metabolites

4.16.1 Lactate

Fifty-seven samples were evaluated for lactate content of meat, but 54 samples were used for the analysis owing to missing values and outliers. Samples were collected at 1, 4 and 24 h pm. GLM procedure revealed significant variation among the treatment groups; at 1 h pm ($R^2 = 0.70$, $P < 0.0001$), at 4 h pm ($R^2 = 0.56$; $P < 0.0001$) and at 24 h pm, the variation was low and insignificant ($R^2 = 0.22$, $P = 0.2120$). Partial correlation between the times of measurement shows positive and significant ($P < 0.05$) correlations between the time levels. Table 4.27 shows the mean values, SD and p- values for muscle lactate content at the various times of measurement.

Table 4.27 Mean, standard deviation and *P*- values for muscle lactate content (µmol/g) at 1, 4 and 24-hours post-mortem

Treatments	N	Lact 1 h pm Mean ± SD	Lact 4 h pm Mean ± SD	Lact 24 h pm Mean ± SD	% Δ Lact (1-4 h pm)	% Δ Lact (1-24 h pm)
1) S NES	7	29.10 ± 8.24^{c}	38.76 ± 10.26^{d}	76.82 ± 9.31^{b}	33.2	164.0
2) H NES	6	29.04 ± 2.51^{c}	48.98 ± 12.12^{cd}	81.03 ± 3.88^{b}	68.7	179.0
3) S 30 B	5	47.68 ± 9.34^{b}	65.10 ± 14.39^{bc}	91.64 ± 19.90^{ab}	36.5	92.2
4) S 60 B	3	58.95 ± 9.64^{a}	81.67 ± 4.70^{ab}	96.24 ± 13.62^{ab}	38.5	63.3
5) S 30 A	5	32.45 ± 4.30^{c}	44.90 ± 4.83^{d}	89.78 ± 19.24^{ab}	38.4	176.7
6) S 60 A	6	31.34 ± 7.44^{c}	47.73 ± 14.35^{cd}	92.24 ± 13.11^{ab}	52.3	194.3
7) H 30 B	6	54.80 ± 13.62^{ab}	73.61 ± 11.08^{ab}	103.19 ± 12.11^{a}	34.3	88.3
8) H 60 B	5	60.80 ± 14.02^{a}	83.89 ± 20.60^{a}	95.51 ± 22.99^{ab}	38.0	57.1
9) H 30 A	5	29.72 ± 4.71^{c}	52.06 ± 10.60^{cd}	84.58 ± 18.17^{ab}	75.2	184.6
10) H 60 A	6	29.87 ± 8.70^{c}	47.66 ± 25.31^{cd}	89.02 ± 19.82^{ab}	59.6	198.0

Key: N, Number of carcasses; SD, standard deviation; Lact, Lactate; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.
^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.16 shows the distribution of the mean values of treatment groups at different times, according to their lactate content.

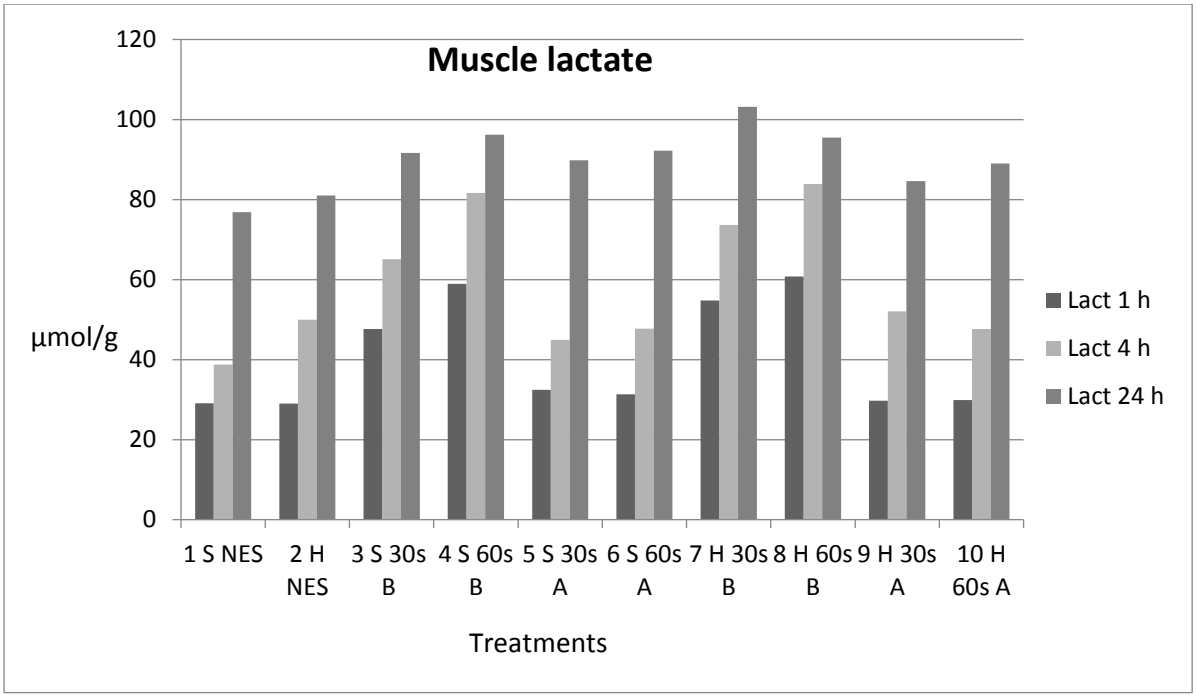

Figure 4.16 Distribution of muscle lactate content at 1, 4 and 24-hours post-mortem for treatment groups

Key: S, small carcass; H, heavy carcass; B, early stimulation; A, late stimulation; 30s = 30 second stimulation; 60s = 60 seconds stimulation

The following illustration shows the ranking of the muscle lactate content according to treatment groups at 1, 4 & 24 h pm in descending order.

Lactate 1 h pm: 8 (H 60 B) > **4** (S 60 B) > **7** (H 30 B) > **3** (S 30 B) > **5** (S 30 A) > **6** (S 60 A) > **10** (H 60 A) > **9** (H 30 A) > **1** (S NES) > **2** (H NES)

Lactate 4 h pm: 8 (H 60 B) > **4** (S 60 B) > **7** (H 30 B) > **3** (S 30 B) > **9** (H 30 A) > **2** (H NES) > **6** (S 60 A) **10** (H 60 A) > **5** (S 30 A) > **1** (S NES)

Lactate 24 h pm: 7 (H 30 B) > **4** (S 60 B) > **8** (H 60 B) > **6** (S 60 A) > **3** (S 30 B) > **5** (S 30 A) > **10** (H 60 A) > **9** (H 30 A) > **2** (H NES) > **1** (S NES)

There was a clear pattern of reaction at 1 h pm (Table 4.27 and the illustration above). Carcasses stimulated before evisceration (8, 7, 4 and 3) (B) had the highest ($P < 0.05$) lactate content at 1, 4 and 24 h pm, followed by carcasses stimulated after evisceration (A) (5, 6, 9 and 10) (A) and controls (1 and 2). Regarding the duration of stimulation, there were mixed reactions. However, Groups 8 and 4 which were stimulated early for 60 seconds had the highest ($P < 0.05$) lactate content at 1 h pm. In terms of carcass weight, there were mixed responses.

At 4 h pm, a similar pattern of reaction to 1 h pm was observed. Carcasses stimulated before evisceration (8, 7, 4 and 3) (B) had the highest ($P < 0.05$) lactate content, followed by carcasses stimulated after evisceration and controls. Regarding the duration of stimulation, similar pattern to 1 h pm was observed. Treatments 8 and 4 had the highest ($P < 0.05$) muscle lactate content which differed significantly from the late stimulated carcasses and controls, but not the 30 s early stimulated carcasses. In terms of carcass weight, there was also a mixed pattern of reaction, which implied little or no influence of carcass weight on muscle lactate content. At this time, the late stimulated carcasses had the highest (56.4%) mean lactate increase rate in the muscle, followed by the NES carcasses (50.9%), while the early stimulated carcasses had the lowest (36.8%) mean lactate increase in the muscle.

At 24 h pm, there was a clear pattern of reaction. Most of the early stimulated carcasses had the highest muscle lactate, followed by the late stimulated and controls. However, there were lower variations among the groups and there were no significant differences among treatments except between Treatment 8 (highest lactate content) and the controls (lowest lactate content). Regarding the duration of stimulation, there were mixed reactions. In terms of carcass weight, there were also mixed reactions. This implied lack of influence of carcass weight on lactate content at 24 h pm. A similar trend to 4 h pm was observed in the rate of muscle lactate increase. The late stimulated carcasses had a much higher rate of mean lactate increase (188.4%) followed by the NES carcasses (171.5%), while the early stimulated carcasses had the lowest mean increase rate (75.2%) from 1 to 24 h pm.

4.16.2 Glucose

A total of 56 meat samples were evaluated for muscle glucose content at 1, 4 and 24 h pm. GLM procedure revealed these variations between the groups: at 1 h pm ($R^2 = 0.42$, $P = 0.0015$), at 4 h pm ($R^2 = 0.62$, $P < 0.0001$), while at 24 h pm ($R^2 = 0.44$, $P = 0.0009$). Repeated measure ANOVA univariate test for the effect of time and treatments revealed a significant difference ($P = 0.0004$) between the treatment groups.

Table 4.28 shows the mean values, SD and p- values of muscle glucose content at different time of measurement.

Table 4.28 Mean glucose values (µmol/g) and standard deviation for the treatment groups at 1, 4 and 24-hours post-mortem

Treatments	N	Glu 1 h pm Mean ± SD	Glu 4 h pm Mean ± SD	Glu 24 h pm Mean ± SD	% Δ Glu (1-4 h pm)	% Δ Glu (1-24 h pm)
1) S NES	7	0.75 ± 0.30^{c}	1.10 ± 0.35^{f}	2.47 ± 0.30^{d}	46.7	229.3
2) H NES	6	0.68 ± 0.39^{c}	0.95 ± 0.45^{f}	3.09 ± 0.69^{cd}	39.7	354.4
3) S 30 B	5	1.42 ± 1.17^{ab}	2.53 ± 1.29^{bcd}	3.33 ± 1.20^{cd}	78.2	134.5
4) S 60 B	4	2.08 ± 1.10^{a}	3.28 ± 0.68^{ab}	3.93 ± 1.11^{abc}	57.7	88.9
5) S 30 A	5	0.83 ± 0.42^{c}	2.26 ± 1.37^{cde}	4.96 ± 2.11^{a}	172.3	497.6
6) S 60 A	6	0.80 ± 0.34^{c}	1.34 ± 0.37^{ef}	2.93 ± 0.43^{cd}	67.5	266.3
7) H 30 B	6	1.80 ± 0.72^{ab}	2.84 ± 0.92^{abc}	3.51 ± 0.72^{bcd}	57.8	95.0
8) H 60 B	5	1.69 ± 0.94^{ab}	3.63 ± 0.98^{a}	4.57 ± 1.06^{ab}	114.8	170.4
9) H 30 A	6	0.67 ± 0.19^{c}	1.22 ± 0.36^{f}	2.51 ± 0.80^{d}	82.1	274.6
10) H 60 A	6	1.13 ± 0.33^{bc}	1.82 ± 0.45^{def}	3.76 ± 0.67^{bc}	61.1	232.7

Key: N, Number of carcasses; SD, standard deviation; Glu, glucose; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.
^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.17 shows the distribution of the mean values of the treatment groups according to their glucose content at 1, 4 and 24 h pm.

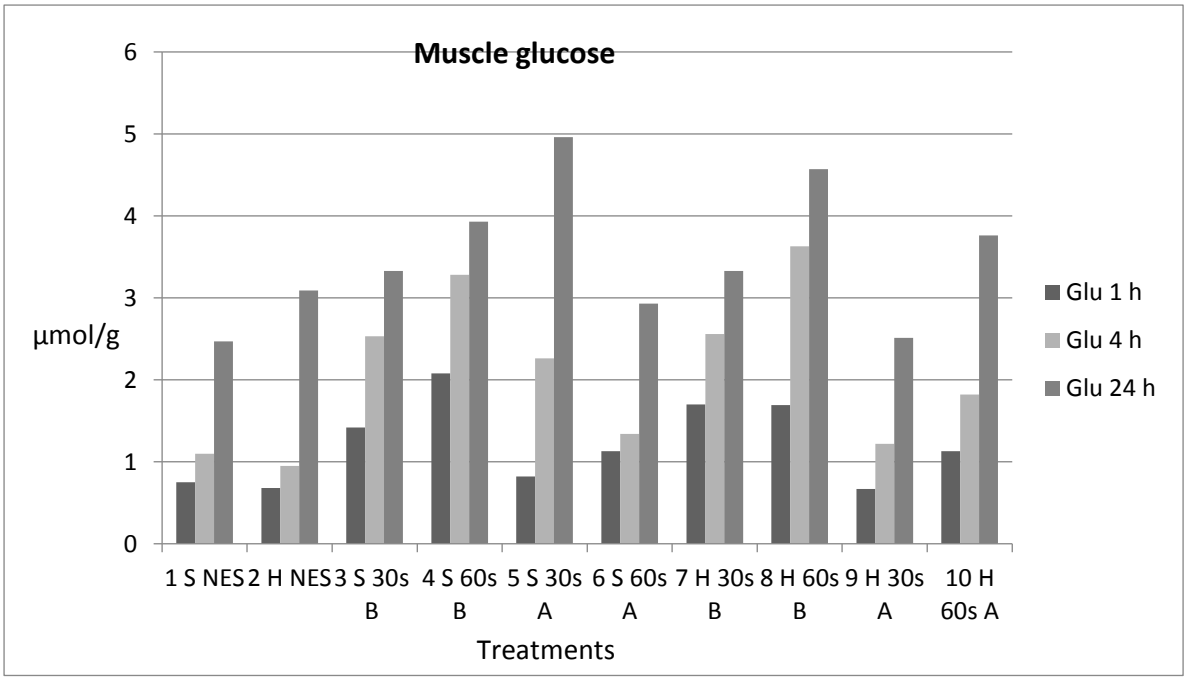

Figure 4.17 Distribution of muscle glucose content for treatment groups at 1, 4 and 24-hour post-mortem

Key: S, small carcass; H, heavy carcass; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation

The illustration below shows the ranking of the muscle glucose content according to the treatment groups at 1, 4 and 24 h pm in descending order.

Glucose 1 h pm: 4 (S 60 B) > **7** (H 30 B) > **8** (H 60 B) > **3** (S 30 B) > **10** (H 60 A) > **5** (S 30 A) > **6** (S 60 A) > **1** (S NES) > **2** (H NES) > **9** (H 30 A)

Glucose 4 h pm: 8 (H 60 B) > **4** (S 60 B) > **7** (H 30 B) > **3** (S 30 B) > **5** (S 30 A) > **10** (H 60 A) > **6** (S 60 A) > **9** (H 30 A) > **1**(S NES) > **2** (H NES)

Glucose 24 h pm: 5 (S 30 A) > **8** (H 60 B) > **4** (S 60 B) > **10** (H 60 A) > **7** (H 30 B) > **3** (S 30 B) > **2** (H NES) > **6** (S 60 A) > **9** (H 30 A) > **1** (S NES)

At 1 h pm, there was a clear pattern of reaction (Table 4.28 and the illustration above). Carcasses stimulated before evisceration (8, 7, 4 and 3) had the highest ($P < 0.05$) muscle glucose content, followed by carcasses stimulated after evisceration and controls. For duration of stimulation, there were mixed reactions, that is, no definite pattern, likewise carcass weight. At 4 h pm, a similar pattern of reaction to 1 h pm was observed for time of stimulation. Carcasses stimulated before evisceration (B) had the highest ($P < 0.05$) muscle glucose content followed by the late stimulated carcasses (A). Regarding the duration of stimulation, there was a mixed pattern of reaction, but the groups stimulated early for 60 seconds (8 and 4) had the highest glucose content (Table 4.28). Regarding carcass weight, there was also a mixed reaction, and this suggested a weak or no influence of carcass weight on muscle glucose content at 4 h pm. Late stimulated carcasses had the highest mean increase (95.8%) in muscle glucose followed by the early stimulated carcasses (77.1%), while the NES had the lowest increase in muscle glucose at 4 h pm. At 24 h pm, there was no clear pattern of reaction in terms of the time of stimulation, likewise the duration of ES and carcass weight. At 24 h pm, the late stimulated carcasses displayed significantly greater rate of increase in muscle glucose content (317%) compared with 122.2% from the early stimulated carcasses and 291.9% for the NES carcasses from 1 to 24 h pm.

4.16.3 Glycogen

Fifty-seven carcasses were evaluated for muscle glycogen content, but 51 values were used for the analysis because of outliers. At 1 h pm, there was significant variation among the treatment groups ($R^2 = 0.55$, $P < 0.0001$); at 4 h pm, there was higher and significant variation ($R^2 = 0.60$; $P < 0.0001$); likewise, at 24 h pm there was significant variation ($R^2 = 0.41$; $P = 0.0056$) among

treatment groups. GLM repeated ANOVA showed significant differences ($P < 0.0001$) among the treatment groups at each time of measurement. GLM univariate test of hypothesis for the combination of the effect of time with treatments also showed significant effect ($P = 0.0021$). Regarding the correlation between the time levels, muscle glycogen at 1 h pm correlated moderately with glycogen at 4 h pm ($r = 0.40$), while it had a weak correlation with the 24 h pm glycogen value. On the other hand, muscle glycogen at 4 h pm correlated strongly ($r = 0.56$) with the 24 h pm glycogen value. Table 4.29 shows the mean values of each treatment group, SD and significant differences for muscle glycogen content at 1, 4 and 24 h pm.

Table 4.29 Mean glycogen values (µmol/g) and standard deviation for treatment groups at 1, 4 and 24-hour post-mortem

Treatments	**N**	**Gly 1 h Mean ± SD**	**Gly 4 h Mean ± SD**	**Gly 24 h Mean ± SD**	**% Δ Gly (1-4h pm)**	**% Δ Gly (1-24h pm)**
1) S NES	5	31.25 ± 6.58^{b}	19.79 ± 9.62^{bc}	9.15 ± 2.33^{bc}	36.7	70.7
2) H NES	5	25.97 ± 8.17^{b}	21.38 ± 10.99^{b}	7.47 ± 2.01^{bc}	17.7	71.2
3) S 30 B	5	22.12 ± 10.82^{b}	5.36 ± 0.82^{e}	3.63 ± 1.17^{c}	75.8	83.6
4) S 60 B	4	23.46 ± 13.60^{b}	9.14 ± 3.99^{de}	5.25 ± 1.94^{c}	61.0	77.6
5) S 30 A	4	29.55 ± 15.25^{b}	19.64 ± 11.40^{bc}	7.30 ± 4.06^{bc}	34.6	75.3
6) S 60 A	6	35.25 ± 8.75^{b}	23.22 ± 8.04^{b}	12.63 ± 6.25^{ab}	34.1	64.2
7) H 30 B	6	20.19 ± 8.08^{b}	10.79 ± 5.80^{cde}	6.30 ± 1.90^{c}	46.6	68.8
8) H 60 B	4	31.67 ± 21.82^{b}	7.61 ± 3.19^{e}	6.08 ± 2.98^{c}	76.0	81.6
9) H 30 A	6	28.86 ± 11.08^{b}	18.57 ± 4.27^{bcd}	8.63 ± 5.21^{bc}	35.7	70.1
10) H 60 A	6	61.69 ± 13.63^{a}	33.15 ± 9.50^{a}	15.60 ± 9.29^{a}	46.3	74.7

Key: N, Number of carcasses; SD, Standard Deviation; Gly, Glycogen; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.
^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.18 shows the distribution of mean values of glycogen content in muscle among the treatment groups at 1, 4 and 24 h pm.

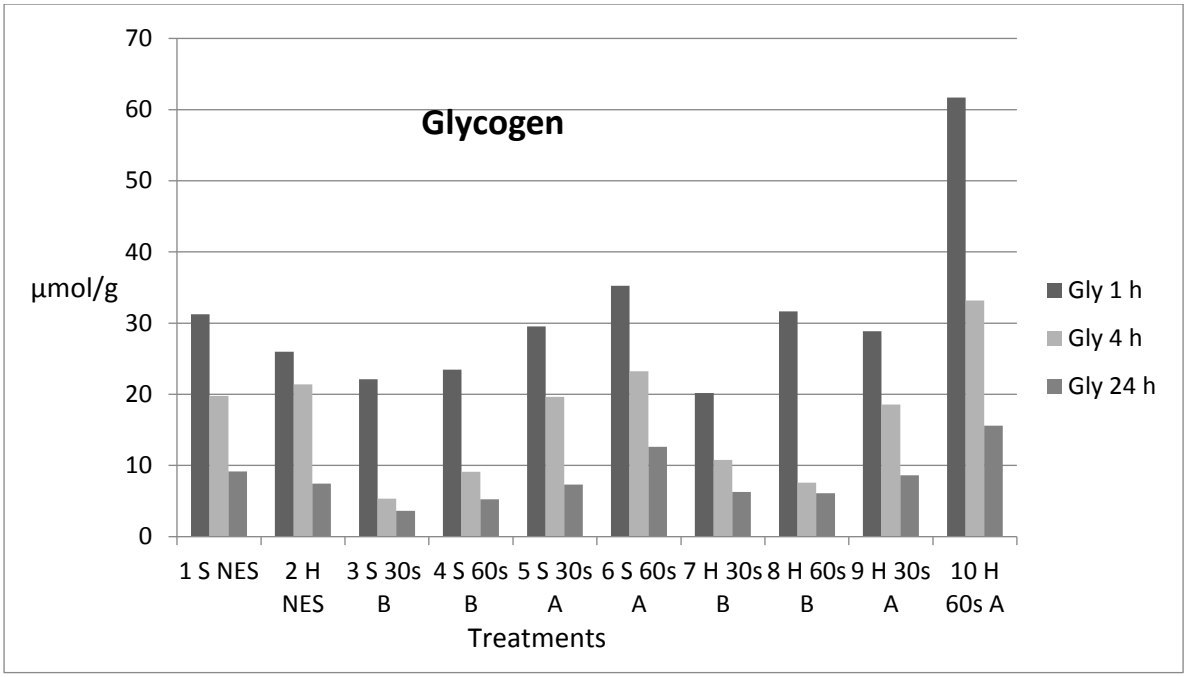

Figure 4.18 Distribution of muscle glycogen content among treatment groups at 1, 4 and 24-hours post-mortem

Key: S, small carcass; H, heavy carcass; B, early stimulation; A, late stimulation; 30s, 30 seconds stimulation; 60s, 60 seconds stimulation.

The following illustration shows the ranking of the muscle glycogen content according to treatment groups at 1, 4 and 24 h pm in descending order.

Glycogen 1 h pm: 10 (H 60 A) > **6** (S 60 A) > **8** (H 60 B) > **1** (S NES) > **5** (S 30 A) > **9** (H 30 A) > **2** (H NES) > **4** (S 60 B) > **3** (S 30 B) > **7** (H 30 B)

Glycogen 4 h pm: 10 (H 60 A) > **6** (S 60 A) > **2** (H NES) > **1** (S NES) > **5** (S 30 A) > **9** (H 30 A) > **7** (H 30 B) > **4** (S 60 B) > **8** (H 60 B) > **3** (S 30 B)

Gly 24 h pm: 10 (H 60 A) > **6** (S 60 A) > **1** (S NES) > **9** (H 30 A) > **2** (H NES) > **5** (S 30 A) > **7** (H 30 B) > **8** (H 60 B) > **4** (S 60 B) > **3** (S 30 B)

At 1 h post-mortem, in terms of time of stimulation, there was a clear pattern of reaction from the treatment groups. Most of the groups that were stimulated after evisceration (5, 6, 9 and 10) (A) and Group 1 (control) had the highest but not significantly different muscle glycogen content. Regarding the duration of stimulation, Groups 10, 6, and 8 (60 s stimulation) had the highest glycogen content, while Groups 3 and 7 (30 s stimulation) had the lowest glycogen content at this time. In terms of carcass weight, there was a mixed pattern of reaction from the treatment groups and there seem to be no influence of carcass weight on muscle glycogen content. From the t-

groupings, Treatment 10 had the highest ($P < 0.05$) glycogen content while other carcass groups were not significantly different from one another.

At 4 h pm, there was a clear pattern of reaction. Late stimulated groups (A) and the control had the highest ($P < 0.05$) muscle glycogen content, while the early stimulated carcasses (B) had the lowest muscle glycogen level. Regarding the duration of stimulation there was no clear pattern of reaction, but Treatments 10 and 6 (late stimulation, 60 s duration) had the highest muscle glycogen content. Regarding carcass weight, there was no definite pattern, and this suggests little or no influence of carcass weight on muscle glycogen content, at 4 h pm. Early stimulation produced the highest (64%) mean muscle glycogen decline followed by 37% decline for the late stimulated carcasses while the NES recorded the lowest (27.2%) mean decline from 1 to 4 h pm.

At 24 h pm, there was also a clear pattern of reaction from the treatment groups. Groups stimulated after evisceration (10, 6, 9 and 5) and the controls had the highest ($P < 0.05$) values of muscle glycogen, while the ones stimulated before evisceration had the lowest values (Table 4.29). Regarding the duration of stimulation, there was no clear pattern of reaction, but similar to 4 h pm, Groups 10 and 6 (late stimulation, 60 s duration) had the highest ($P < 0.05$) values of muscle glycogen. Regarding carcass weight, there was also no clear pattern of reaction and this implied lack or little influence of carcass weight on glycogen level at 24 h pm. Treatments 7, 8, 4 and 3 had numerically close values which were not significantly different, from one another, but differed significantly from most of the other groups. Early stimulation produced the highest (77.9%) mean muscle glycogen decline, followed by late stimulated carcasses (71.1%) and controls (70.9%) from 1 to 24 h pm.

4.16.4 Creatine phosphate

Fifty-seven samples were evaluated for muscle craetine phosphate (CP) but 56 were used for analysis at 1, 4 and 24 h. At 1 h pm, there was a moderate and significant variation ($R^2 = 0.42$; $P = 0.0013$) among the treatment groups. At 4 h pm, there was lower but moderate significant variation ($R^2 = 0.33$; $P = 0.0218$), while at 24 h pm, there was low and insignificant variation ($R^2 = 0.20$; $P = 0.2680$). GLM repeated measure ANOVA for test of hypothesis between subject effects showed significant difference ($P = 0.0039$) among the treatment groups. Univariate test for the effect of time levels also showed significant difference ($P < 0.0001$) between the time levels, likewise the interaction of time and treatment ($P = 0.0008$), which was also confirmed by a MANOVA test. Partial correlation between 1 and 4 h pm measurements showed a strong and significant correlation ($r = 0.63$). However, between 1 hour and 24 hour measurements, there was

a weak and insignificant correlation (r = 0.28). The 4 and 24 h measurement also correlated positively and significantly (r = 0.57). Table 4.30 shows the mean values and significant differences among treatments for CP at 1, 4 and 24 h pm.

Table 4.30 Mean values, standard deviation and *P*- values for creatine phosphate (µmol/g) at 1, 4 and 24-hour post-mortem

Treatments	N	CP 1 h Mean ± SD	CP 4 h Mean ± SD	CP 24 h Mean ± SD	% decline (1h to 4h)	% decline (4 to 24h)	% decline (1 to 24h)
1 S NES	7	6.48 ± 2.11^{a}	3.88 ± 1.22^{a}	2.01 ± 0.15^{ab}	40.1	48.2	69.0
2 H NES	6	3.28 ± 2.50^{c}	2.67 ± 1.78^{bc}	1.57 ± 0.78^{b}	23.8	41.2	52.1
3 S 30 B	5	3.56 ± 0.98^{bc}	2.35 ± 0.60^{c}	2.01 ± 0.51^{ab}	34.0	14.5	43.5
4 S 60 B	5	4.02 ± 1.22^{bc}	2.58 ± 0.35^{c}	2.42 ± 0.45^{a}	35.8	6.2	39.8
5 S 30 A	4	4.20 ± 0.35^{bc}	2.85 ± 0.77^{abc}	2.30 ± 0.60^{a}	32.1	19.3	45.2
6 S 60 A	6	3.69 ± 0.73^{bc}	2.96 ± 0.86^{abc}	1.85 ± 0.40^{ab}	19.8	37.5	49.9
7 H 30 B	6	2.71 ± 0.64^{c}	2.07 ± 0.29^{c}	1.94 ± 0.20^{ab}	23.6	6.3	28.4
8 H 60 B	5	2.92 ± 0.53^{c}	2.45 ± 0.41^{c}	2.13 ± 0.49^{ab}	16.1	13.1	27.1
9 H 30 A	6	4.06 ± 1.19^{bc}	2.80 ± 0.48^{abc}	2.07 ± 0.34^{ab}	31.0	26.1	49.0
10 H 60 A	6	5.09 ± 1.72^{ab}	3.73 ± 0.87^{ab}	2.18 ± 0.66^{a}	26.7	41.6	57.2

Key: N, Number of carcasses; SD, standard deviation; CP, creatine phosphate; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60 = 60 seconds stimulation.

^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

There was a declining trend in CP content in the muscle from 1 h to 24 h pm (Table 4.30) Figure 4.19 shows the CP content of muscles among treatment groups at 1, 4 and 24 h pm.

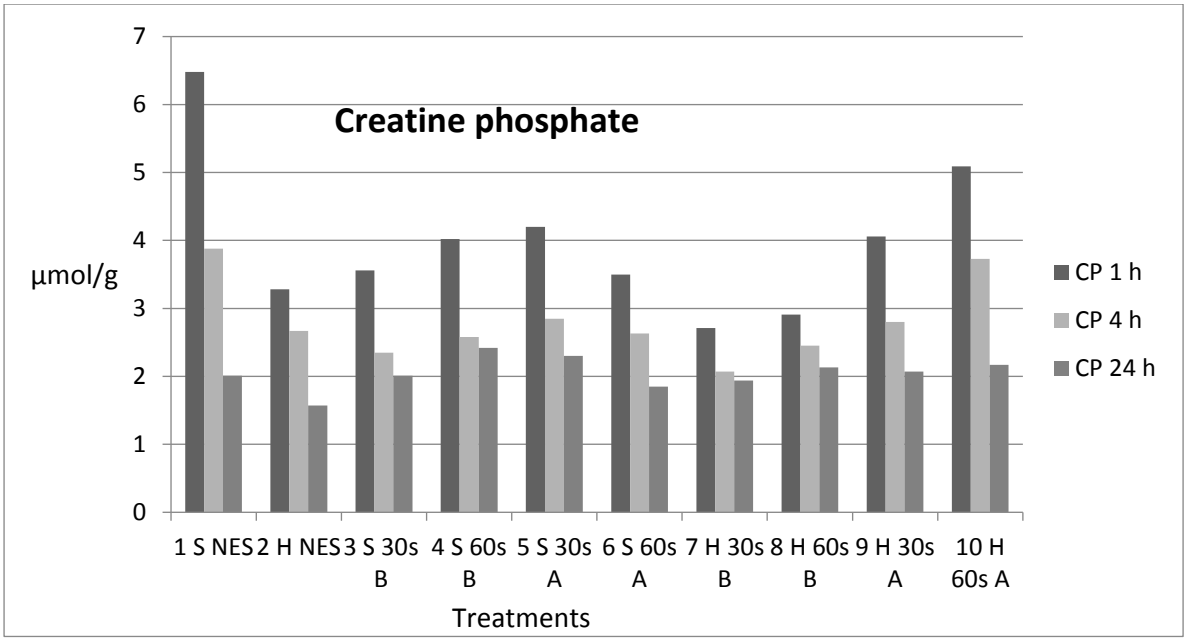

Figure 4.19 Graphical distribution of muscle creatine phosphate among the treatment groups at 1, 4, and 24-hour post-mortem

Key: S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation

The illustration below shows the ranking of muscle CP among treatment groups at 1, 4 and 24 h post-mortem in descending order.

Creatine phosphate 1 h pm: 1 (S NES) > **10** (H 60 A) > **5** (S 30 A) > **9** (H 30 A) > **4** (S 60 B) > **6** (S 60 A) > **3** (S 30 B) > **2** (H NES) > **8** (H 60 B) > **7** (H 30 B)

Creatine phosphate 4 h pm: 1 (S NES) > **10** (H 60 A) > **6** (S 60 A) > **5** (S 30 A) > **9** (H 30 A) > **2** (H NES) > **4** (S 60 B) > **8** (H 60 B) > **3** (S 30 B) > **7** (H 30 B)

Creatine phosphate 24 h pm: 4 (S 60 B) > **5** (S 30 A) > **10** (H 60 A) > **8** (H 60 B) > **9** (H 30 A) > **3** (S 30 B) > **1** (S NES) > **7** (H 30 B) > **6** (S 60 A) > **2** (H NES)

At 1 h post-mortem, regarding the time of stimulation, the reaction pattern was not clear. However, most of the late stimulated carcass groups (A) (10, 5, and 9) had the highest amount of muscle CP (Table 4.30). Most of the early stimulated carcass groups (B) (3, 7 and 8) had lower, but not significantly different muscle CP at this time. This implied that ES before evisceration brought about faster CP decline, as expected. Regarding duration of stimulation, there was no clear pattern of reaction from the treatment groups. In terms of carcass weight, there were mixed reactions in relation to CP content in the muscle. However, the early stimulated heavier carcasses

(7 and 8) had the lowest amount of CP, which differed ($P < 0.05$) only from Treatments 1 and 10. From the t- groupings, Treatments 1 and 10 had the highest CP content.

AT 4 h pm, regarding the time of stimulation, a similar pattern (to 1 h pm) was observed as carcasses stimulated before evisceration had the lowest muscle CP. In terms of duration of stimulation, a similar scenario and pattern at 1 h pm was observed. Regarding the influence of carcass weight on CP, there were mixed reactions, similar to 1 h pm. At this time, the early stimulated carcasses, especially the lighter ones, had a higher rate of CP decline compared with the late stimulated carcasses from 1 to 4 h pm. AT 24 h pm, regarding the time of stimulation, there was no clear pattern of reaction (mixed). In terms of duration of stimulation, there was also a mixed reaction (no clear pattern). Likewise, there were mixed reactions on the effects of carcass weight on CP from the groups. NES carcasses (1 and 2) had the highest average decline (mean 60.5%) in muscle CP at 24 h pm, followed by late stimulated carcasses (mean 50.3%) and the early stimulated carcasses (mean 34.7%) respectively (Table 4.30). Of the early stimulated carcasses, Treatments 7 and 8 (heavier carcasses) had the lowest mean muscle CP (mean 27.7%) decline rate from 1 to 24 h pm.

4.16.5 Adenosine tri-phosphate

Fifty-seven samples were evaluated for ATP, but 53 were used for analysis owing to outliers. Samples were taken for analysis at 1, 4 and 24 h pm. Repeated measures by GLM showed a moderate and significant variation ($R^2 = 0.50$, $P = 0.0002$) among the groups at 1 h pm. At 4 h pm, there was a higher variation among the groups ($R^2 = 0.58$, $P < 0.0001$) and at 24 h pm, there was a smaller but significant variation ($R^2 = 0.36$, $P = 0.0131$). GLM repeated measure univariate test of hypothesis for between subject effects showed significant difference between the times ($P < 0.0001$) of measurement, and also the combined effect of time by treatments ($P < 0.0001$). Partial correlation between the time levels also revealed strong, positive and significant correlations between the time levels (1, 4 and 24 h pm). Table 4.31 shows the mean values, SD and significant differences among the treatment groups.

Table 4.31 Mean adenosine tri-phosphate (µmol/g), standard deviation and *P*-values for treatment groups at 1, 4 and 24-hour post-moretm

Treatments	N	ATP 1 h pm Mean ± SD	ATP 4 h pm Mean ± SD	ATP 24 h pm Mean ± SD	% Δ ATP (1- 4 h pm)	% Δ ATP (1-24 h pm)
1) S NES	6	7.90 ± 0.53^{bc}	7.06 ± 1.02^{bcd}	4.15 ± 0.84^{b}	10.6	47.5
2) H NES	6	11.06 ± 2.99^{a}	9.96 ± 2.40^{a}	5.82 ± 1.76^{a}	9.9	47.4

3)	**S 30 B**	5	8.06 ± 1.62^{bc}	5.88 ± 2.30^{cde}	4.15 ± 1.20^{b}	27.1	48.5
4)	**S 60 B**	5	8.46 ± 1.67^{bc}	5.38 ± 1.01^{de}	4.21 ± 0.45^{b}	36.4	50.2
5)	**S 30 A**	4	10.61 ± 2.03^{a}	7.35 ± 2.42^{bc}	3.67 ± 0.29^{b}	30.7	65.4
6)	**S 60 A**	6	9.71 ± 1.43^{ab}	7.71 ± 1.21^{bc}	4.33 ± 1.09^{b}	20.6	55.4
7)	**H 30 B**	5	6.76 ± 1.10^{c}	4.35 ± 0.89^{e}	3.51 ± 0.56^{b}	35.7	48.1
8)	**H 60 B**	5	6.52 ± 0.81^{c}	4.56 ± 1.06^{e}	3.64 ± 0.97^{b}	30.1	44.2
9)	**H 30 A**	6	9.54 ± 1.21^{ab}	7.87 ± 0.83^{b}	4.68 ± 0.98^{ab}	17.5	50.9
10)	**H 60 A**	5	8.20 ± 0.90^{bc}	6.65 ± 1.52^{bcd}	3.52 ± 0.61^{b}	18.9	57.1

Key: N, Number of carcasses; SD, standard deviation; ATP, adenosine tri-phosphate; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

[a,b,c,d] Means in the same column without a common superscript letters differ ($P < 0.05$).

Similar to CP, there was a declining trend in the muscle ATP content from 1 to 24 h pm for all the treatment groups. Figure 4.20 shows the distribution of muscle ATP content for treatment groups at 1, 4 and 24 h pm.

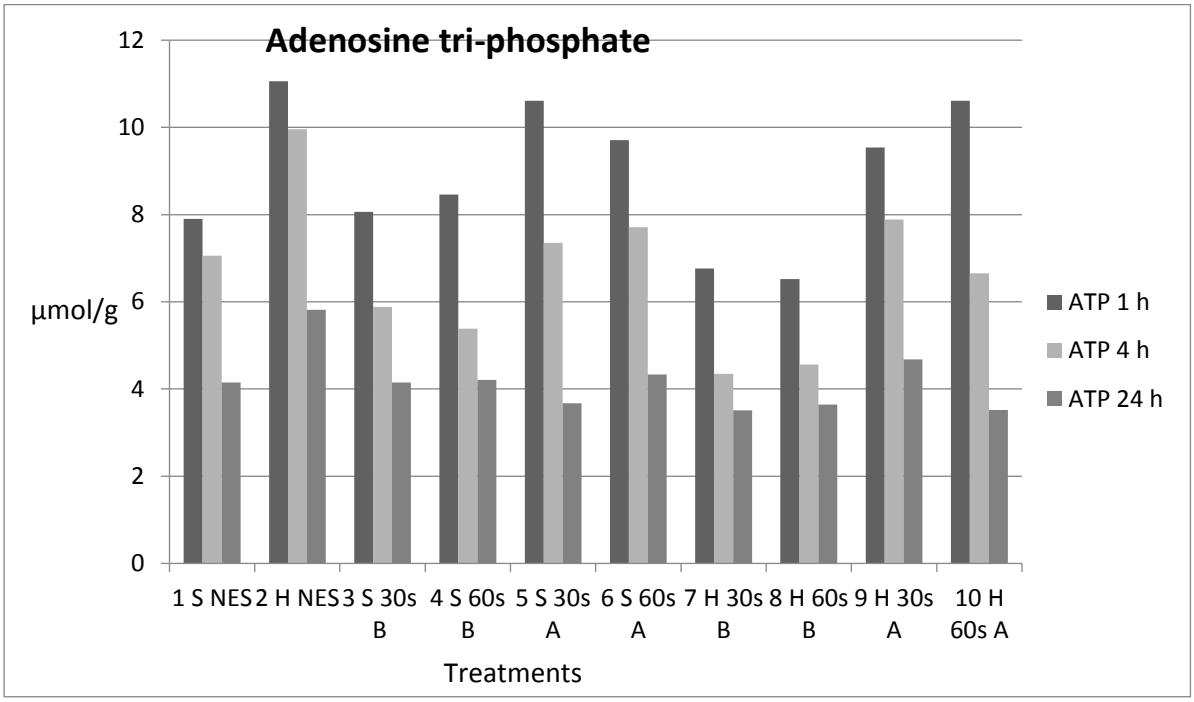

Figure 4.20 Graphical distribution of muscle adenosine tri-phosphate according to treatment groups at 1, 4 and 24-hour post-mortem

Key: S, small carcass; H, heavy carcass; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation

The following illustration shows the ranking of ATP according to treatments at different time levels pm in descending order.

Adenosine tri-phosphate 1 h pm: 2 (H NES) > **5** (S 30 A) > **6** (S 60 A) > **9** (H 30 A) > **4** (S 60 B) > **10** (H 60 A) > **3** (S 30 B) > **1** (S NES) > **7** (H 30 B) > **8** (H 60 B)

Adenosine tr-phosphate 4 h pm: 2 (H NES) > **9** (H 30 A) > **6** (S 60 A) > **5** (S 30 A) **1** (S NES) > **10** (H 60 A) > **3** (S 30 B) > **4** (S 60 B) > **8** (H 60 B) > **7** (H 30 B)

Adenosine tri-phosphate 24 h pm: 2 (H NES) > **9** (H 30 A) > **6** (S 60 A) > **4** (S 60 B) > **1** (S NES) > **3** (S 30 B) > **5** (S 30 A) > **8** (H 60 B) > **10** (H 60 A) > **7** (H 30 B)

At 1 h pm, most of the carcass groups stimulated after evisceration (5, 6, 9, and 10) and control (2) had the highest amounts of muscle ATP, while the ones stimulated before evisceration (3, 7and 8), especially the heavy carcasses (7 and 8), with the exception of Treatment 4 (which had marginally higher ATP), had the lowest ($P < 0.05$) muscle ATP content, compared with the late stimulated carcasses and controls. Regarding the duration of stimulation, there was no clear pattern of reaction among the groups. In terms of carcass weight, there was also no clear pattern of reaction but light and late stimulated carcasses (Groups 5 and 6) had the highest amount of muscle ATP while early stimulated heavy carcass groups (8 and 7) had the lowest amount of muscle ATP at this time.

At 4 h pm, regarding the time of stimulation, there was a clear pattern of reaction. Carcasses stimulated after evisceration and the controls had the highest ($P < 0.05$) muscle ATP content while carcasses stimulated before evisceration, especially the heavy carcasses had the lowest ($P < 0.05$) muscle ATP contents (Table 4.31). In terms of duration of stimulation, there was no clear pattern of reaction among treatment groups (mixed reactions). Regarding carcass weight, there was also a mixed reaction. There was no significant difference between the smaller and heavier early stimulated carcasses. Likewise, there were no significant differences between the heavy and smaller late stimulated carcasses (Table 4.31). At 4 h pm, the NES groups had the lowest average decline in muscle ATP (mean 10.1%), followed by the late stimulated carcasses (mean 21.9%), while the early stimulated carcasses (mean 32.3%) had the highest mean muscle ATP decline.

At 24 h pm, there were mixed reactions from the treatment groups in terms of time of simulation. Regarding the duration of stimulation, there was also mixed reactions from the groups (no definite pattern). In terms of carcass weight, there were no significant differences among treatment groups (Table 4.31). As stated earlier, there was low variation among treatments groups at 24 h pm and

this suggested a weak influence of ES on exhaustion of muscle ATP at this time. Moreover, at 24 h pm, the late stimulated carcasses had the overall highest mean decline (57.2%) in muscle ATP, while the early stimulated (47.8%) and the NES (control) carcasses (47.5%) had numerically close muscle ATP decline.

4.16.6 Glucose-6-phosphate

Fifty-seven carcasses were evaluated for G-6-P but 54 were used for analyses due to outliers. Analysis revealed low but significant variation (R^2 = 0.30, P = 0.0484) among the treatment groups at 1 h pm. At 4 h pm, there was a slightly higher and significant variation (R^2 = 0.32, P = 0.0296) among the groups, while at 24 h pm, there was much lower and insignificant variation (R^2 = 0.14, P = 0.6055). MANOVA test of hypothesis for number of time effect showed significant differences (P < 0.0001) between the times of measurement. Likewise, the interaction of time and treatment showed a significant difference between groups (P < 0.05). Partial correlation between time levels of measurement showed that the only strong and significant correlation was between the 4 and 24 h measurements (r = 0.56; P < 0.0001). Other correlations were weak and insignificant. Table 4.32 shows the mean values, SD and the significant differences between groups for G-6-P at different times of measurements.

Table 4.32 Means and standard deviation for glucose-6-phosphate (µmol/g) at 1, 4 and 24-hours post-mortem

Treatments	N	G-6-P 1 hpm Mean ± SD	G-6-P 4 hpm Mean ± SD	G-6-P 24 hpm Mean ± SD	% Δ G-6-P (1-4 h pm)	% Δ G-6-P (1-24 h pm)
1) S NES	7	1.47 ± 0.57^{ab}	1.70 ± 0.77^{b}	6.48 ± 1.99^{a}	15.7	340.8
2) H NES	6	1.50 ± 0.26^{ab}	1.81 ± 0.46^{b}	7.71 ± 0.96^{a}	20.7	414.0
3) S 30 B	5	1.17 ± 0.30^{bc}	4.03 ± 0.33^{a}	6.62 ± 3.76^{a}	244.4	465.8
4) S 60 B	4	1.01 ± 0.12^{c}	3.43 ± 0.80^{ab}	5.64 ± 1.89^{a}	239.4	458.4
5) S 30 A	5	1.32 ± 0.29^{ab}	3.09 ± 0.83^{ab}	7.79 ± 2.35^{a}	134.1	490.2
6) S 60 A	5	1.08 ± 0.47^{bc}	2.01 ± 0.70^{b}	8.11 ± 0.92^{a}	86.1	650.9
7) H 30 B	6	1.16 ± 0.36^{bc}	3.29 ± 1.51^{ab}	5.14 ± 1.69^{a}	183.6	343.1
8) H 60 B	5	1.09 ± 0.39^{bc}	4.08 ± 2.43^{a}	6.92 ± 3.46^{a}	274.3	534.9
9) H 30 A	5	1.71 ± 0.32^{a}	1.88 ± 0.52^{b}	7.08 ± 3.79^{a}	9.9	314.0
10) H 60 A	6	1.08 ± 0.26^{bc}	1.83 ± 0.78^{b}	7.82 ± 2.86^{a}	69.4	624.1

Key: N, Number of carcasses; SD, standard deviation; G-6-P, glucose-6-phospate; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation. [a,b,c,d] Means in the same column without a common superscript letter differ ($P < 0.05$).

As shown in Table 4.32, there was an increasing trend of G-6-P from 1 to 24 h pm. Figure 4.21 shows the distribution of G-6-P at different times of measurement.

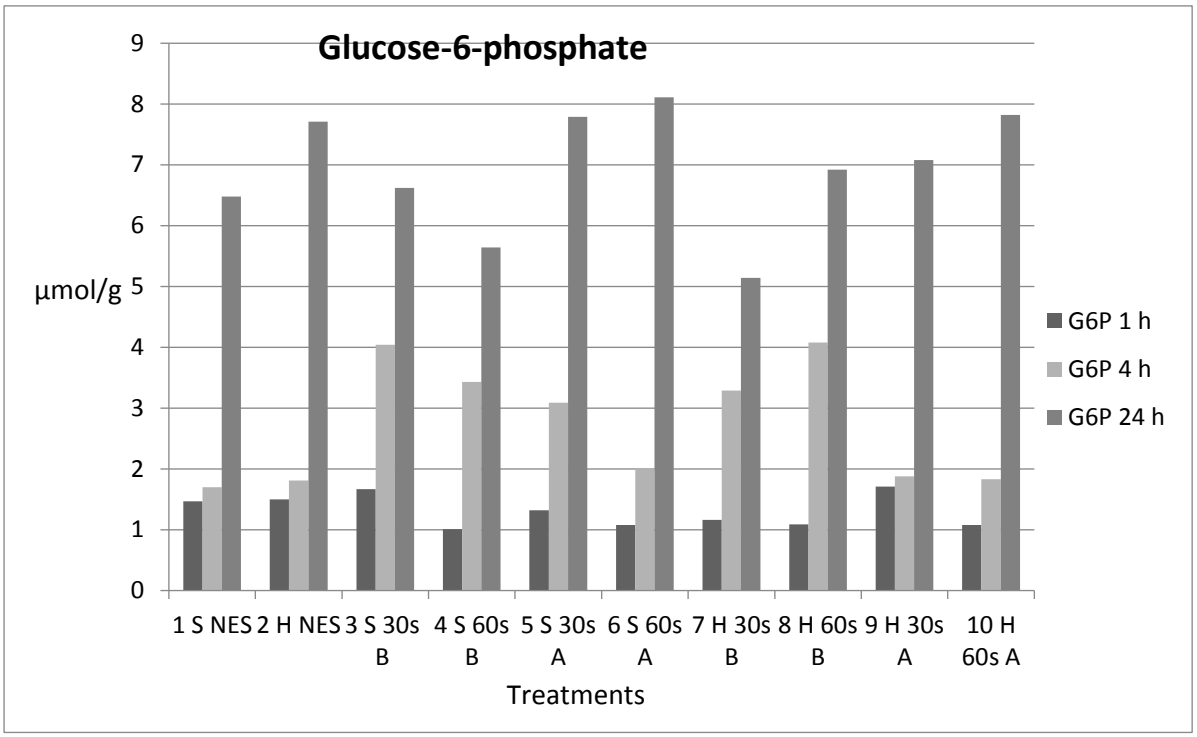

Figure 4.21 Graphical distribution of muscle glucose-6-phosphate according to treatment groups at 1, 4 and 24-hours post-mortem

Key: S, small carcass; S, heavy carcass; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation

The following illustration shows the ranking of G-6-P according to treatment groups at 1, 4, and 24 h pm

Glucose-6-phosphate 1 h pm: 9 (H 30A) > **2** (H NES) > **1** (S NES) > **5** (S 30 A) > **3** (S 30 A) > **7** (H 30 B) > **8** (H 60 B) > **6** (S 60 A) > **10** (H 60 A) > **4** (S 60 B)

Glucose-6-phosphate 4 h pm: 8 (H 60 B) > **3** (S 30 B) > **4** (S 60 B) > **7** (H 30 B) > **5** (S 30 A) > **6** (S 60 A) > **9** (H 30 A) > **10** (H 60 A) > **2** (H NES) > **1** (S NES)

Glucose-6-phosphate 24 h pm: 6 (S 60 A) > **10** (H 60 A) > **5** (S 30 A) > **2** (H NES) > **9** (H 30 A) > **8** (H 60 B) > **3** (S 30 B) > **1** (S NES) > **4** (S 60 B) > **7** (H 30 B)

At 1 h pm, regarding the time of stimulation, there were mixed reactions from the treatment groups and there was no clear pattern of reaction. There were no significant differences among most treatments although, the early stimulated carcasses generally displayed numerically lower values of G-6-P. Regarding duration of stimulation, there was no clear pattern of reaction. In terms of carcass weight, there were mixed reactions from the groups and this implied lack of influence of carcass weight on muscle G-6-P content at 1 h pm.

At 4 h pm, there was a clear pattern of reaction from the treatment groups regarding the time of stimulation. Carcass groups (8, 7, 4 and 3) that were stimulated before evisceration had the highest but not significantly different G-6-P content, while the carcasses stimulated after evisceration (5, 6, 9 and 10) especially the heavy ones (9 and 10) had the lowest but not significantly different muscle G-6-P content, followed by the controls (Table 4.32). In terms of duration of stimulation, there was no clear pattern of reaction from the treatment groups, likewise, carcass weight. Early ES produced the highest (mean 235.4%) mean increase in muscle G-6-P compared with 74.9% mean increase in the late stimulated carcasses, while there was 18.2% average G-6-P increase in the NES carcasses from 1 to 4 h pm.

At 24 h pm, contrary to 4 h pm, groups that were stimulated after evisceration (6, 10, 5 and 9) had the numerically highest G-6-P content, while the early stimulated carcasses (8, 7, 4 and 3) had the lowest values of muscle G-6-P content. In terms of duration of stimulation, there was no clear pattern of reaction from the groups. Regarding carcass weight, there was also no clear pattern of reaction in terms of muscle G-6-P content. However, there was no significant difference among all groups. Additionally, late stimulation produced the highest (519. 8%) mean G-6-P increase compared with early stimulation (450.6%), while the NES recorded 377.4% increase from 1 to 24 h pm.

4.16.7 Glycolytic potential

Glycolytic potential (GP) is a term that is used to estimate the ability of muscle to generate lactic acid and it is calculated as: GP = 2 x (glucose + glycogen + G-6-P) + lactate. Forty-seven samples were analysed for this parameter. At 1 h pm, there was a high and significant variation (R^2 = 55%, P = 0.0002) among the groups. At 4 h pm, there was a lower and insignificant variation (R^2 = 24%, P = 0.2529) among the treatment groups. At 24 h pm, similar variation to 4 h pm (R^2 = 0.24, P = 0.2635) was observed among the treatment groups. MANOVA test of hypothesis for number of times effect showed significant difference (P < 0.0001) between the times of measurement. MANOVA test of hypothesis for the interaction of time and treatments also showed significant

difference (P = 0.0386) among the treatment groups. Table 4.33 shows the mean and SD of GP for treatment groups at various times of measurement.

Table 4.33 Means, standard deviation and *P*-values for Glycolytic potential at 1, 4, and 24-hours post-mortem

Treatments	**N**	**GP 1 h pm Mean ± SD**	**GP 4 h pm Mean ± SD**	**GP 24 h pm Mean ± SD**	**% Δ GP (1 to 4h pm)**	**% Δ GP (1 to 24h pm)**
1) S NES	5	97.08 ± 19.81^{c}	85.41 ± 27.66^{b}	115.70 ± 6.62^{b}	-12.0	19.2
2) H NES	5	84.36 ± 16.81^{c}	99.09 ± 31.02^{ab}	118.20 ± 9.94^{ab}	17.5	40.1
3) S 30 B	5	97.11 ± 26.18^{c}	88.95 ± 22.23^{ab}	118.79 ± 28.90^{ab}	-8.4	22.3
4) S 60 B	3	104.83 ± 18.27^{bc}	108.91 ± 2.56^{ab}	122.87 ± 12.01^{ab}	3.9	17.2
5) S 30 A	4	94.29 ± 30.31^{c}	91.69 ± 26.51^{ab}	125.22 ± 15.81^{ab}	-2.8	32.8
6) S 60 A	5	100.28 ± 11.41^{c}	101.78 ± 19.90^{ab}	140.33 ± 12.21^{ab}	1.5	39.9
7) H 30 B	6	101.22 ± 18.26^{c}	107.45 ± 16.66^{ab}	133.10 ± 12.23^{ab}	6.2	31.5
8) H 60 B	4	133.85 ± 48.85^{ab}	120.89 ± 23.03^{a}	138.38 ± 25.92^{ab}	-9.7	3.4
9) H 30 A	4	79.88 ± 17.30^{c}	93.79 ± 20.70^{ab}	120.95 ± 33.71^{ab}	17.4	51.4
10) H 60 A	6	157.66 ± 19.26^{a}	121.27 ± 30.07^{a}	143.37 ± 24.86^{a}	-23.1	-9.1

Key: N, Number of carcasses; SD, standard deviation; GP, glycolytic potential; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.22 shows the distribution of GP at 1, 4, and 24 h pm for the treatment groups.

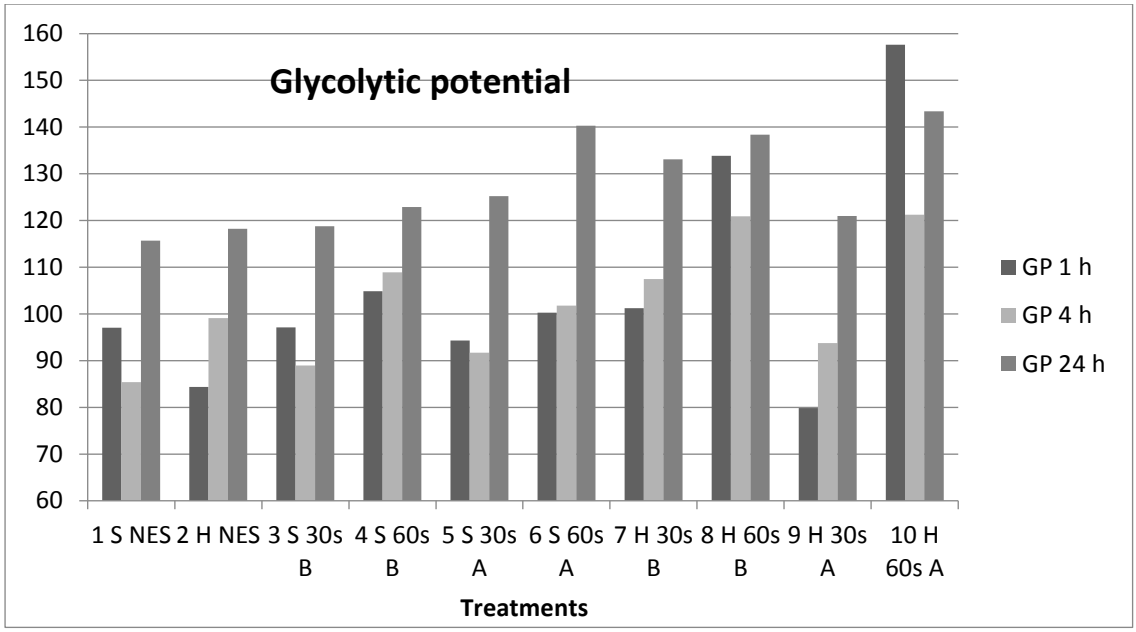

Figure 4.22 Bar chart of glycolytic potential at 1, 4 and 24-hour post-mortem for treatment groups

Key: S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30 = 30 seconds stimulation; 60, 60 seconds stimulation.

The following illustration shows the ranking of GP at 1, 4 and 24 h pm in descending order.

Glycolytic potential 1 h pm: 10 (H 60 A) > **8** (H 60 B) > **4** (S 60 B) > **7** (H 30 B) > **6** (S 60 A) > **3** (S 30 B) > **1** (S NES) > **5** (S 30 A) > **2** (H NES) > **9** (H 30 A)

Glycolytic potential 4 h pm: **10** (H 60 A) > **8** (H 60 B) > **4** (S 60 B) > **7** (H 30 B) > **6** (S 60 A) > **2** (H NES) > **9** (H 30 A) > **5** (S 30 A) > **3** (S 30 B) > **1** (S NES)

Glycolytic potential 24 h pm: 10 (H 60 A) > **6** (S 60 A) > **8** (H 60 B) > **7** (H 30 B) > **5** (S 30 A) > **4** (S 60 B) > **9** (H 30 A) > **3** (S 30 B) > **2** (H NES) > **1** (S NES)

At 1 h pm, from the illustration above, there was a clear pattern of reaction. Most of the early stimulated carcasses (8, 7 and 4) had the highest GP (save Treatment 3), followed by the late stimulated carcasses and controls. Regarding the duration of stimulation, the reaction pattern was not so clear. But carcasses stimulated for 60 seconds (10, 8, 6 and 4) had the highest ($P < 0.05$) GP followed by carcasses stimulated for 30 s and controls. Regarding carcass weight, there were mixed reactions, however, large carcass sides (10 and 8) stimulated for 60 seconds had the highest ($P < 0.05$) GP (Table 4.33 and illustration above).

At 4 h pm, regarding the time of stimulation, a similar trend to 1 h pm was observed (see Table 4.33 and the illustration of the rankings above). Most of the carcasses that were stimulated before evisceration (8, 4 and 7), had the highest GP values, followed by the carcasses stimulated after

evisceration and the controls. In terms of duration of stimulation, a similar trend was observed to 1 h pm, where carcasses stimulated for 60 seconds (10, 8, 4 and 6) produced the highest GP values. Regarding carcass weight there was mixed reactions from the groups but the heavy carcass weights (10 and 8), stimulated for 60 s had the highest ($P < 0.05$) GP values at 4 h pm. In addition, there were mixed reactions in terms of change in GP values from 1 to 4 h pm. Some carcasses had a reduction in GP values while some carcasses had an increase in GP values (Table 4.33). Carcasses in group 10, with the highest ($P < 0.05$) GP and unusually high initial muscle glycogen had the highest decline (-23.1%) in GP from 1 to 4 h pm.

At 24 h pm, time of stimulation produced mixed reactions from the groups. In terms of duration of stimulation, there was also a mixed reaction but Groups 10, 6 and 8 (stimulated for 60 s) had the highest GP values. This is consistent with 1 and 4 h pm measurements, where the 60 s stimulation produced the highest GP values. In terms of carcass weight, there was no definite pattern from the groups. Generally, most of the groups were not significantly different except Treatment 10, which had the highest ($P < 0.05$) GP. At 24 h pm, there was a mixed reaction in terms of GP increase. However, similar to 4 h pm, carcasses in Group 10 had a decline (-9.1) in GP value (Table 4.33). Also, carcasses stimulated late had a mean higher GP (28.8%) compared with the early stimulated carcasses (18.6%), while the control groups had the highest mean increase (29.7%).

Tables 4.34 and 4.35 summarize the mean value and the significance of the main effects on GP values.

Table 4.34 Mean values and standard deviation of main effects on glycolytic potential at 1, 4 and 24-hours post-mortem

Main effects		**GP 1 h pm** **Mean ± SD**	**GP 4 h pm** **Mean ± SD**	**GP 24 h pm** **Mean ± SD**
CW	**S**	98.36 ± 20.10	94.29 ± 22.07	124.71 ± 18.21
	H	113.20 ± 38.59	109.06 ± 25.44	131.40 ± 22.40
ES t	**0 (NES)**	90.72 ± 18.57	92.25 ± 28.63	116.95 ± 8.07
	B	107.93 ± 30.49	105.55 ± 20.78	128.59 ± 20.97
	A	112.84 ± 36.93	104.13 ± 26.18	134.03 ± 23.05
ES d	**0 (NES)**	90.72 18.57	92.25 ± 28.63	116.95 ± 8.07
	30s	94.19 ± 22.63	96.39 ± 20.93	125.12 ± 22.24
	60s	127.62 ± 35.20	113.72 ± 23.06	138.00 ± 20.11

Key: CW, carcass weight; S, small carcasses; H, heavy carcasses; ES t, stimulation time; ES d, stimulation duration; 0 (NES), non-stimulated carcasses; B, early stimulation; A- late stimulation; 30s, 30 seconds stimulation; 60s, 60 seconds stimulation; SD, standard deviation.

Table 4.35 Significance of carcass weight, stimulation time time and stimulation duration on glycolytic potetial at 1, 4 and 24-hour post-mortem

Effects	***P* value F ratio SEM**	***P* value F ratio SEM**	***P* value F ratio SEM**
Main effects	**GP 1 h pm**	**GP 4 h pm**	**GP 24 h pm**
CW	0.186 1.818 7.088	0.077 3.313 7.157	0.345 0.916 5.957
ES time (ES t)	0.880 0.023 8.020	0.588 0.298 8.099	0.539 0.385 6.741
ES dura (ES d)	0.000 14.967 9.346	0.035 4.802 9.596	0.090 3.024 7.987
Interactions	**GP 1 h pm**	**GP 4 h pm**	**GP 24 h pm**
CW x ES t	0.122 2.430 8.691	0.120 2.452 8.777	0.012 6.426 7.305
CW x ES d	0.599 0.278 7.983	0.567 0.329 7.758	0.618 0.250 6.710
ES t x ES d	0.004 8.562 7.206	0.007 7.551 7.277	0.296 1.103 6.327
CW x ESt x ESd	0.399 0.715 10.645	0.770 0.085 10.749	0.524 0.408 10.003

Key: CW, carcass weight; ESt, ES time (early/late); ESd, ES duration; $p = 0.05$; SEM, standard error of means.

Heavier carcasses produced numerically higher GP at 1, 4 and 24 h pm compared with the smaller carcasses, but the differences were not statistically significant (Tables 4.34 and 4.35). Late stimulation also produced numerically higher GP compared with early stimulation and controls, but the differences were not statistically significant. However, 60 s stimulation produced significantly higher ($P < 0.05$) GP at 1 and 4 h pm and a high tendency to be significant at 24 h pm (Table 4.35). The interaction of heavier carcasses and early stimulation also produced significantly higher GP at 24 h pm. Likewise, the interaction of early stimulation and longer duration of stimulation (60 s) produced significantly higher GP at 1 and 4 h pm.

4.17 Proteolytic enzymes

4.17.1 Micro calpain (calpain-1)

Fifty-seven carcasses were evaluated for calpain-1 but 55 were used for analyses at 1 and 24 h pm because of outliers. At 1 h pm, there was moderate and significant variation (R^2 = 0.36; P = 0.0116) among the groups. At 24 h pm, there was similar significant variation among the groups (R^2 = 0.35; P = 0.0150). Partial correlation between the two levels of measurement showed a moderate significant (P < 0.05) positive relationship (r = 0. 33; P = 0.0239). The interaction of time and treatments among the groups also showed a significant difference using GLM MANOVA (P = 0.0005). Table 4.36 shows the t- test (LSD) in pairwise comparisons using the means of the treatment groups.

Table 4.36 Means and standard deviation of calpain-1 at 1 and 24 h pm

Treatments	N	Calpain-1 1 h pm Mean ± SD	Calpain-1 24 h pm Mean ± SD	% calpain-1 pm decline
1) S NES	7	1.20 ± 0.26^{b}	1.07 ± 0.13^{ab}	10.83
2) H NES	6	1.23 ± 0.17^{b}	1.06 ± 0.26^{ab}	13.82
3) S 30 B	5	1.58 ± 0.23^{a}	1.29 ± 0.27^{a}	18.35
4) S 60 B	4	1.57 ± 0.21^{a}	1.09 ± 0.08^{ab}	30.57
5) S 30 A	5	1.50 ± 0.15^{ab}	0.93 ± 0.26^{bc}	38.00
6) S 60 A	6	1.57 ± 0.43^{a}	0.98 ± 0.17^{b}	37.58
7) H 30 B	6	1.65 ± 0.22^{a}	1.03 ± 0.44^{b}	37.57
8) H 60 B	4	1.48 ± 0.11^{ab}	0.62 ± 0.33^{c}	58.11
9) H 30 A	6	1.50 ± 0.26^{ab}	1.20 ± 0.31^{ab}	20.00
10) H 60 A	6	1.51 ± 0.27^{b}	1.16 ± 0.15^{ab}	23.18

Key: N, Number of carcasses; SD, Standard Deviation; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation

^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

There was a declining trend in calpain-1 from 1 to 24 h pm in all the treatment groups (Table 4.36). The control groups (1 and 2) and Treatment 10 had the lowest (P < 0.05) calpain-1 content at 1 h pm, while the other groups were not significantly different. At 24 h pm, all groups were numerically close in calpain-1 content, except Group 8, which was significantly lower (P < 0.05). Muscles in the control group (1 and 2) had the lowest mean decline (12.3%) followed by the late stimulated groups (5, 6, 9 and 10) with average of 29.7%, while the early stimulated groups (3, 4,

7 and 8) had the highest mean decline (36.2%). Figure 4.23 shows the distribution of the mean values of calpain-1 at 1 and 24 h pm.

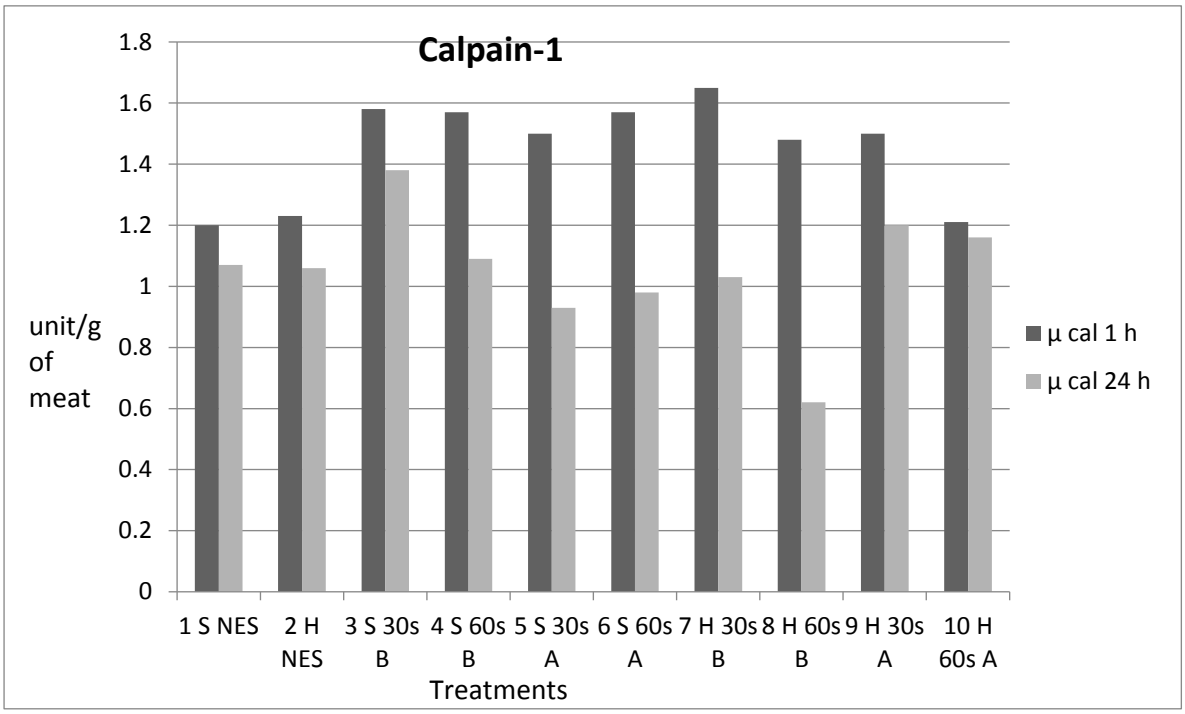

Figure 4.23 Distribution of mean values of calpain-1 according to treatment groups at 1 and 24-hours post-mortem

Key: S, small carcass; H, heavy carcass; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation

The following illustration shows the ranking of muscle calpain-1 concentration according to treatment groups at 1 and 24 h pm in descending order:

Calpain-1 1 h pm: 7 (H 30 B) > **3** (S 30 B) > **4** (S 60 B) > **6** (S 60 A) > **10** (H 60 A) > **5** (S 30 A) > **9** (H 30 A) > 8 (H 60 B) **2** (H NES) > **1** (S NES)

Calpain-1 24 h pm: **3** (S 30 B) > **9** (H 30 A) > **10** (H 60 A) > **4** (S 60 B) > **1** (S NES) > **2** (H NES) > **7** (H 30 B) > **6** (S 60 A) > **5** (S 30 A) > **8** (H 60 B)

At 1 h pm, regarding the time of stimulation, there was a definite pattern of reaction from the treatment groups. Most of the carcasses stimulated before evisceration (3, 4 and 7) and the late stimulated carcasses (5, 6 and 9) had numerically close values with no significant difference, while the non-ES groups (1 and 2) had similar and significantly lower calpain-1 content compared with most of the early ES treatments (3, 4 and 7). Regarding the duration of stimulation, there was a mixed pattern of reaction among the groups. In terms of the influence of carcass weight on

calpain-1, there was no definite pattern of reaction between the small and heavier electrically stimulated carcasses.

At 24 h pm, regarding the time of stimulation, there was a mixed pattern of reaction from the treatments. In duration of stimulation, there was also a mixed pattern of reaction but the carcass groups stimulated for 30 s (3 and 9) had the highest muscle calpain-1 content, while treatment 8 (heavy carcasses with 60 s early stimulation) was still erratically low ($P < 0.05$) compared with the other treatments. In terms of carcass weight, there was a mixed pattern of reaction and no definite pattern was observed. There were no statistical differences between most of the treatment groups except for Treatment 8, which has the lowest concentration (Table 4.36).

4.17.2 M calpain (Calpain-2)

Fifty-seven samples were evaluated for calpain-2 but 56 were used for the analyses. At 1 h pm, there was a moderate and significant ($R^2 = 0.44$; $P = 0.0008$) variation among the treatment groups. At 24 h pm, there was also a moderate and significant ($R^2 = 0.45$; $P = 0.0005$) variation. GLM repeated measure ANOVA revealed significant difference ($P < 0.0001$) among the groups. Partial correlation between the time levels of measurement revealed a strong and significant ($r = 0.62$; $P < 0.0001$) positive correlation between the 1 and 24 h muscle calpain-2. GLM Multiple analysis of variance reveals there is no significant difference ($P = 0.9596$) in terms of the interaction of time and treatment effect. Table 4.37 shows the t- test (LSD) among the treatment groups at 1 and 24 h pm.

Table 4.37 Mean and standard deviation and of calpain-2 at 1 and 24-hours post-mortem

Treatments	**N**	**Calpain-2 1 h pm Mean ± SD**	**Calpain-2 24 h pm Mean ± SD**	**% Δ calpain-2**
1) S NES	6	1.04 ± 0.14^{a}	1.10 ± 0.09^{a}	5.77
2) H NES	6	0.90 ± 0.06^{abc}	0.90 ± 0.20^{bc}	0
3) S 30 B	5	0.80 ± 0.03^{cd}	0.80 ± 0.05^{cd}	0
4) S 60 B	5	0.85 ± 0.07^{bc}	0.87 ± 0.06^{bc}	2.35
5) S 30 A	5	0.93 ± 0.13^{abc}	1.01 ± 0.05^{ab}	8.60
6) S 60 A	6	0.97 ± 0.15^{ab}	0.98 ± 0.17^{ab}	-1.03
7) H 30 B	6	0.67 ± 0.06^{d}	0.71 ± 0.06^{d}	5.97
8) H 60 B	5	0.81 ± 0.09^{cd}	0.79 ± 0.05^{cd}	-2.47
9) H 30 A	6	0.92 ± 0.19^{abc}	0.92 ± 0.18^{bc}	0
10) H 60 A	6	0.90 ± 0.19^{abc}	0.92 ± 0.21^{bc}	2.22

Key: N, Number of carcasses; SD, standard deviation; S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

[a,b,c,d] Means in the same column without a common superscript letter differ ($P < 0.05$).

There was no clear pattern of reaction in the content of calpain-2 in the muscle at the two times of measurement among the treatment groups (Table 4.37). In some treatments there were slight increases in calpain-2 content, while in others, there were slight decreases from 1 to 24 h pm. Figure 4.24 shows the graphical distribution of calpain-2 among the treatment groups at 1 and 24 h pm.

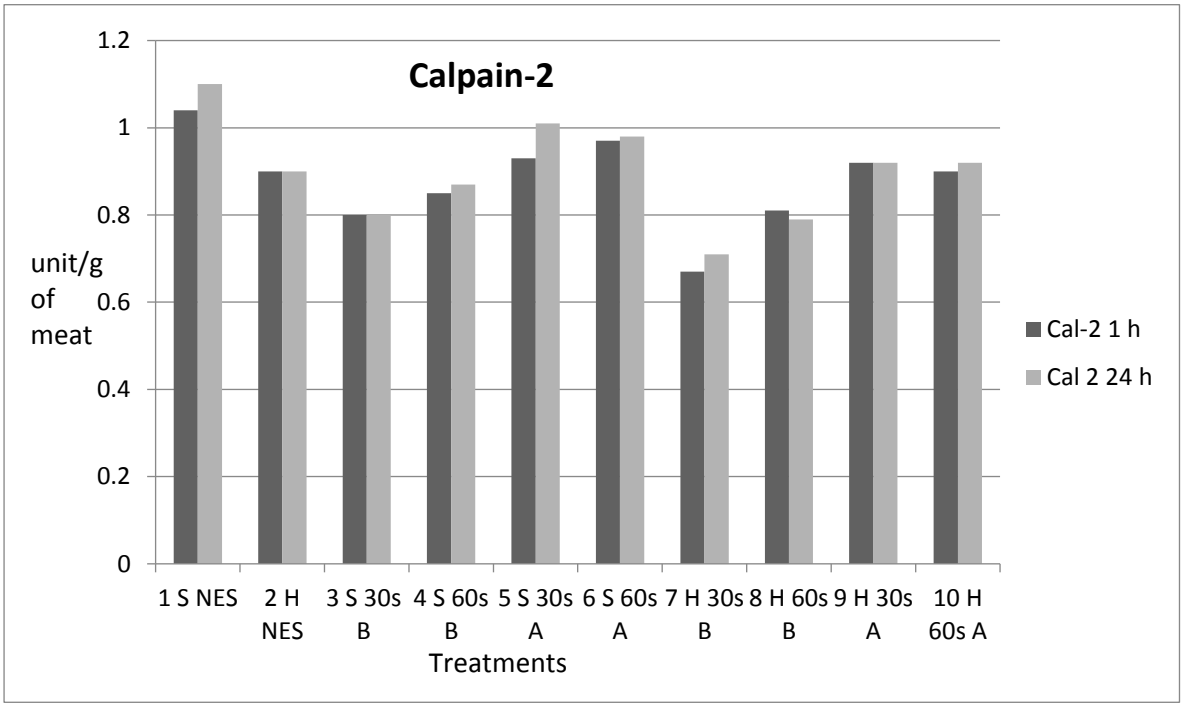

Figure 4.24 Distribution of calpain-2 according to treatment groups at 1 and 24-hours post-mortem

Key: S, small carcass; H, heavy carcass; B, early ES; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

The following illustration shows the ranking of the content of calpain-2 in the muscle among the treatment groups at 1 and 24 h pm in descending order.

Calpain-2 1 h pm: **1** (S NES) > **6** (S 60 A) > **5** (S 30 A) > **9** (H 30 A) > **10** (H 60 A) > **2** (H NES) > **4** (S 60 B) > **8** (H 60 B) > **3** (S 30 B) > **7** (H 30 B)

Calpain-2 24 h pm: **1** (H NES) > **5** (S 30 A) > **6** (S 60 A) > **10** (H 60 A) > **9** (H 30 A) > **2** (H NES) > **4** (S 60 B) > **3** (S 30 B) > **8** (H 60 B) > **7** (H 30 B)

There was a clear pattern of reaction in the time of stimulation at 1 h pm. Carcass groups stimulated after evisceration (A) (6, 5, 9 and 10), save Treatment 1 (NES) had the numerically highest calpain-2 content, while the muscles of the early stimulated carcasses (3, 4, 7 and 8) had the lowest. However, the numerical differences were very low and not significant among most of the treatment groups. In terms of the duration of stimulation, there was a mixed pattern of reaction, as seen from the illustration. Regarding the influence of carcass weight, there was also a mixed pattern of reaction.

There was a clear pattern of reaction from the treatment groups at 24 h pm, regarding the time of stimulation, there was a clear pattern of reaction from the treatment groups, similar to 1 h pm. Groups that were stimulated after evisceration (5, 6, 10 and 9) and controls had the numerically highest values of calpain-2, while the early stimulated carcasses (4, 3, 8 and 7) had the lowest values of muscle calpain-2. In terms of duration of stimulation, there was a mixed pattern of reaction from the treatment groups. Regarding carcass weight, there was also a mixed pattern of reaction. Erratic values were observed and there was no definite pattern in the percentage calpain-2 decline in all treatment groups. Some treatment groups increased in value, while some decreased, and others did not change at 24 h pm. The numerical changes in calpain-2 from all treatments were also very low.

4.17.3 Calpastatin

Fifty-seven carcasses were evaluated for muscle calpastatin content but 56 were used for the analysis due to an outlier value. Analysis was done on 1 and 24 h pm samples. For the 1 h pm samples, there was low and insignificant (R^2 = 0.18; P = 0.3701) variation among the treatment groups. At 24 h pm, there was a much lower and insignificant (R^2 = 0.096; P = 0.8351) variation. Partial correlation between the time levels showed a strong, positive and significant correlation (r = 0.55; P = 0.0001). GLM MANOVA revealed no significant difference (P = 0.1397) among the groups in terms of the interaction of time and treatment. Repeated measures ANOVA also confirmed no significant (P = 0.8741) difference among the groups. Table 4.38 shows the t- test (LSD) pairwise comparison among the mean values of the treatment groups for muscle calpastatin content at 1 and 24 h pm

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Table 4.38 Mean and standard deviation for muscle calpastatin content at 1 and 24-hours post-mortem

Treatments	N	Calpas 1 h pm Mean ± SD	Calpa 24 h pm Mean ± SD	% Δ calpas decline
1) S NES	7	2.46 ± 0.29^{ab}	2.22 ± 0.23^{a}	9.76
2) H NES	6	2.53 ± 0.38^{ab}	1.98 ± 0.58^{a}	21.74
3) S 30 B	5	2.77 ± 0.22^{a}	1.84 ± 0.45^{a}	33.57
4) S 60 B	5	2.19 ± 0.41^{b}	1.74 ± 0.46^{a}	20.55
5) S 30 A	5	2.47 ± 0.20^{ab}	1.79 ± 0.43^{a}	27.53
6) S 60 A	6	2.57 ± 0.34^{ab}	1.88 ± 0.52^{a}	26.85
7) H 30 B	6	2.36 ± 0.38^{ab}	2.03 ± 0.20^{a}	13.98
8) H 60 B	4	2.67 ± 0.52^{a}	1.86 ± 0.12^{a}	30.34
9) H 30 A	6	2.60 ± 0.48^{ab}	1.98 ± 0.73^{a}	23.85
10) H 60 A	6	2.44 ± 0.09^{ab}	1.92 ± 0.46^{a}	21.31

Key: N, Number of carcasses; SD, standard deviation; Calpas, Calpastatin; S, small carcasses; H, heavy carcasses; A, late stimulation; B, early stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.
^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

There were low variations among the treatment groups at 1 and 24 h pm (Table 4.38). There was also a declining trend in muscle calpastatin content from 1 to 24 h pm. Figure 4.25 shows the graphical distribution of calpastatin according to treatment groups at 1 and 24 h pm.

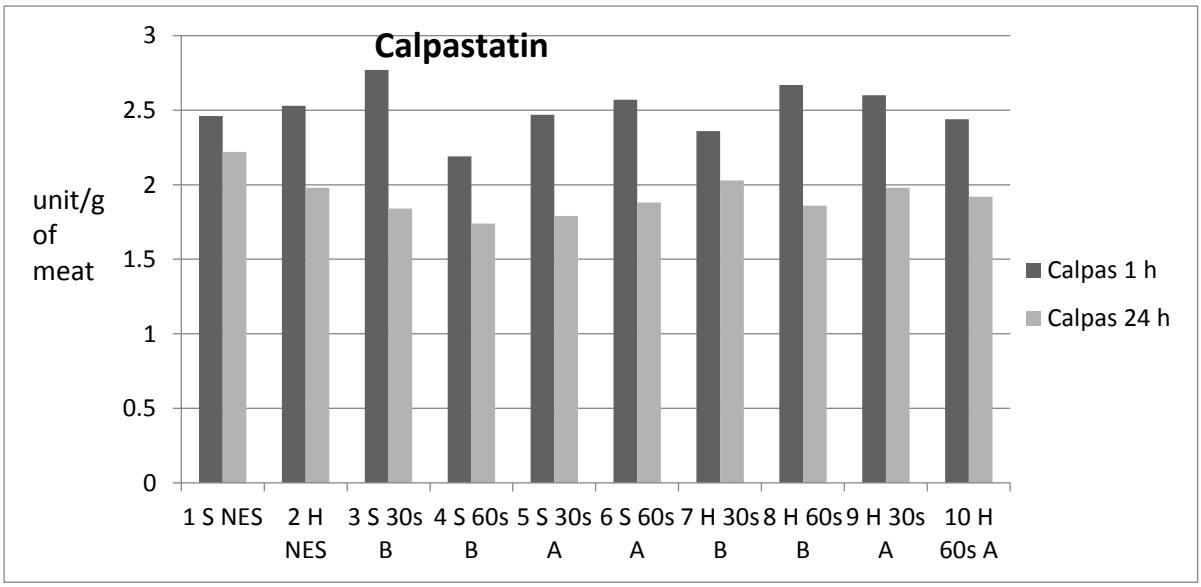

Figure 4.25 Distribution of mean values of muscle calpastatin content according to treatment groups at 1 and 24-hours post-mortem

Key: S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation

The following illustration shows the ranking of muscle calpastatin content according to treatment groups at 1 and 24 h pm in descending order.

Calpastatin 1 h pm: 3 (S 30 B) > **8** (H 60 B) > **9** (H 30 A) > **6** (S 60 A) > **2** (H NES) > **5** (S 30 A) > **1** (S NES) > **10** (H 60 A) > **7** (H 30 B) > **4** (S 60 B)

Calpastatin 24 h pm: 1 (S NES) > **7** (H 30 B) > **2** (H NES) > **9** (H 30 A) > **10** (H 60 A) > **6** (S 60 A) > **8** (H 60 B) > **3** (S 30 B) > **5** (S 30 A) > **4** (S 60 B)

There were mixed reactions from the treatment groups regarding the time of stimulation at 1 h pm. There was no definite reaction pattern, but early stimulated carcass groups (3 and 8) had slightly higher, but not significantly different muscle calpastatin content from other groups. In terms of duration of stimulation, there was also a mixed reaction. Likewise, there was a mixed reaction regarding the effect of carcass weight on muscle calpastatin content. This suggests lack of or little influence of stimulation time, duration of stimulation and carcass weight on muscle calpastatin content at this time. There were low numerical differences among all treatment groups (Table 4.38).

At 24 h pm, regarding the time of stimulation, there was a mixed pattern of reaction among the treatments. Likewise, in duration of stimulation, there was no definite reaction pattern. In term of carcass weight, there was also no definite pattern of reaction. There were no significant differences among all the treatment groups at 24 h pm (Table 4.38). The non-stimulated (average15.8%) controls had the least mean muscle calpastatin decline, while the early (average 24.6%) and late (average 24.8%) stimulated carcasses had numerically similar mean muscle calpastatin decline at 24 h post-mortem.

4.17.4 Calpastatin to calpain-1 ratio

Fifty-seven samples were evaluated but 55 were used for analysing the ratio of calpastatin to calpain-1 content at 1 and 24 h pm. At 1 h pm, there was moderate and significant ($R^2 = 0.36$; $P = 0.0102$) variation among the groups. At 24 h pm, there was lower and insignificant ($R^2 = 0.29$; $P = 0.0579$) variation among the treatment groups. Partial correlation using repeated measures ANOVA revealed a moderate positive correlation and significant difference ($r = 0.41$; $P = 0.0048$) between the two time levels of measurements. GLM MANOVA also revealed a

significant (P = 0.0019) difference in the interaction of time and treatment effect among the groups. Table 4.39 below shows the t- test (LSD) for calpastatin/calpain-1.

Table 4.39 Mean and standard deviation for calpastatin to calpain-1 at 1 and 24-hours post-mortem for treatment groups

Treatments	N	**Calpas/cal-1 h pm Mean ± SD**	**Calpas/cal-1 24 h pm Mean ± SD**
1) S NES	7	2.11 ± 0.40^{a}	2.11 ± 0.42^{ab}
2) H NES	6	2.06 ± 0.12^{ab}	1.87 ± 0.30^{abc}
3) S 30 B	5	1.77 ± 0.23^{abc}	1.33 ± 0.11^{c}
4) S 60 B	5	1.49 ± 0.15^{c}	1.64 ± 0.29^{bc}
5) S 30 A	5	1.65 ± 0.15^{bc}	1.96 ± 0.44^{ab}
6) S 60 A	6	1.72 ± 0.44^{abc}	1.95 ± 0.62^{ab}
7) H 30 B	5	1.46 ± 0.23^{c}	1.92 ± 0.73^{ab}
8) H 60 B	4	1.65 ± 0.55^{bc}	2.42 ± 0.65^{a}
9) H 30 A	6	1.77 ± 0.40^{abc}	1.62 ± 0.42^{bc}
10) H 60 A	6	2.08 ± 0.39^{a}	1.67 ± 0.44^{bc}

Key: N, Number of carcasses; SD, standard deviation; Calpas/cal-1, Calpastatin divided by calpain-1; S, small carcasses; H, heavy carcasses; A, late stimulation; B, early stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation. ^{a,b,c,d} Means in the same column without a common superscript letter differ ($P < 0.05$).

Figure 4.26 shows the graphical distribution of calpastatin to calpain-1 ratio at 1 and 24 h pm according to the treatment groups.

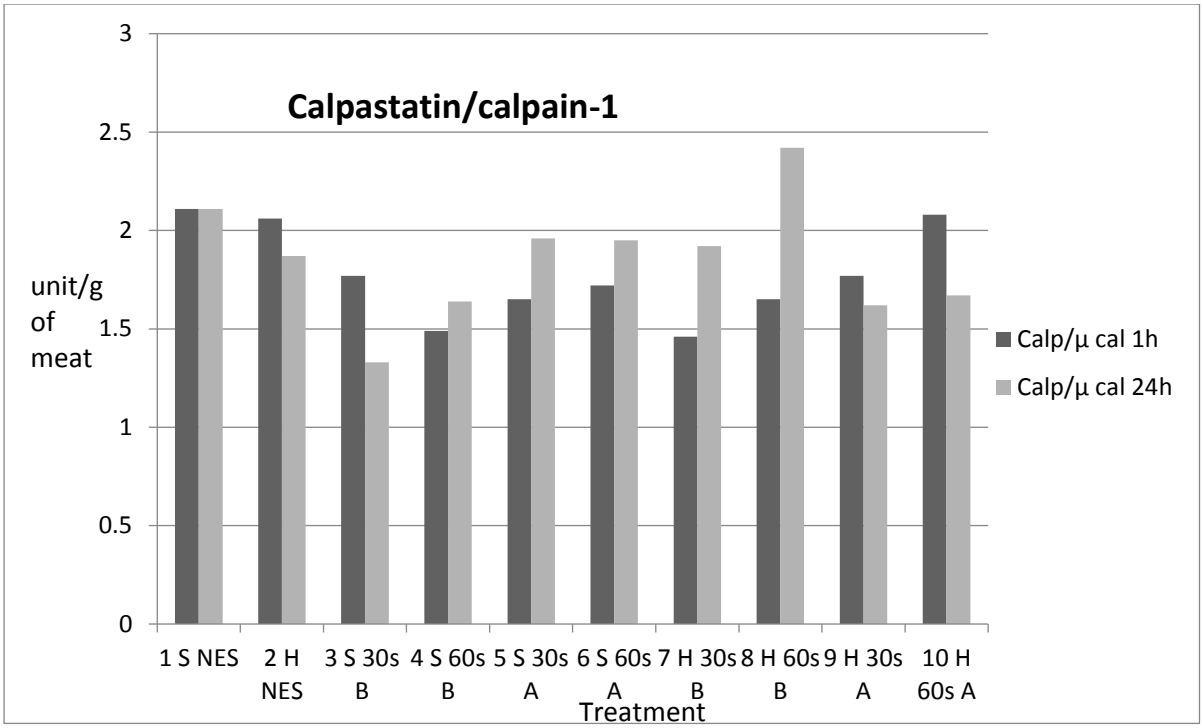

Figure 4.26 Graphical distribution of calpastatin to calpain-1 ratio at 1 and 24-hours post-mortem for treatment groups

Key: S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation

The following illustration shows the ranking of calpastatin/calpain-1 ratio according to treatments at 1 and 24 h pm in descending order.

Calp/calpain-1 1h pm: **1** (S NES) > **10** (H 60 A) > **2** (H NES) > **3** (S 30 B) > **9** (H 30 A) > **6** (S 60 A) > **5** (S 30 A) > **8** (H 60 B) > **4** (H 60 B) > **7** (H 30 B)

Calp/calpain-1 24h pm: 8 (H 60 B) > **1** (S NES) > **5** (S 30 A) > **6** (S 60 A) > **7** (H 30 B) > **2** (H NES) > **10** (H 60 A) > **4** (S 60 B) > **9** (H 30 A) > **3** (S 30 B)

There was a clear pattern of reaction from the treatment groups in terms of time of stimulation at 1 h pm (see Table 4.39 and the illustration above). Apart from Treatment 3, groups stimulated after evisceration (A) (10, 9, 5 and 6) and the controls (1 and 2) had the highest muscle calpastatin/calpain-1 ratio while the groups stimulated before evisceration (B) (8, 4 and 7), had the lowest ratio. Regarding duration of stimulation, there was no definite pattern of reaction from the treatment groups. In terms of carcass weight, there was also a mixed reaction from the groups and no clear pattern of reaction. As stated earlier, the variation and numerical differences among all the treatment groups were low. However, the early stimulated groups (4 and 7) had the lowest values ($P < 0.05$) irrespective of carcass weight and duration of stimulation.

At 24h pm, regarding the time of stimulation, there was no clear pattern of reaction from the treatment groups. In terms of duration of stimulation, there was also a mixed pattern of reaction from the treatment groups. In terms of carcass weight, there was a mixed pattern of reaction from the treatment groups as seen in the illustration above and this shows a lack of influence of weight on calp/μ-cal ratio. From the t- test, Groups 8 and 3 had the highest and lowest ratio respectively and they differed significantly ($P < 0.05$) from each other and also differ from the other groups ($P < 0.05$). All other groups had numerically close values, that is, low variation.

4.17.5 Ratio of calpastatin to calpain-1 plus calpain-2

Fifty-seven samples were evaluated for this parameter, but 55 samples were used for analysis owing to outliers. Samples were analysed for 1 and 24 h pm collection for all groups. At 1 h pm, there was low and insignificant ($R^2 = 0.20$; $P = 0.2699$) variation among the treatment groups. At 24 h pm, there was also a low and insignificant ($R^2 = 0.29$; $P = 0.0575$) variation among the treatment groups in terms of calpastatin/calpain-1 + calpain-2. Partial correlation between the two time levels reveal a significant, moderate and positive correlation ($r = 0.31$; $P = 0.0335$). GLM MANOVA also revealed significant ($P < 0.0066$) difference among the treatment groups in term of the interaction of time and treatment. Table 4.40 shows the mean values, SD and *P*-values for the treatment groups in term of calpastatin/calpain-1 + calpain-2 at 1 and 24 h pm.

Table 4.40 Means and standard deviation for thebration of calpastatin to calpain-1 plus calpain-2 at 1 and 24-hours post-mortem for treatment groups

Treatments	**N**	**Calpas/cal 1+2 1 h Mean ± SD**	**Calpa/cal 1+2 24 h Mean ± SD**
1) S NES	7	1.14 ± 0.16^{a}	1.05 ± 0.13^{ab}
2) H NES	6	1.18 ± 0.08^{a}	1.01 ± 0.22^{ab}
3) S 30 B	5	1.17 ± 0.13^{a}	0.84 ± 0.10^{b}
4) S 60 B	5	0.93 ± 0.10^{ab}	0.90 ± 0.17^{b}
5) S 30 A	4	0.99 ± 0.02^{ab}	1.00 ± 0.09^{ab}
6) S 60 A	6	1.03 ± 0.17^{ab}	0.96 ± 0.26^{ab}
7) H 30 B	6	1.02 ± 0.15^{ab}	1.25 ± 0.38^{a}
8) H 60 B	5	1.04 ± 0.29^{ab}	1.23 ± 0.34^{a}
9) H 30 A	6	1.09 ± 0.23^{ab}	0.92 ± 0.27^{b}
10) H 60 A	5	1.11 ± 0.08^{ab}	0.85 ± 0.19^{b}

Key: N= Number of carcasses; SD = Standard Deviation; Calpas/cal 1+2 = Calpastatin divided by calpain-1 + calpain-2; S = smaller carcasses; H = heavier carcasses; B = early stimulation; A = late stimulation; 30 = 30 s stimulation; 60 = 60 s stimulation. [a,b,c,d] Means in the same column without a common superscript letter differ ($p < 0.05$).

Figure 4.27 shows the graphical distribution of calpastatin/calpain-1+ calpain-2 at 1and 24 h according to treatment groups.

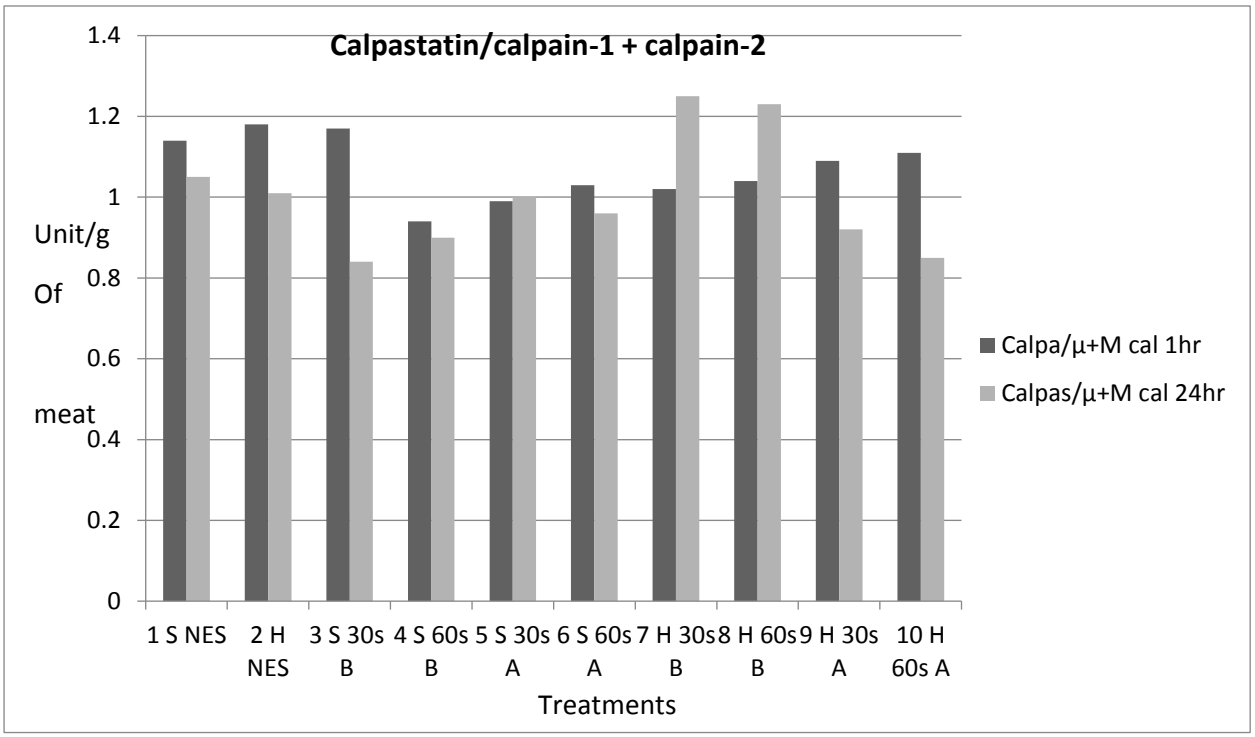

Figure 4.27 Distribution of the ratio calpastatin to calpain-1 plus calpain-2 according to treatment groups at 1 and 24-hours post-mortem

Key: S, small carcasses; H, heavy carcasses; B, early stimulation; A, late stimulation; 30, 30 seconds stimulation; 60, 60 seconds stimulation.

The following illustration shows the ranking of calpastatin/calpain-1 + calpain-2 ratio according to treatment groups at 1 and 24 h pm in descending order.

Calpas/calpain-1 + calpain-2: 2 (H NES) > **3** (S 30 B) > **1** (S NES) > **10** (H 60 A) > **9** (H 30 A) > **8** (H 60 B) > **6** (S 60 A) > **7** (H 30 B) > **5** (S 30 A) > **4** (S 60 B)

Calpas/calpain-1 + calpain-2: 7 (H 30 B) > **8** (H 60 B) > **1** (S NES) > **2** (H NES) > **5** (S 30 A) > **6** (S 60 A) > **9** (H 30 A) > **4** (S 60 B) > **10** (H 60 A) > **3** (S 30 B)

There was a mixed pattern of reaction, that is, no definite pattern from the treatment groups at 1 h pm regarding the time of stimulation (see Table 4.40 and the above illustration). In terms of

duration of stimulation, there was also no definite pattern of reaction. Likewise, the effect of carcass weight, there was no definite pattern of reaction. There were low numerical differences among all treatment groups and there were no significant differences among the electrically stimulated groups.

At 24 h pm, regarding the time of stimulation, there was a mixed pattern of reaction from the groups. However, Group 7 and 8 (heavy carcasses), that were stimulated before evisceration, had the highest values, followed by the controls, and other groups. In terms of duration of stimulation, there was also a mixed reaction. Likewise, for the effect of carcass weight, there was a mixed reaction but heavy carcass groups (7 and 8) had the highest ratios. Similar to the 1 h post-mortem values, there were low numerical differences and low variation among all treatment groups at 24 h pm.

4.18 Important correlations

Table 4.41a-c shows the attributes that had at least moderate correlations ($r \geq 0.30$/ -0.30 to 0.49/ -0.49) to strong correlations ($r \geq 0.50$/ $-$ 0.50) with the important meat quality parameters that could be appraised for quality assurance. These qualities included shear force (SF) 3 and 14 dpm; % drip loss (DL) 3 and 14 dpm; chroma (C*) 2 and 14 dpm; lightness (L*) 2 and 14 dpm and hue angle (H*) 2 and 14 dpm. Correlations of r values less than 0.30/ -0.30 were regarded as weak and unimportant, and therefore excluded from these tables. Of these moderate to strong correlations, some important and statistically significant attributes were selected for regression equations, which enabled the reaserch team to predict these meat quality attributes abattoir level before the carcasses were dispatched, and in display for a longer period. These predictive values would help at the abattoir (at 3 dpm) and the butchers who might want to store the carcasses for a longer period at 14 dpm.

Table 4.41a List of important correlations for meat shear force, drip loss, chroma, lightness, and hue angle

Attributes	**SF d 3**	*Attributes*	**SF d 14**	*Attributes*	**%DL d 3**	*Attributes*	**%DL d 14**
pH 45 min	**r = 0.30**	**pH 3 h**	**r = 0.40**	**pH 45 min**	**r = -0.39**	**pH 45 min**	**r = -0.41**
p value	0.0002	*p*	<0.0001	*p*	<0.0001	*p*	<0.0001
pH 3 h	**0.43**	**pH 6 h**	**0.48**	**pH 3h**	**-0.56**	**pH 3 h**	**-0.53**
p	<0.0001	*p*	<0.0001	*p*	<0.0001	*p*	<0.0001
pH 6 h	**0.54**	**pH 12 h**	**0.36**	**pH 6 h**	**-0.65**	**pH 6 h**	**-0.60**
p	<0.0001	*p*	<0.0001	*p*	<0.0001	*p*	<0.0001

pH 12 h	**0.45**	**T 45 min**	**-0.40**	**pH 12 h**	**-0.62**	**pH 12 h**	**-0.45**
p	<0.0001	*p*	<0.0001	*p*	<0.0001	*p*	<0.0001
pH 24 h	**0.40**	**SF d 3**	**0.58**	**pH 24 h**	**-0.53**	**T 45 min**	**0.36**
p	<0.0001	*p*	<0.0001	*p*	<0.0001	*p*	<0.0001
T 45 min	**-0.33**	**%DL d 3**	**-0.36**	**T 45 min**	**0.40**	**SF d3**	**-0.46**
p	<0.0001	*p*	<0.0001	*p*	<0.0001	*p*	<0.0001
SF d 14	**0.58**	**MFL d 14**	**0.30**	**T 12 h**	**0.31**	**%DL d 3**	**0.78**
P	<0.0001	*p*	0.0003	*p*	0.0001	*p*	<0.0001
%DL d 3	**-0.50**	**a* d 2**	**-0.37**	**SF d 3**	**-0.50**	**WHC d 3**	**-0.57**
p	<0.0001	*p*	< .0001	*p*	<0.0001	*p*	<0.0001
%DL d 14	**-0.46**	**b* d 14**	**-0.35**	**%DL d 14**	**0.79**	**L* d 2**	**0.54**
p	**<0.0001**	*p*	0.0001	*p*	<0.0001	*p*	<0.0001
L* d 14	**-0.30**	**C* d 2**	**-0.32**	**WHC d 3**	**-0.50**	**L* d 14**	**0.59**
p	0.0003	*p*	<0.0001	*p*	<0.0001	*p*	<0.0001
a* d 2	**-0.43**	**Lact 4 h**	**-0.38**	**SL (mm)**	**0.30**	**a* d 2**	**0.30**
p	<0.0001	*p*	0.0041	*p*	0.0002	*p*	0.0004
b* d 2	**-0.39**	**Glu 4 h**	-0.43	**L* d 2**	**0.55**	**b* d 2**	**0.56**
p	<0.0001	*p*	0.0009	*p*	<0.0001	*p*	<0.0001
C* d 2	**-0.43**	**CP 4 h**	**0.30**	**L* d 14**	**0.63**	**C* d 2**	**0.44**
p	<0.0001	*p*	0.024	*p*	<0.0001	*p*	<0.0001
Lact 4 h	**-0.46**	**ATP 4 h**	**0.32**	**a* d 2**	**0.31**	**Hue d 2**	**0.30**
p	0.0004	*p*	0.0184	*p*	0.0002	*p*	0.0003
Lact 24 h	**-0.38**	**G-6-P 4 h**	**-0.45**	**b* d 2**	**0.58**	**Hue d 14**	**0.37**
p	0.0034	*p*	0.0006	*p*	<0.0001	*p*	<0.0001
Glu 4 h	**-0.47**	**Cal-1 1 h**	**-0.33**	**C* d 2**	**0.44**	**Lact 1 h**	**0.56**
P	0.0003	*p*	0.0143	*p*	<0.0001	*p*	<0.0001
Glu 24 h	**-0.37**	**Calpas/cal**	**0.41**	**Hue d 14**	**0.36**	**Lact 4 h**	**0.47**
P	0.0051	**-1 1h pm**	0.0017	*p*	<0.0001	*p*	0.0005
ATP 4 h	**-0.43**	**Calpas/cal-**	**0.36**	**Lact 1 h**	**0.34**	**Lact 24 h**	**0.31**
P	0.001	**1+2 1h pm**	0.007	*p*	0.0125	*p*	0.024
G-6-P 4 h	**-0.37**			**Lact 4 h**	**0.36**	**Glu 4 h**	**0.48**
p	0.0054			*p*	0.0068	*p*	0.0003
Cal-1 24 h	**0.45**			**Glu 1 h**	**0.34**	**Glu 24 h**	**0.45**
p	0.0006			*p*	0.0109	*p*	0.0008
Calpas 24 h	**0.40**			**Glu 4 h**	**0.53**	**CP 1 h**	**-0.45**
p	0.0023			*p*	<0.0001	*p*	0.001

Calpas/cal-1 1 h pm	**0.32** 0.0147			**Glu 24 h** *p*	**0.52** <0.0001	**CP 4 h** *p*	**-0.52** <0.0001
Calpas/cal-1+2 1 h pm	**0.32** 0.015			**ATP 4 h** *p*	**-0.52** <0.0001	**ATP 4 h** *p*	**-0.48** 0.0004
				ATP 24 h *p*	**-0.32** 0.0189	**G-6-P 4 h** *p*	**0.51** 0.0002
				G-6-P 4 h *p*	**0.61** <0.0001	**Cal-1 24 h** *p*	**-0.37** **0.0075**
				Cal-1 24 h *p*	**-0.44** 0.0008		

Table 4.41b List of important correlations for meat shear force, drip loss, chroma, meat lightness and hue angle

Attribute	**C* d 2**	*Attribute*	**C* d 14**	*Attribute*	**L* d 2**	*Attribute*	**L* d 14**
pH 45 min *p*	**r = -0.49** <0.0001	**a* d 14** *p*	**r = 0.93** <0.0001	**pH 45 min** ***p***	**r =-0.51** <0.0001	**pH 45 min** *p*	**r =-0.50** <0.0001
pH 3 h *p*	**-0.51** <0.0001	**b* d 14** *p*	**0.88** <0.0001	**pH 3 h** *p*	**-0.58** <0.0001	**pH 3 h** *p*	**-0.55** <0.0001
pH 6 h *p*	**0.54** <0.0001	**H* d 2** *p*	**-0.40** <0.0001	**pH 12 h** *p*	**-0.59** <0.0001	**pH 6h** *p*	**-0.52** <0.0001
pH 12 h *p*	**-0.56** <0.0001	**H* d 14** *p*	**-0.49** <0.0001	**pH 24 h** *p*	**-0.48** <0.0001	**pH 12 h** *p*	**-0.59** <0.0001
pH 24 h *p*	**-0.52** <0.0001			**T 45 min** *p*	**0.35** <0.0001	**pH 24 h** *p*	**-0.56** <0.0001
SF d 3 *p*	**-0.43** <0.0001			**T 3 h** *p*	**-0.30** 0.0003	**SF d 3** *p*	**-0.30** 0.0003
SF d 14 *p*	**-0.32** <0.0001			**%DL d 3** *p*	**0.55** <0.0001	**%DL d 3** *p*	**0.63** <0.0001
%DL d 3 *p*	**0.44** <0.0001			**%DL d 14** *p*	**0.54** <0.0001	**%DL d 14** *p*	**0.59** <0.0001
%DL d 14 *p*	**0.44** <0.0001			**WHC d3** *p*	**-0.50** **<0.0001**	**WHC d 3** *p*	**-0.53** <0.0001
WHC d 3 *p*	**-0.49** <0.0001			**WHC d 14** *p*	**-0.43** <0.0001	**WHC d 14** *p*	**-0.38** <0.0001

L* d 2	**0.36**			**MFL d 14**	**0.31**	**L* d 2**	**0.86**
p	<0.0001			*p*	0.0002	*p*	<0.0001
L* d 14	**0.46**			**L* d 14**	**0.86**	**a* d 14**	**-0.30**
p	**<0.0001**			*p*	<0.0001	*p*	0.0003
a* d 2	**0.92**			**a* d 14**	**-0.31**	**b* d 2**	**0.68**
p	<0.0001			*p*	<0.0002	*p*	<0.0001
b* d 2	**0.91**			**b* d 2**	**0.58**	**b* d 14**	**0.30**
p	<.0001			*p*	<0.0001	*p*	0.0003
Lact 4 h	**0.33**			**C* d 2**	**0.36**	**C* d 2**	**0.46**
p	0.0173			*p*	<0.0001	*p*	<0.0001
Glu 1 h	**0.32**			**H* d 2**	**0.65**	**H* d 2**	**0.56**
p	0.0179			*p*	<0.0001	*p*	<0.0001
Glu 4 h	**0.38**			**H* d 14**	**0.61**	**H* d 14**	**0.67**
P	0.0052			*p*	<0.0001	*p*	<0.0001
Glu 24 h	**0.36**			**Lact 1 h**	**0.58**	**Lact 1 h**	**0.48**
p	0.0084			*p*	<0.0001	**p**	0.0002
CP 1 h	**-0.38**			**Glu 1 h**	**0.36**	**Lact 4 h**	**0.48**
p	0.0055			*p*	0.0059	*p*	0.0002
ATP 4 h	**-0.39**			**Glu 4 h**	**0.55**	**Glu 1 h**	**0.34**
p	0.0036			*p*	<0.0001	**p**	0.0093
G-6-P 4 h	**0.37**			**Glu 24 h**	**0.46**	**Glu 4 h**	**0.41**
p	0.0076			*p*	0.0005	***p***	0.0014
Cal-1 24 h	**-0.41**			**Gly 4 h**	**-0.30**	**Glu 24 h**	**0.36**
p	0.0022			*p*	0.0281	**p**	0.0063
Cal-2 24 h	**-0.30**			**CP 1 h**	**-0.33**	**CP 1 h**	**-0.32**
p	0.0316			*p*	0.0141	*p*	**0.0165**
Calpas24 h	**-0.31**			**CP 4 h**	**-0.36**	**ATP 4 h**	**-0.40**
p	0.0234			*p*	0.0057	*p*	0.0024
Calpas/cal	**-0.32**			**ATP 4 h**	**-0.45**	**G-6-P 4 h**	**0.37**
-1 1 h p	0.0196			*p*	0.0006	*p*	0.0051
Calpas/cal	**-0.30**			**G-6-P 4 h**	**0.48**	**Cal-1 24 h**	**-0.44**
1+2 1 h	0.00291			*p*	0.0002	*p*	0.0006
				Cal-2 1 h	**-0.31**		
				p	0.0182		
				Cal-2 24 h	**-0.40**		
				p	0.0027		

Table 4.41c List of important correlations for meat shear force, drip loss, chroma, meat lightne and hue angle

Attribute	**H* d 2**	*Attribute*	**H* d 14**			
%DL d 14	**r = 0.30**	**pH 24 h**	**r = -0.32**			
p	0.0003	*p*	<0.0001			
L* d 2	**0.65**	**%DL d 3**	**0.36**			
p	<0.0001	*p*	<0.0001			
L* d 14	**0.56**	**%DL d 14**	**0.37**			
p	<0.0001	p	<0.0001			
a* d 2	**-0.50**	**WHC d 3**	**-0.35**			
p	<0.0001	*p*	<0.0001			
a* d 14	**-0.58**	**L* d 2**	**0.61**			
p	<0.0001	*p*	<0.0001			
C* d 14	**-0.40**	**L* d 14**	**0.68**			
p	<0.0001	*p*	<0.0001			
H* d 14	**0.70**	**a* d 14**	**-0.76**			
p	<0.0001	*p*	<0.0001			
Lact 1 h	**0.32**	**b* d 2**	**0.31**			
p	0.0193	*p*	0.0002			
		C* d 14	**-0.49**			
		p	<0.0001			
		H* d 2	**0.70**			
		p	<0.0001			
		Lact 1 h	**0.35**			
		p	0.009			

Key: d 2, day 2 post-mortem; d 3, day 3 post-mortem; d 14, day 14 post-mortem; SF, shear force; DL, drip loss; C*, chroma; L*, lightness; H*, hue angle; a*, meat redness; b*, meat yellowness; T, carcass temperature; h, hours post-mortem; MFL, myofibril fragment length, WHC, water holding capacity; ATP, adenosine-tri-phosphate; Lact, muscle lactate; G-6-P, glucose-6-phosphate; Glu, glucose; Gly, glycogen; CP, ctreatine phosphate; Cal-1, calpain-1; Cal-2, calpain-2; Calpas, calpastatin

4.19 Regressions

Regression analysis was carried out to determine the level of influence of the strongly correlated dependent and independent variables on key quality attributes (shear force, drip loss and colour attributes) at 3 dpm (abattoir/butchery level) and 14 dpm (retail/supermarket level). This was

done for quality assurance at the various levels of processing. The results are provided in Table 4.42 below.

Table 4.42 Important regressions for key quality parameters

Parameter	**Quality variable**	***P* value**
Shear force day 3 pm	pH 3 h pm	0.6582
	pH 24 h pm	0.0664
	Temperature 45 min	0.8042
	% DL 3 d pm	0.7154
	C* day 2 pm	**0.0052**
Shear force day 14 pm	pH 3 h pm	0.3346
	Temperature 45 min pm	0.6651
	Shear force 3 d pm	**< 0.0001**
	% DL 3 d pm	0.3462
	Chroma day 2 pm	0.1870
% Drip loss day 3 pm	pH 3 h pm	0.6176
	pH 24 h pm	**0.0041**
	Temperature 45 min pm	0.5346
	L* day 2 pm	0.1426
	C* day 2 pm	**0.0206**
% Drip loss day 14 pm	pH 3 h pm	0.8257
	pH 14 d pm	0.7774
	Temeperature 45 min pm	0.1156
	% DL 3 d pm	**< 0.0001**
	L* day 2 pm	0.4274
	C* day 2 pm	0.8939
Chroma day 2 pm	**pH 3 h pm**	**0.0455**
	pH 24 h pm	0.1662
	% DL day 3 pm	**0.0206**

	L* day 2 pm	**0.0224**
Chroma day 14 pm	**H* day 2 pm**	**< 0.0001**
Hue angle day 2 pm	**L* day 2 pm**	**< 0.0001**
Hue angle day 14 pm	pH 24h pm	0.0669
	% DL 3 dpm	**< 0.0001**
	L* day 2 pm	**< 0.0001**

Key: % DL, % drip loss; L*, meat lightness; C*, chroma; H*, hue angle; highlighted (bold) *P* values and variables shows significant relationship at $P < 0.05$.

As shown in the regression analysis (Table 4.42), the quality parameters that showed significant ($P < 0.05$) relationship/s with the quality variables are highlighted in bold letters. That is, C* 2 dpm could be used to predict SF 3 dpm ($P = 0.0052$). Likewise, SF 3 dpm could be used to predict SF at 14 dpm ($P < 0.0001$). Percentage drip loss at 3 dpm could be predicted by the values of at pH 24 h pm ($P = 0.0041$) as well as C* 2 dpm ($P = 0.0206$). Percentage drip loss at 14 dpm could be safely predicted by percentage drip loss at 3 dpm ($P < 0.0001$). Chroma value at 2 dpm could be predicted by pH 3 h pm ($P = 0.0455$) as well as L* 2 dpm ($P = 0.0224$). Chroma value at 14 days pm could be predicted by H* at 2 dpm ($P < 0.0001$). Analysis also showed that hue angle (H*) 14 dpm could be predicted with pH 24 h pm ($P = 0.0669$) as well as percentage DL 3 dpm ($P < 0.0001$) and L* value at 2 d pm ($P < 0.0001$).

CHAPTER 5

DISCUSSION

5.1 Muscle pH and its decline

pH in muscles normally decrease from around 7.2 at slaughter to approximately 5.4-5.8, which is regarded as the ultimate pH (pH_u) (Smulders et al., 1992; England et al., 2017). In this study and from previous literature, there were significant differences in pH at the various times of measurement (45 min pm, 3 h, 6 h, 12 h and 24 h pm) as the pH declines (Agbeniga and Webbb, 2018). Electrical stimulation (ES) is known to bring about a rapid pH decline by increasing the glycolytic rate, thereby causing faster rigor onset, so that rapid chilling can be carried out without the risk of cold shortening (Davey & Chrystall, 1980; Davey, Gilbert & Carse, 1976). The same trend was observed for the treatments administered in this study, in which low voltage electrical stimulation LVES (110 V) was applied. This is in accord with the work of Eikelenboom, Smulders and Ruderus (1985) and Kondos and Taylor (1987) in which LVES brought about a similar pH fall, compared with high voltage electrical stimulation (HVES). There was a clear pattern of pH decline in terms of time of stimulation, as observed in the ranking of the treatments. Carcasses that were stimulated immediately after bleeding (early stimulation/before evisceration) (Treatments 3, 4, 7 and 8) had the fastest pH decline and the lowest pH at all time of measurement. On the other hand, carcasses stimulated after evisceration (late stimulation) (5, 6, 9 and 10) and the controls (1 and 2), had the slowest pH decline and the highest pH at all time of measurement (Table 4.1; Figure 4.1). This reaction pattern is in accord with the work of Hwang and Thompson (2001), using LVES and HVES, and Wahlgren, Devine and Tomberg (1997) in which HVES was used. The results of this study show that LVES achieved similar results.

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In terms of the duration of stimulation, the results revealed a mixed reaction from the treatment groups. There was no definite pattern of reactions at all time of measurement. This is no surprise, as currently there is no consensus on the optimum duration of application of ES, be it HVES or LVES. Mckeith et al. (1981) concluded that 60 seconds stimulation with voltage of 550 V was ideal for improving palatability, while Strydom and Frylinck (2014) demonstrated that 15 seconds at 150 V could be beneficial in terms of tenderness development, which is a function of pH decline and glycolytic rate (Wulf et al., 2002), compared with 45 and 90 sec duration. According to Gursansky et al. (2010), LVES could be as efficient as HVES as long as the duration was not less than 40 seconds as opposed to 10 seconds. Pearce et al. (2006) also revealed that duration of 34 seconds at 300 V resulted in 60% of carcasses hitting the pH/temperature window (pH6 below 35 °C). In this study, it was concluded that 30 seconds duration would be ideal, as it would reduce the processing time and cost of electricity, in addition better meat quality such as reduced shear force (in combination with heavy carcass weight) (Table 4.6). Shorter ES duration (30 s) brought about marginally lower drip loss and heavy carcass weight brought about higher drip loss (Table 4.9). This therefore implies that shorter duration of ES would be ideal for larger and heavier carcasses to minimize drip loss. Regarding the effects of carcass weight on pH decline, there were mixed reactions from the treatment groups. However, out of the early stimulated carcasses (with the lowest pH decline), heavy carcass groups (7 and 8) had the lowest pH from 3 to 24 h pm. This result accords with the recent findings of Warner et al. (2014) and the review of Strydom and Rosenvolt (2014) and Jacob and Hopkins (2014), that heavier feedlot carcasses exhibits faster pH decline and stands the risk of high temperature rigor. Likewise, Hopkins et al. (2007) indicated that heavier un-stimulated beef carcasses exhibited faster pH decline compared with lighter carcasses.

Key factors in the decline of pH are obviously the time of application of ES and carcass weight. Application of ES immediately after slaughter (before evisceration) (7 min pm) brought about the fastest pH decline and lowest pH values at all times of measurement. This was irrespective of duration of stimulation or carcass weight. However, the last two groups that had the lowest ultimate pH values were heavy carcasses (Groups 7 and 8). These groups also showed significant faster pH decline ($P < 0.05$) compared with the late stimulated and control groups (Table 4.1). A high and significant variation (73%) was observed among the treatment groups at 45 min pm. This variation decreased to 37% at 24 h pm. These variations could be attributed mainly to the time of stimulation followed by carcass weight, which brought about a difference between the heavy and smaller early stimulated carcasses. Multiple analysis of variance (MANOVA) revealed

significant differences between the times ($P < 0.0001$) of measurement, and also the number of time and treatments interaction effects ($P < 0.0001$). This was also expected owing to the large and significant variations among the treatment groups at the various times of measurement.

All pH readings correlated positively and strongly with one another and with meat shear force, drip loss, WHC, lightness, chroma, and energy metabolites, but correlates weakly/moderately with the proteolytic enzymes (Table 4.41). This reflected the influence and relationship (positive or negative) of pH on these variables and some were included in the regression equation to predict important quality variables, for example, shear force, drip loss and chroma at the abattoir and display level, for quality assurance. The time of evaluation and prediction of these quality attributes was targeted at 3 dpm for the abattoir and butchery and 14 days for the retail shops and supermarkets. This would enable the butchers and the retail shops to ascertain the quality of their products and enable them to place appropriate prices and possibly to know how the products could be processed, based on quality assessment. The results (Table 4.42) showed that pH 24 h pm could be used to predict meat drip loss at 3 dpm. The lower the pH_u, the more the drip loss (Offer and Cousins, 1992; Uytterhaegan et al., 1994). Similarly, pH 3 h pm could be used to predict meat chroma value at 2 dpm. That is, the faster the pH decline at 3 h pm, the better the chroma value at 2 dpm, as reported by some researchers (Hwang and Thompson, 2000), Pommier (1992), Powell et al. (1996) and Li et al. (2011).

5.2 Carcass temperature and its decline due to treatments

There was a clear pattern of reaction from the treatment groups in terms of temperature decline. As observed from the rankings and the charts, in terms of carcass weight, heavy carcass groups (2, 7, 8, 9 and 10) had the highest carcass temperatures at all time of measurement (i.e. 45 min, 3, 6, 12, and 24 h pm), while the lighter sides had the lowest temperatures and the fastest temperature decline (Table 4.2; Figure 4.2). This was expected and is in accord with the work of Klont et al. (1999) and Smith et al. (1976). A recent report by Warner et al. (2014a) also recorded that heavier feedlot carcasses across Australia, exhibited high temperature rigor (i.e. temperature above 35 °C when pH is 6) (Thompson 2002) in 75% of carcasses sampled. A similar finding was reported by Hopkins et al. (2007) in carcasses that were not electrically stimulated. According to Warner et al. (2014a) plasma insulin and days of feed were responsible for the high rigor temperature in the heavy carcasses. As reviewed by DiGiacomo, Leury & Dunshea (2014a), increased fat deposition elevates circulating glucose and insulin through insulin resistance. They added that insulin resistance reduces the ability to dissipate heat and this may lead to high rigor temperature in heavier and fatter carcasses. Thompson et al. (2006) reported that hot carcass

weight had a linear effect on temperature decline, especially Temperature at pH6, which affects glycolytic rate. Chilling rate and carcass temperature decline are affected by carcass size, shape, fatness, temperature, relative humidity and flow pattern of air (Smulders et al., 1992). Fat thickness plays an important role in reducing cold shortening (Dolezal et al., 1982; Smith et al., 1976) and increased enzymatic activities by allowing the carcasses to chill more slowly (Smith et al., 1976). The slower chilling from heavy carcasses is due to insulation from its fat or to increased body mass. Smith and Carpenter (1973) suggested that a subcutaneous fat depth of 0.62 cm at the 12^{th} rib is the minimum to prevent cold shortening in beef carcasses. Hopkins, Holman, and Van de Ven (2015) suggested that as a result of change in mean carcass sizes over experimental years, there could be variation in abattoirs in temperature decline.

Regarding the duration of stimulation, there was no definite pattern of reaction and this was expected. There is no known literature on the effects of the duration of ES on carcass temperature decline. Regarding the time of stimulation, there was also no clear pattern of reaction at the various times of measurement, except at 3 h pm when carcasses stimulated after evisceration (10, 9, 6 and 5) had the highest temperature readings (rankings after Figure 4.2). Again, there is no plausible explanation for this. Hwang and Thompson (2001) reported that stimulation type and time of application had little or no effects on carcass temperature. This supports the current findings as there were no significant differences in carcass temperature due to time of application and duration of ES among the groups. The GLM also revealed a declining trend in variation for temperature from 45 min (68%) to 41% at 24 h pm among the groups. This variation could be attributed mainly to carcass weight as it exerted the strongest effects on the results compared with ES duration and time. Treatments 7, 8, 9 and 10 had the highest initial and the ultimate temperature values because of their heavy weights but were significantly ($P < 0.05$) different from one another (Table 4.2). However, they were numerically close in value. Certain regulations (James, 1996; EU Parliament, 2004; FAO, 1991; South African Meat Safety Act, 2000) requires that all meat temperatures in the carcass must be reduced to below 7 °C, before being processed or transported from the chillers. All groups in this study abided by this regulation as their 24-hour temperatures were below 7 °C.

There were significant differences ($P < 0.05$) among the different times of measurement, irrespective of the treatments (see analysis in Chapter 4). This was expected. As heat dissipates from carcasses, the carcasses become colder and the higher the weight, the slower the temperature decline (Agbeniga and Webb, 2018). The interaction between time and treatment effects was also found to be significant ($P < 0.0001$) and this was expected. From the correlations (Table 4.41),

temperature at 45 min pm correlated with shear force 3 and 14 dpm. This was contrary to the reports of authors such as Hwang and Thompson (2001b) and Warner et al. (2009). However, authors such Dransfied. (1993) and Morton et al. (1999) supports this and suggests that calpain-1 is likely to undergo autolysis at this initial high temperature and faster pH decline, resulting in increased tenderness. Initial carcass temperature also correlated with % drip loss at 3 and 14 dpm. This was in accord with the report of Thompson et al. (2005), who cautioned that a high initial carcass temperature at low pH could lead to protein denaturation, which increases drip loss.

5.3 pH and temperature interactions

Many studies have been conducted to investigate the importance of proper balance between pH and temperature in the first 24 h pm to achieve desired qualities in meat, especially, colour, drip loss and tenderness. Warner, Kearney, Thompson and Polkinghorne (2009), Warner et al. (2014), Jacob and Hopkins (2014) and Thompson (2002) indicated that carcasses that pass through the hot/heat shortening window (temperature above 35 °C at pH 6) (Thompson, 2002) (Figure 2.3) are likely to have tough meat due to sarcomere shortening and exhaustion of proteolytic enzymes, which reduces ageing potential and tenderization (Devine et al., 1999). Meat from such a carcass is likely to have reduced tenderization, lower WHC and reduced colour stability (Kim et al., 2014). Electrical stimulation should be properly regulated and applied efficiently to achieve optimum results, in objective carcass and meat quality. Thompson et al. (2005) cautioned that if pH declined too rapidly at high temperature, protein might be denatured, which could result in meat toughening and increased drip loss. The most tender beef after 14 days of ageing was achieved when carcass temperature at pH 6.0 was between 29 and 30 °C, according to Hwang and Thomspon (2001b). Also, according to these authors, the rate of pH decline had the highest impact on meat eating quality. Recent findings by Hopkins et al. (2007), Warner et al. (2014) and Jacob and Hopkins (2014) indicated that a high proportion of carcasses from feedlot cattle, especially the heavy ones, exhibited high rigor temperature and PSE-like (pale, soft and exudative) meat conditions.

In this study, Treatments 4, 7 and 8 (early stimulated light and heavy carcasses) passed through the hot/heat shortening window between 1 and 3 h pm. These carcass groups had the lowest shear force at 3 and 14 dpm. The reason points to the notion that higher temperature at the initial chilling period favoured enzymatic activities (proteolysis). This is contrary to the reports of Dransfield (1994) and Dranfield et al. (1992), who suggested that micro calpain was likely to undergo autolysis at this high temperature and low pH, resulting in reduced tenderness. In another study, Simmons, Cairney and Daly (1997) reported that heat shortening occurred in some of the

carcasses that went through the hot/heat shortening window, but not all the carcasses had tough meat. However, most of the reports on high rigor temperature did not take a longer ageing period into account (Kim et al., 2014). Most authors measured tenderness at 1 to 3 dpm and found improved tenderness early post-mortem in high rigor temperature meat (Hwang et al., 2004). In this study, lower shear force was recorded in the early stimulated carcasses at 3 and 14 dpm. As shown in the calpain-1 readings of these carcasses (early stimulated), there was more decline in calpain-1 in the first 24 h pm (Table 4.36) in the early stimulated carcases (especially the heavy carcasses) compared with the late stimulated carcasses. This implies that LVES did not result in huge decline in calpain-1, compared with HVES, as stated by Morton et al. (1999), who reported about 80% decline. This decline was reflected in the lower shear force of the early stimulated carcasses (Table 4.4), which persisted till 14 dpm. It was also reflected in the higher drip loss in the early stimulated carcasses, especially in the heavier ones (7 and 8), ($P < 0.05$) (Table 4.8) (Thompson et al., 2005; Devine, 2009). Likewise, it was reflected in the colour attributes in which there was higher L* in the early stimulated carcasses (Table 4.16), especially in the heavier carcasses at 3 and 14 dpm. A similar trend was observed in C* values, but early stimulated and the heavier carcasses in particular, (Table 4.21), exhibited higher ($P < 0.05$) C* values but only at 3 dpm. (The reasons for the effects of pH and temperature interactions are discussed in sections 5.5, 5.6.2, 5.9 and 5.12). The low shear force of the carcasses (3, 4, 7 and 8) that were stimulated early pm, is also contrary to the report of Hwang and Thompson (2001a) which stated that early application of ES was associated with rapid pH decline, which led to exhaustion of the protease system (lower micro calpain and higher calpastatin activities), to produce a high meat shear force. The WHC for these carcass groups (4, 7 and 8) was also the lowest at 3 and 14 dpm (Table 4.8). This is no surprise as drip loss correlated negatively strongly with WHC (Table 4.41).

5.4 Subcutaneous fat (mm)

Analysis revealed a clear pattern of reaction regarding carcass weight and subcutaneous fat. Heavy carcass groups (2, 9, 8, 7 and 10), had the highest depth of subcutaneous fat (Table 4.3). This was no surprise as heavy carcasses are expected to have more subcutaneous fat. The fat allows the carcasses to chill more slowly thereby increasing enzyme activities (Smith et al., 1976), which increases meat tenderness. Subcutaneous fat thickness also helps in reducing cold shortening and as a result, improves tenderness (Dolezal et al., 1982; Smith et al., 1976). The study of Smith and Carpenter (1973) suggested that a subcutaneous fat of 0.62 cm at the 12^{th} rib is the minimum to prevent cold shortening. This was evident in this study as heavy carcass groups (2, 9, 8 and 7) had subcutaneous fat cover of over 0.62 cm and they were not cold shortened.

Groups 7 and 8 had the lowest shear force at 14 dpm, compared with the other groups. This could be attributed partly to their heavy weight. These heavy carcass groups also had the lowest temperature decline rate in this study, owing to the thick fat cover. This shows a strong correlation/relationship between fat thickness, carcass weight, temperature decline, cold shortening, enzymatic activities and tenderness. (Some of these correlations are further discussed below). There was no definite pattern of reaction from the treatment groups with regards to the effects of time of stimulation and the duration of stimulation on subcutaneous fat. Subcutaneous fat is an intrinsic part of the carcass and it is not known to be affected by electrical stimulation. Currently, there is no known literature on the effects of time and duration of electrical stimulation on subcutaneous fat cover and this leaves us with no plausible explanation.

5.5 Effects of treatments on meat shear force

Meat tenderness, which is a function of shear force, is the most important meat quality attribute after cooking (Miller et al., 1995). Low voltage electrical stimulation has been shown to improve tenderness in many research (Aalhus, Jones, Lutz, Best & Robertson, 1994; Taylor & Marshall, 1980; Agbeniga & Webb, 2018). Ducastaing et al. (1985) and Uytterhaegen et al. (1992) found that stimulation resulted in early reduction of calpain-1 activity, which was related to improved tenderness. This is in accord with the result of the current study. Some researchers also reported little or no effect of ES on tenderness (Rodbotten, Lea & Hildrum, 2001), and even negative effects (Unruh et al., 1986). In this study, NES carcasses had the highest shear force values, while the electrically stimulated carcasses, especially the early stimulated ones had the lowest shear force values at 3 and 14 dpm (Table 4.4). Although other factors affect meat shear force values such as sarcomere length and myofibril fragment length. These factors are discussed later in this chapter.

It was observed from the illustration of the rankings in Chapter 4 that carcasses stimulated before evisceration (Treatments 3, 4, 7 and 8) had the lowest shear force values at 3 and 14 dpm. Time of stimulation had the greatest influence on shear force at 3 and 14 dpm (Table 4.5 and 4.6). Early stimulation produced significantly ($P < 0.05$) lower shear force at 3 dpm and a high tendency to be significant ($P = 0.06$) at 14 dpm, compared with late stimulated carcasses and NES controls. This is contrary to the report of Hwang and Thompson (2001), which stated that stimulation at 3 min pm resulted in significantly higher shear force compared with the carcasses stimulated at 40 min pm, regardless of the type of stimulation. This was attributed to initial decrease of calpain activity combined with a relatively high calpastatin level immediately after slaughter. Unruh et al. (1986) and Pommier et al. (1987) also reported tougher meat for carcasses stimulated early pm

compared with late stimulation. Similarly, Wahlgren et al. (1997) reported that if there was a rapid pH decline as a result of ES within 2 min pm, meat was not as tender after three days of ageing at 4 °C compared with ES at 30 min pm. Bendall (1976) suggested that the response of bovine muscles to ES decreases significantly after 50 min pm in beef carcasses and even sooner for lamb carcasses. The author concluded on 30 min pm as ideal time to carry out ES. Kim et al. (2013) also concluded that LVES at 90 min pm did not have effects on proteolysis, instrumental and sensory tenderness of important muscles in the carcass.

However, Meat and Livestock Australia (MLA) and Sheep Industry Cooperative Research Centre reported that carcasses stimulated with LVES at sticking point produced 62% more bleed-out, a lighter and redder colour and improved tenderness (MLA, 2008). Also, according to the report of Adeyemi & Sazili (2014), carcasses are now subjected to ES immediately post-mortem (MLA & AMPC, 2011), to enhance tenderness and bleed-out. Wiklund et al. (2001) also reported that LVES on red deer during bleeding produced lower shear force for up to 3 weeks pm. On this note and from the results of this study, the researchers can conclude that LVES immediately after slaughter applied to the whole carcass is ideal for enhancing beef tenderness at 3 and 14 dpm. Although, the most tender meat in this trial came from carcass groups 7 and 8 (heavy carcasses, early stimulated) and they passed through the hot/heat shortening window at 1 to 3 h pm. This presented an advantage by early activation of the proteolytic enzymes, which brought about enhanced tenderness.

There were mixed reactions from the treatment groups and no significant influence of duration of ES on meat shear force was seen (Table 4.6). However, the interaction of heavy carcass weight and longer duration of ES (60 s) produced significantly lower shear force at 3 dpm (Table 4.6). As stated in section 5.1, there is no consensus on optimum duration for the application of ES, but care should be taken not to over- or under-stimulate as this could result in reduced tenderness, colour defects, increased drip loss and reduced shelf life rather than improved quality (Hwang et al., 2003; Hildrum et al., 1999). Strydom and Frylinck (2014) demonstrated that a short duration of ES (15 s at 150 V, 17 Hz, 5 ms pulse width) could be more beneficial to initial tenderness, tenderness development, drip loss and colour, compared with 45 and 90 sec stimulation, where pre-slaughter stress is minimized. The work of Gursansky, O'Halloran, Egan, & Devine (2010) reported a very effective influence on tenderness of LVES (45 V) applied for 40 s at about 5 to 10 min after slaughter on beef carcasses (*Bos Indicus* and *Bos Taurus*) and chilled conventionally, compared with 10 s stimulation. Similarly, the work of Hwang & Thompson (2001b) on *B. Indicus* and *B. Taurus* showed that 40 s LVES applied within 3 min of slaughter or later (40 or 60

min pm) could be similar in effectiveness to HVES. In this vein, it could be concluded that 30 s stimulation compared with 60 s would be ideal as this would reduce processing time, and the cost of electricity usage without detrimental effects to meat and carcass quality.

There were mixed reactions and no significant difference in shear force in terms of carcasses weight at 3 and 14 dpm (Table 4.4 and 4.6). However, heavy carcass groups, stimulated early pm (7 and 8) had the lowest shear force (Table 4. 4). Similarly, of the two control groups that were not stimulated (1 and 2), the heavy carcass group (Group 2) was significantly ($P < 0.05$) more tender than the lighter carcass group (Group 1) at 3 and 14 dpm. Currently, there are few studies on the effects of carcass weight and size and dimension on the efficacy of ES on carcasses. Hopkins et al. (2007) reported in their study that, most of the heavier carcasses had higher rates of pH decline which were too fast and did not comply with the pH/temperature window of MSA. Hopkins, Ponnampalam, Van de ven and Warner (2014) also concluded that there was an obvious interplay between temperature, pH and enzyme activity and if conditions allowed for early activation of calpains before denaturation, with calcium having an integral role, there would be a commensurate improvement in tenderness. This was supported by Strydom et al. (2005). There was more calpain decline (35%) in the early stimulated carcasses compared with the late stimulated carcasses (25%) and this could be a major player in the improved tenderness in the early stimulated carcasses (Table 4.36). This was also supported by the work of Morton et al. (1999), who reported that carcasses with the most rapid pH decline and the highest loss of calpain-1 at 24 h pm had significantly lower shear force. The early stimulated carcasses, especially the heavier ones had fastest pH decline, lowest pH_u, and the highest initial carcass temperature, with the highest calpain-1 loss at 24 h pm (Table 4.1; Table 4.2; Table 4.36). It could therefore be concluded that the main reason that these carcass groups had the lowest shear force at 3 and 14 dpm was their rapid pH fall at higher initial carcass temperature and higher proteolytic activity, as indicated by the higher loss of calpain-1 (Dutson et al., 1978; Moeller et al., 1976).

The correlations (Table 4.41a) show the meat quality variables that are closely correlated with shear force. All the pH values were closely and positively correlated with shear force. This is supported by Kastner et al. (1993) and Marsh et al. (1987). Dransfield (1993) and Koohmaraie and Geesink (2006) also affirmed that ES accelerated pH decline which could increase calpain proteolytic activity. Lactate 4 and 24 h pm, glucose 4 and 24 h pm, ATP 4 h pm and G-6-P 4 h pm were negatively and moderately correlated to shear force at 3 dpm (Table 4.41a). At 14 dpm, the concentration of lactate, glucose, CP, ATP and G-6-P at 4 h pm also correlated moderately with meat shear force (Table 4.41). These energy metabolites increase or decrease over time post-

mortem and these affects tenderness (Monin & Sellier, 1985). Lactate, for example, is a direct function of pH decline. The higher the lactate in muscle, the lower the pH fall, and the lower the shear force (Hamilton et al., 2000; Van Laack et al., 2001; Wulf et al., 2002).

These relationships and correlations were also affirmed by the rankings following Table 4.4, in which the carcass groups with the lowest shear force and fastest pH decline (7, 8, 4 and 3) had the highest lactate content at 1, 4 and 24 h pm. These carcasses were all stimulated before evisceration. These carcass groups also had the highest glucose content in the muscles at 1 and 4 h pm (Table 4.28) but at 24 h pm, there were mixed reactions because most of the glucose in the muscle had been exhausted (Gregory, 1998). A similar pattern was observed for glycogen, in which the carcass groups (7, 8, 4 and 3) with the lowest shear force had the lowest glycogen levels at 1, 4 and 24 h pm (Table 4.29) due to their strong glycolytic potential. A similar pattern was observed for CP and ATP 1 and 4 h pm but at 24 h pm, there were mixed reactions owing to the exhaustion of CP and ATP, and at which time, rigor is completed (Gregory, 1998). A pattern that was observed for G-6-P at 1h pm was that all the carcasses that were stimulated for 30 s had the highest content of G-6-P, while the groups stimulated for 60 s had the lowest content of G-6-P but at 4 and 24 h pm, there were mixed reactions. This suggests that longer duration of stimulation exhausts G-6-P faster than shorter duration at the early pm period. Also, carcasses stimulated before evisceration had the lowest G-6-P content at 24 h pm. This reaffirms the fact that all the energy metabolites are inter-related (Gregory, 1998).

Some correlations and relationships were also observed for proteolytic enzymes and shear force (Figure 4.41a). At 1 and 24 h pm, there were mixed reactions in terms of calpain-1. However, the heavier, early stimulated carcasses (7 and 8) had the highest average calpain-1 decline (47.84%). This shows the effects of the combination of time of ES (Hwang & Thompson, 2001; Hwang et al., 2004) and heavy carcass weight (Sanudo et al., 2004). Kim et al. (2014) found that, early activation and subsequent exhaustion of proteolytic enzymes (especially the calpain system) early post-mortem, beacause of increased muscle metabolism at high rigor temperature resulted in increased tenderization. This was observed in the early stimulated carcasses, especially the heavier ones, in the current study. The amount of calpain-1 decline, early pm, in this study could be linked to the time of stimulation (early stimulation) in those carcass groups (see rankings and Table 4.36). Most of the carcasses with the fastest pH decline (Groups 7, 8 and 4) had the highest calpain decline at 24 h pm. In this study, we also found no significant difference between the 1 and 24 h calpain-2 and this accords with the work of Whipple et al. (1990) and Zamora et al. (1996), which reported that calpain-2 levels remained unchanged for up to 4 dpm. Morton et al.

(1999) also reported that carcasses with high levels of calpain-2 at slaughter produced tough meat. This agrees with the result of this study in which carcass groups (5, 6, 9 and 10) with the highest units of calpain-2 had the toughest meat at 3 and 14 dpm (Table 4.37 and rankings), although the numerical differences were low. The duration of stimulation and carcass weight had no visible influence on calpain-1 activities pm.

Regarding calpastatin and meat shear force, there were very low variations among the treatment groups at 1 h pm (R^2 = 0.18) and 24 h pm (R^2 = 0.096). Calpastatin is known to modulate the activity of calpains (Koohmaraie, 1992). The pattern of reaction at 1 h pm was skewed, with Groups 3 and 8 (which had the lowest shear force) having the highest calpastatin units per gram of meat sample. The other two groups with the lowest shear force (7 and 4), had the lowest amount of calpastatin at 1 h pm (Table 4.38 and the ranking that follows). These four treatment groups were stimulated early and were expected to display similar or numerically close values because they were stimulated early and had the lowest shear force. According to Morton et al. (1999) and Koohmaraie et al. (1991a, b), there was an increase in calpastatin activity early post mortem but at 24 h pm, just like calpain, there was a considerable decline in extractable calpastatin. This agrees with the results in Groups 3 and 8, which had the highest calpastatin activities at 1 h pm, but declined considerably at 24 h pm. Also, in this study, the early (mean 24.6%) and late (mean 24.8%) stimulated carcasses had a similar decline in calpastatin. However, the result in Groups 4 and 7 (which had the lowest calpastatin activities at 1 h pm), which also had the lowest shear force in this study, did not agree with the report of Morton et al. (1999), which showed a strong positive correlation between shear force and calpastatin activities in lamb. The reason is not clear. According to Morton et al. (1999), calpastatin activities were expected to be high for all carcasses early pm and then decline slowly as the pH dropped, and shear force decreased. These authors reported that muscle with high pH at 1 h pm had significantly slower declines in calpain-1 and calpastatin levels, which could result in touch meat. The results suggest there was more autolysis of calpain-1 in the early stimulated carcasses owing to faster pH decline and higher carcass temperature, and so calpain-1 could not bind most of the calpastatin, which resulted in similar levels of calpastatin in the early and late stimulated carcasses at 24 h pm. There were mixed reactions and no definite patterns from the treatment groups regarding the influence of carcass weight and the duration of stimulation.

Therefore, in conclusion, the 3 and 14 day shear force could be related to calpain-1 activities at 1 and 24 h pm and the rate pH and temperature decline over 24 h. Regarding the lowest shear force from the early stimulated heavy carcasses at 14 dpm, Dolezal et al. (1982) on beef and Smith et

al. (1976) on lamb reported that fat thickness and carcass weight played important role in reducing cold shortening and allowing carcasses to chill more slowly to increase enzyme activities and hence, tenderness. As reported in the pH-temperature interaction, Treatments 4, 7, and 8 had the lowest shear force after passing through the heat/rigor shortening window between 1 and 3 h pm. Lawrie's (1998) review also pointed out that pre-rigor meat could be held or conditioned at high temperature to increase tenderness by avoiding cold shortening. In addition, the reports of Dutson (1978) and Moeller et al. (1976) reaffirmed that when muscles entered rigor at high temperature, the activity of proteolytic enzymes was said to be stimulated, which could lead to improved tenderness. However, the lowest shear force from the treatment groups at 3 dpm (7.99 kg) (Treatment 7) and at 14 dpm (5.60 kg) (Treatment 7) was slightly high, but acceptable. A strongly suspected reason for the high shear force value was the Zilmax that was administered, which is known to increase the instrumental shear force of meat. Hope-Jones, Strydom, Frylinck and Webb (2010) showed that ES, combined with post-mortem ageing could improve, but not fully overcome the adverse effects of beta-agonists on beef loin tenderness. Koohmaraie et al. (1991) reported that tenderness was negatively affected in cattle fed beta-agonists owing to an increase in calpastatin activity.

5.6 Cooking loss

In terms of cooking loss, there were no definite pattern of reactions regarding the time of stimulation and duration of stimulation at 3 and 14 dpm. Some researchers reported on the lack of influence of ES on cooking loss of meat. For example, Li et al. (2011) using LVES (80 V for 30 s) 30 min pm, found no significant difference ($P > 0.05$) in cooking loss between stimulated and un-stimulated meat in bovine *longissimus* muscle. Geesink, Mareko, Morton and Bickerstaffe (2001) reported no significant difference in cooking loss of beef treated with LVES (75 V, 15 Hz) after intense (80 s) or mild (20 s) stimulation, 30 min pm. Channon, Walker, Kerr, and Baud (2003) found no significant impact of LVES on pork cooking loss. Hawrish and Wolfe (1983) reported that LVES (50-100 V) applied immediately post-mortem did not affect cooking loss in beef muscles, compared with un-stimulated ones, likewise, the work of Savell et al. (1978b). All these reports are in accord with the current study, in which there were low numerical differences among the different treatments, including the non-stimulated groups, although, the statistical analysis indicated significant differences (3 and 4 vs 2, 7 and 10 at 3 dpm) between some of the treatment groups using pairwise comparison (Table 4.7). Analysis showed very low variation among the treatment groups at three days ($R^2 = 0.18$) and 14 days ($R^2 = 0.06$) pm and the values for each treatment group were numerically close on both days of measurement (Table 4.7).

However, at day 3 pm, small carcass weight groups (3, 4, 5 and 6) showed slightly higher cooking loss, while heavy carcass groups (2, 7, 9 and 10, excluding 8) had the lowest cooking loss. At 14 dpm, the numerical differences among carcasses were much lower as reflected in the variation (R^2 = 0.06). Furthermore, on the influence of carcass weight on cooking loss, the report of Davel et al. (2003), using LVES (20 V, 45 Hz for 45 s) applied on sheep carcasses, showed no effect of cooking loss as related to slaughter weight and carcass fat.

Temperature at 6 h pm (r = -0.37) and muscle glycogen at 24 h pm (r = -0.34) showed at least moderate correlations with percentage cooking loss (Table 4.41). This implies that as the carcass temperature decreased at 6 h pm, there was a corresponding increase in cooking loss at 3 dpm. Likewise, as the glycogen content of meat decreased at 24 h pm, there was a corresponding increase in cooking loss at 3 dpm and vice versa. All other variables were weakly correlated to cooking loss. There was a decrease ($P < 0.05$) in cooking loss from day 3 to day 14 pm. This was expected as the meat lost more water in form of drip/purge as it aged between these times (Bertram et al., 2007; Rosenvold et al., 2008).

5.7 Drip loss

In terms of drip loss, there was a definite pattern of reactions from the treatment groups regarding the time of stimulation. Carcass groups stimulated before evisceration (3, 4, 7 and 8) had the highest ($P < 0.05$) drip loss, followed by the carcasses stimulated after evisceration (5,6, 9 and 10) and controls (1 and 2) respectively, on days 3 and 14 dpm (Tables 4.8, 4.9 and 4.10). This accords with the report of Hwang & Thompson (2000), which stated that the time of stimulation had more impact on pH decline and consequently on drip loss. Additionally, the interaction of early stimulation and heavier carcass weight brought about significant higher ($P < 0.05$) drip loss at 14 dpm and a high tendency to be significantly high ($P = 0.077$) at 3 dpm (Table 4.10). According to Hopkins et al. (2007) and Warner et al. (2014), heavy carcasses, especially stimulated early post-mortem exhibits faster glycolysis leading to more rapid pH decline, which leads to higher drip loss. Faster pH decline at higher carcass temperature is known to cause sarcoplasmic and myofibrillar (especially myosin) protein denaturation (Jacob & Hopkins, 2014). Denaturation subsequently leads to the shortening of myosin head and reduction in filament spacing, thereby inducing water to be expelled from the muscle cells to the extracellular space (Offer et al., 1989; Offer, 1991). This corroborates the findings of the present study, as heavy carcasses (7 and 8) had the highest ($P < 0.05$) drip loss of the early stimulated carcasses at 3 and 14 dpm. It also accords with the findings of Fujita, MacFarlane, Forsyth, Yoshizawa-Fujita, Murata and Nakamura et al. (2007) and Puolanne and Halonem (2010), who reported that ES induced faster pH decline, which

was detrimental to drip loss, because it accelerated the decrease in net charges on the surface of the myofilaments. The lactate formed as a result of glycolysis acted as an anionic chaotrope, which impaired the interaction between water and proteins, which decreased WHC. Offer and Cousins (1992) also reported that the formation of actomyosin at rigor mortis would decrease inter-filamental space, leading to more drip. Uytterhaegan et al. (1994) and Gagniere et al. (1999) reported that faster glycolysis, which leads to rapid pH decline, leads to higher drip loss and generally leads to reduced WHC. Rosenvold et al. (2008) suggested that drip arises from myosin degradation pre- and post rigor, which is an inevitable consequence of tenderization. One could therefore conclude that early stimulation with LVES which brought about the lowest shear force, also brought about significantly ($P < 0.05$) higher drip loss, which was attributed mainly to protein denaturation (Offer et al., 1989).

There were mixed reactions from the treatment groups and no significant differences in stimulation duration. However, 60 seconds stimulation produced slightly higher drip loss compared with 30 s stimulation (Table 4.9). Electrical stimulation could pose the risk of under- or over- stimulation as a result of the intensity of the stimulation, which could lead to higher drip loss (Hildrum et al., 1999). The control groups had the lowest percentage drip loss at both times of measurements.

Another plausible explanation for the increase in drip loss in the heavier carcasses is the higher myofibrillar protein density (Bertram et al., 2002; Dickerson & Widdowson, 1960) compared with lighter carcasses. Increase in myofibrillar protein density reduces intra-myofibrillar myowater content which increases inter-myofibrillar water content as muscles gain weight and size (Bertram et al., 2007). Pearce et al. (2011) indicated that extra-myofibrillar water rises with protein deposition in muscles and suggested that a high growth rate give rise to a higher proportion of glycolytic fibres (Bertam et al., 2002; Dransfield & Sosnicki, 1999), which has larger extra-myofibrillar fluid space and could be related to higher drip loss.

Many variables correlated moderately/strongly with drip loss, to support the current findings (Table 4.41a). Almost all pH values correlate strongly and negatively with drip loss at 3 and 14 dpm, with pH 6 h pm ($r = -0.65$ and $r = -0.60$, respectively) having the highest correlation. This implies that, the faster the pH decreases, the higher the drip loss. There were moderate positive correlations of drip loss with temperature 45 min pm 3 dpm ($r = 0.41$) and 14 dpm ($r = 0.36$). This implies that a decrease in initial temperature corresponds with a concomitant decrease in percentage drip loss. There was also a strong negative correlation between drip loss at 3 dpm and

shear force at 3 dpm (r = -0.50) and also between drip loss at 14 dpm and shear force at 3 dpm (r = -0.46). This implies that as drip loss increases, shear force decreases and vice versa as reported by Dutson (1978) and Moeller et al. (1976). Meat lightness (L*) at 2 and 14 dpm also correlated positively and strongly with drip loss at 3 and 14 dpm (Table 4.41a), and this implies that the higher the drip, the lighter the meat even at 14 dpm. There was also a moderate positive correlation (r = 0.44) between chroma (colour saturation) 2 dpm with 3 dpm (r = 0.44) and 14 dpm (r = 0.44) drip loss, and this implies that as the drip loss increases, chroma also increases especially early pm. This was reflected in the regression analysis (Table 4.42), in which drip loss 3 dpm could be predicted with C* 2 dpm and pH 24 h pm. This result partially agrees with the work of Unruh et al. (1986) who reported detrimental effects of LVES (50V) on colour and its stability. Sleper et al. (1983) also reported higher values of L* on ES meat at the initial storage period, but this decreased after 24 h pm and onwards. Similar reports were made by Shaw et al. (2005) and Toohey et al. (2008), in which faster pH decline by ES brought about the opening-up of muscle structures which hastened the oxygenation of myoglobin to oxymyoglobin thereby bringing about a redder colour, but this bright colour disappeared after the initial storage period as seen in this trial (Table 4.21). The correlation of drip loss with chroma at 14 day pm in this study does not leave any plausible explanation, but as seen in the results, chroma increased for most of the treatment groups from 2 to 14 dpm, except Groups 7 and 8, which had a slight decrease. Drip loss also increased for all the treatment groups from 2 to 14 dpm and this gives an indication of why they correlated positively. Water holding capacity also correlated strongly and negatively (r = -0.49 and -0.57 at 3 and 14 dpm respectively) with drip loss in this study and this was expected. Drip loss is inversely proportional to WHC (Bekhit et al., 2007). It is concluded that shorter LVES of 30 s or less should be adequate for all carcasses, especially for heavier carcasses that are stimulated before evisceration, in order to reduce drip loss.

5.8 Water holding capacity

There was a definite and clear pattern of reaction from the treatment groups in terms of time of stimulation. The reaction is similar to that of percentage drip loss but is inversely proportional, that is, carcasses that were stimulated before evisceration (3, 4, 7 and 8), had the lowest (P = 0.001) WHC while the late stimulated carcass groups (5, 6, 9 and 10) and the controls, had the highest WHC at 3 and 14 dpm (Table 4.12 and 4.13). The variations among the treatment groups at both times of measurement were low (R^2 = 0.29 at 3 and 14 dpm), and the mean values for the treatment groups were numerically close, unlike drip loss, which had wider variation among the treatment groups (Table 4.11). Water holding capacity, just like drip loss, showed a positive

correlation with pH in the conversion of muscle to meat as reviewed by Rosenvold et al. (2008). Early stimulated carcasses (3, 4, 7 and 8) with the lowest pH, at all time of measurements corresponded with the lowest WHC at 3 and 14 dpm and vice versa for the late stimulated carcasses with the highest pH (5, 6, 9 and 10). This is in accord with the work of Offer (1988) and of Huff-Lonergan & Lonergan (2005), who reported on lower WHC as meat pH reduces. In this study, the faster pH decline caused by the early LVES corresponded with the reduced WHC because the anaerobic conversion of glycogen results in hydrogen ion build-up through lactic acid formation (Lawrie, 1998; McGeehin, Sheridan & Butler, 2001; Puolanne & Halonem, 2010), creating anionic chaotrope which breaks the bond between protein and myowater, leading to reduced WHC. The carcasses with the lowest WHC (3, 4, 7 and 8) also entered rigor when the temperature was high, and that was why they passed through the hot/heat shortening window (Figure 4.3). This accords with the work of Offer (1991) who reported that at a fast decreasing pH and an elevated temperature, myosin heads denature and lose the ability to bind water, leading to myofibrillar lateral shrinkage, which reduces WHC.

pH is generally accepted a key factor in controlling the ability of muscles to hold water during conversion to meat (Bendall & Swatland, 1988). It was therefore clear in this study that ES, which is a major factor of influence, played a major role in increasing the rate of pH decline through early stimulation, thereby reducing the WHC of meat. Some researchers reported similar results to the findings of this study. Beckit et al. (2007) using LVES (90 V) on venison (red deer) revealed a decreased WHC with increasing rigor temperature and pH decline ($P < 0.001$). The venison meat also became more tender over time pm. Similarly, Farouk and Swan (1998) reported pm increase in drip loss with increasing rigor temperature in beef. Buts et al. (1986) also reported reduced WHC using LVES on veal carcasses immediately pm. The most plausible reason is protein denaturation as a result of faster pH decline and higher initial carcass temperature, especially around 35 °C or above (Hamm, 1961; Scopes, 1964).

At 3 dpm, heavier carcasses had slightly lower, but significantly ($P < 0.05$) different WHC compared with the lighter carcasses, but at 14 dpm, there were no differences in WHC between the carcass weights (Tables 4.12 and 4.13). Although, there was an expectation of lower WHC in all the heavier carcasses in which extra-myofibrillar water increases with protein deposition in muscles (Pearce et al., 2011). The results of this study are in partial agreement with Pearce et al. (2011) in the sense that the early stimulated heavy carcasses (Groups 7 and 8) were among the groups with the lowest WHC and were significantly ($P < 0.05$) lower than most of the other

carcass groups. However, there were no major numerical differences in WHC between the heavy and light carcass groups (Table 4.11).

There is no consensus on optimum duration of application of ES, be it LVES or HVES. In this study, carcasses stimulated for 60 s produced slightly lower, but significantly different WHC ($P < 0.05$) compared with carcasses stimulated for 30 s and the controls at 3 dpm. At 14 dpm, there was also a high tendency for a significant difference ($P = 0.052$; Table 4.12 and 4.13). According to Strydom & Frylinck (2014), a short duration of 15 s using 150V applied during bleeding produced the best WHC compared with 45 s and 90 s. Pearce et al. (2006) also reported that a duration of 34 s using 300V was the best, resulting in 60% of the carcasses hitting the pH/temperature window (pH 6 between 18-25 °C). As stated earlier, the late stimulated carcasses produced the highest water holding capacity, especially the ones stimulated for 30 s at 3 and 14 dpm (9 and 5) (Table 4.12). Therefore, 30 s or less ES would be recommended because it will make room for faster processing time and less protein denaturation.

Analysis revealed a negative correlation ($r = - 0.49$) between WHC day 3 and chroma day 2 (Table 4.41b). This was opposite in reaction to drip loss (Bekhit et al., 2007). Temperature/pH and time relationship affected both WHC and drip loss. As drip loss decreased, WHC increased, strongly and negatively correlated. Similarly, negative correlation of L* value at day 2 with WHC day 3 and 14 ($r = -0.50$ and -0.43 respectively) was recorded. L* value day 14 also correlated negatively with WHC day 3 and 14 ($r = -0.58$ and -0.38, respectively). Furthermore, a negative correlation ($r = -0.35$) of WHC day 3 with hue angle (meat discoloration) day 14 was also recorded. This was expected because, just like chroma, hue angle is a derivative of a* and b* values.

In conclusion, the treatment effect that showed the most influence on meat WHC in this study was the time of stimulation. In this case, stimulation before evisceration (early stimulation; 3, 4, 7 and 8) produced the lowest WHC followed by carcasses stimulated late (5, 6, 9 and 10) and NES (1 and 2). Heavier carcasses also had lower WHC compared with the lighter carcasses, while longer duration of stimulation produced lower WHC compared with shorter duration of stimulation.

5.9 Effects of treatments on sarcomere length

Analysis showed a very low variation ($R^2 = 0.066$) among treatment groups regarding sarcomere length and the differences were numerically low. All carcasses exhibited numerically close values and there were no significant differences among carcasses. Similar results were reported by Fabiansson and Reutersford (1985) in which low voltage electrically stimulated (1.81μm) and

NES (1.79 μm) carcasses had similar SL and there was no significant difference. These authors also recorded lower SF in ES carcasses compared with the NES carcasses. The values of SL (Table 4.14) in this study shows there was no cold shortening in any of the carcasses. Sarcomere length is related to muscle toughening and it has been said to be a major determinant of meat tenderness (Locker, 1960; Marsh & Leet, 1966; Locker & Daines, 1975; Locker & Hagyard, 1963; Smulders et al., 1990). These researchers reported a strong positive correlation between sarcomere length and tenderness. Devine et al. (1999) reported that the longer the sarcomere, the more tender the meat, even after 14 days of ageing. Wiklund et al. (2001) reported a longer, but not significantly different SL between ES (1.90 μm) and NES (1.70 μm) in red deer carcasses using LVES (90-95 V) during bleeding for 55 s. A sarcomere length of 1.44-1.77 μm is generally indicative of muscle shortening (Devine et al., 2002; Pearce et al., 2009), therefore the current result of average SL of 1.95 μm could be regarded as normal sarcomere range, which indicates lack of cold or heat shortening.

Hwang and Thompson (2001) reported that carcasses stimulated at 3 min pm had the shortest SL using LVES (70 V) and HVES (800 V)) and were significantly ($P < 0.05$) tougher (higher meat shear force) than carcasses stimulated at 40 min pm. The current finding is contrary to this report because carcasses stimulated early pm had the longest sarcomeres, even though the numerical differences were low. Early stimulated carcasses with the lowest shear force (3, 4, 7 and 8) had slightly longer but insignificantly different sarcomere length (Table 4.14). Smulders et al. (1990) also reported on the manipulation of glycolysis by ES in conjunction with an appropriate chilling temperature to produce a wide range of sarcomere length. Some researchers (Koohmaraie, 1996; Gursansky et al., 2010; Smulders et al; 1989; Pearce et al., 2006) reported that the SL of NES meat was in normal range and probably did not contribute to lower tenderness. Other researchers, such as Dutson et al. (1980), Koh et al. (1987) and Uytterhaegen et al. (1992) reported improved tenderness without any change in SL, while researchers such as Buts et al. (1986) reported that ES brought about reduced SL. The variations among the treatments were very low (Table 4.14). Nevertheless, meat samples from the carcass groups stimulated before evisceration and with the lowest shear force values, especially the heavier carcasses (8 and 7) had the longest sarcomeres ($P < 0.05$), compared with the late stimulation groups and NES (controls).

There was no clear pattern of reaction among the treatment groups on the effect of ES duration and and carcass weight on SL. A major factor to be considered is the rate of pH decline, which affects SL and accounts for the similar SL in this study. Tenderness is highly dependent on sarcomere shortening in slow glycolysing muscles, but completely independent in muscles with

more rapid pH decline, which was the case in this study (Buts et al., 1986). Also, contraction is impeded by low muscle pH, therefore reducing the proportion of myosin cross-bridges in the strong binding state (Goll et al., 1997). At this time (early pm), ATP levels decreases slowly so that muscle pH is less than 6.3 before intracellular calcium concentration reaches a level high enough to initiate contraction. Another factor is that all the animals are from the feedlot with similar feeding regime. According to Warner et al. (2014) and Hopkins et al. (2007), most of the feedlot cattle that were investigated had faster rates of pH decline compared with animals on different production systems. The similarity in sarcomere length from all the carcasses can therefore be attributed to the faster pH decline due to ES and the feedlot system in which the animals were raised. From the correlations (Table 4.41), the researchers recorded a moderate positive correlation (r = 0.30) of sarcomere length and drip loss at 3 dpm. This shows that as sarcomere length increases, the drip loss also increases within 3 dpm. There was also a moderate positive correlation between G-6-P 1 h pm (r = 0.42) and 4 h pm (r = 0.42) with sarcomere length. This indicates that the initial rate of increase in glucose-6-phosphate early pm in the muscle corresponds with longer SL. There is no known reference to this. It could be linked to the increase in glycolysis early pm due to ES but needs to be investigated. Moreover, there was a moderate negative correlation of calpain-1 at 24 h pm (r = -0.32) with sarcomere length. This indicates that the lower the calpain-1 activity at 24 h pm, the longer the sarcomere length. This is in line with some studies (Dransfield, 1993; Hwang & Thompson, 2001). Hwang and Thompson (2001) indicated that ES possibly caused greater acceleration of autolysis/proteolytic activities of calpain-1 during stimulation and in the early pm period. These studies also suggested that residual calpain-1 levels at 24 h pm may be affected by pH/temperature profile for the fast glycolysing muscles.

It could be concluded that, there was no sign of cold shortening in all the carcasses including controls as all the sarcomeres were longer than 1.90 μm. Carcass weight and duration of stimulation did not exert any influence on sarcomere length in this study.

5.10 Effects of treatments on myofibril fragment length

Analysis showed a very low variation in MFL at 3 dpm (R^2 = 0.058, P = 0.4801), but at 14 dpm a slightly higher variation was observed among the treatments (R^2 = 0.11, P = 0.0495). At 3 dpm, the pairwise comparison (Table 4.15) and the rankings that followed showed a clear pattern of reaction. Carcasses stimulated before evisceration (8, 7, 4 and 3) and Group 1 (control) had the highest MFL, followed by carcasses stimulated late (9, 6, 10 and 5) and control group 2 (NES). Groups 7 and 8 with heavy carcasses had the longest MFL. At 14 dpm, a similar pattern was

observed. That is, carcasses stimulated late had the shortest MFL at both times of measurement. There was also a decrease in MFL from 3 to 14 dpm as anticipated, indicating myofibrillar breakdown (Martin et al., 2006; Sonaiya et al., 1982; Toohey et al., 2008). In addition, early stimulated carcasses had an average MFL decrease of 24%, while the carcasses stimulated late had an average MFL decrease of 32% and the controls, an average MFL decrease of 30%. Electrical stimulation has been reported to enhance tenderness by accelerating proteolysis of myofibrillar proteins which are responsible for the structural integrity of myofibrils (Hollung et al., 2007; Rosenvold et al., 2008). In this study, early stimulated carcasses (8, 7, 3 and 4), with the fastest glycolytic rate and the lowest shear force were expected to have the lowest myofibrillar length or the highest myofibrillar fragmentation index (MFI) (Hopkins & Taylor, 2004; Penny & Dransfield, 1979), but the reverse was the case. Instead, carcasses stimulated late and the control (1 and 2) had the shortest MFL or the highest MFI but the least tender meat. This work agrees partially with that of Strydom et al. (2011), in which ES (400 V) samples with more tender meat, had longer MFL compared with NES samples with tougher meat. They suggested the involvement of other tenderizing mechanisms.

It is generally accepted that degradation of myofibrillar and cytoskeletal proteins during pm ageing plays an important role in tenderization (Ho et al., 1996; Koohmaraie et al., 1987; Robson et al., 1997). Koohmaraie (1988), however reported that contractile proteins (actin and myosin) were barely changed during ageing at refrigeration temperature. Ho et al. (1996) reported that ES slightly accelerates the degradation of cytoskeletal protein pm, which may improve tenderness. They indicated, however, that the improved tenderness seemed to be caused by mechanical disruption rather than enhanced proteolysis. Some researchers also showed that the combination of LVES with high temperature early conditioning effectively hastened cytoskeletal degradation (Rhee, Ryu, Imm, & Kim., 2000). Dutson et al. (1980) and Marsh et al. (1974), also suggested a powerful association between physical disruption of myofibrillar complex and increase in tenderness. However, the theory of physical myofibrillar disruption could be ruled out in this study because most of the studies on physical disruption have been on HVES (300V and above) (e.g. Takahashi et al., 1987; Will et al., 1980) and MVES (145V – 250V) (Ho et al., 1996; Sorinmade et al., 1982). In this study, physical disruption of the myofibrillar complex did not seem to play a part because of the LVES that was used, and this could be ruled out as an influencing factor.

Most of the studies on MFL have been on HVES and there has been a lot of conflicting results, even in the measurement of MFI with different techniques. Some used myofibrillar fragmentation

(Olson et al., 1976) and some used amino acid determination (Field & Chang, 1969), protein solubility (Claeys et al., 1994), non-protein nitrogen (Davey & Gilbert, 1966), and gel electrophoresis (Olson et al., 1977). These measurement techniques brought about different reports, while some authors recorded no effect of ES on protein solubility (Bruce et al., 1990). In this study, a VIA imaging system was used to measure hundreds of fragments from each sample (Culler et al., 1978) and averages of MFL's were taken for each sample. Some researchers also showed that there was no significant difference between stimulated and NES carcasses in terms of MFI after days of ageing (Toohey et al., 2008). These authors indicated, however, that MFI increased from 1 to 5 days of ageing. This study shows that early stimulation increased glycolytic and proteolytic activity, but resulted in longer MFL compared with late stimulation, which is contrary to Hwang and Thompson (2001b), Dranfield (1993), Geesink and Koohmaraie (1999) and others. Devine et al. (1999), on the effects of manipulation of rigor temperature and ageing on MFL, indicated that MFL alone was not a complete indicator of the extent of tenderization during proteolytic ageing. The results of this study showed that LVES applied later pm (45 min) produced shorter MFL (with higher shear force), compared with the carcasses stimulated early pm (with lower shear force). This suggests that other mechanisms may be involved, apart from ES and its concomitant proteolytic activities, in influencing MFL.

In terms of the effects of carcass weight and duration of stimulation on MFL, there were mixed reactions among the treatment groups 3 and 14 dpm. However, the heavier, early stimulated carcasses (7 and 8) had some of the lowest percentage degradation of MFL, although they exhibited the lowest shear force (Table 4.15). From the faster pH and slower temperature decline in the heavier, early stimulated carcasses (Figure 4.3), it could be deduced that the faster glycolytic rate played a role in the lower MFL degradation in these carcasses. A common feature of carcasses, especially heavy carcasses that undergo faster glycolysis is acceleration of calpain exhaustion through autolysis at faster pH decline and higher temperature (Kim et al., 2014; Warner et al., 2014). This process is known to facilitate an increase in meat toughness and limited ageing potential or reduced tenderization. As stated, other tenderizing activities or mechanisms could have been involved in the heavier carcasses. More lysozomal enzymes might have been released in the heavier carcasses due to the lower pH at higher carcass temperature (Li et al., 2011). This condition can enhance the disruption of the lysosomal membrane (O'Halloran et al., 1997) thereby releasing the lysosomal enzymes as ATP depletes and muscles enter rigor. However, these enzymes still have to act on the myofibrillar protein and it is still unclear why the

effect was not significant on the degradation of the MFL's even at 14 dpm. This calls for further investigation.

In terms of the correlations, analysis showed a moderate to weak positive correlation (r = 0.30) between MFL at 14 dpm and shear force at 14 dpm. This shows that the lower MFL at 14 dpm correlates weakly with lower shear force at 14 days for all the treatment groups (Devine et al., 1999). MFL does not indicate absolute tenderness values according to Devine et al. (1999). The researchers also recorded moderate negative correlations of ATP 1 h (r = -0.32) and 4 h pm (r = -0.37) pm with MFL 14 dpm respectively. Moderate positive correlation was recorded with glucose 1 h (r = 0.31) and 24 h pm (r = 0.30) with MFL 14 dpm respectively. This indicates the ability of ES to bring about a faster biochemical reaction in the glycolytic pathway and a concomitant proteolytic activity (Asghar & Henrickson, 1982; Hwang & Thompson, 2001). Lastly, a positive moderate correlation of MFL day 14 pm with L* value day 2 (r = 0.31) pm was recorded. This could be related to the protein denaturation and myofibrillar lattice shrinkage as a result of fast declining pH due to ES (Offer & Trinick, 1983; Swatland, 1993; Li et al., 2011). Wiklund et al. (2001) suggested that ES accelerates the loss of metmyoglobin reductase activity so that after one week of ageing, metmyoglobin accumulation is increased. All the proteolytic enzyme values had weak to very weak correlations with MFL in this study.

5.11 Effects of treatments on Meat Lightness

Analysis revealed moderate and significant variation (R^2 = 0.47) at 2 dpm and at 14 dpm (R^2 = 0.40) among the treatment groups. There was also a general increase in L* value from 2 day pm to 14 dpm in all treatment groups. This was due to reduced rate of oxygen consumption as meat aged (Ledward et al., 1986) and myofibrillar lattice shrank (Offer & Trinick, 1983; Swatland, 1993). Higher L* could also be attributed to light scattering and the denaturation of myoglobin, which is common in high rigor temperature meat (Kim et al., 2014). Buts et al. (1986) suggested that ES brought about increased lightness due to accelerated denaturation of protein, which accords with the results of George et al. (1980), who found irregular bands of denatured sarcoplasmic proteins deposited in fibres of stimulated muscles, analogous to pale, soft, exudative meat (PSE) in pig muscles. Li et al. (2011) reported that LVES increased L* values of bovine *longissimus* as the meat aged. Partial correlation between the two times (2 and 14 dpm) of measurement was high (r = 0.755, P < 0.0001) and significant. In terms of the time of stimulation, the pairwise comparison showed a definite and clear pattern of reaction. At 2 day pm, carcasses stimulated before evisceration (8, 7, 4 and 3) had the highest L* values followed by the late stimulated carcasses and NES respectively. At 14 dpm, similar pattern was observed but the

control groups (1 and 2) had higher L* values than the late stimulated carcasses (Chapter 4, Table 4.16 and Figure. 4.11). These results indicate a link between the shear force values, drip loss and L* values. They also indicate that faster pH decline and high initial carcass temperature in the early stimulated carcasses brought about higher L*. Seideman, Cross, Smith and Durlan (1984) indicated that meat with lower ultimate pH values and faster pH decline had more open structures and scattered more light compared with dark firm and dried (DFD) meat with high ultimate pH, which does not scatter light. Jeremiah et al. (1991) and Wulf et al. (1997) also reported that objective colour measurement of *longissimus dorsi* was closely related to beef tenderness and this allowed carcasses to be easily grouped into different tenderness and palatability class.

Abril et al. (2001) reported that samples were darker as the ultimate pH values increased. In other words, the lower the pH_u, the lighter the meat. Their findings also reported that pH was the most influential variable in separating the treatment groups according to their colour attributes. Scopes (1970) and Bendall & Swatland (1988) on pork also showed a strong correlation between pH and myosin filament separation. They concluded that myosin filament separation and myofibrillar shrinkage, which brings about fluid extrusion, is highly dependent on pH. They indicated that the meat would be darker with less fluid extrusion in DFD meat because of the high pH_u values (> 6.4). This affirms the results in the current study, which showed a similar trend of fast pH decline/low pH_u in the early stimulated carcasses, which had the highest drip loss, lowest shear force and the lightest (L*) meat.

There was no definite pattern of reaction from the treatment groups with regards to the influence of carcass weight on meat lightness. This shows the lack of or negligible influence of carcass weight on meat lightness at both 2 and 14 dpm. From the literature review, there has been little or no research on the influence of carcass weight on the efficacy of ES regarding colour attributes. However, Wilson et al. (1995) found a tendency for lighter L* values in heavier carcasses. A recent report by Warner et al. (2014c) also stated that heavier carcasses from feedlot cattle exhibited higher L* at grading, owing to high temperature rigor. There was no definite pattern of reaction regarding the influence of the duration of ES on meat lightness value. However, the treatment groups that were stimulated early pm for 60 seconds (8 and 4) had the highest L* values at both 2 and 14 dpm. Treatment 8 had significantly ($P < 0.05$) higher values compared with treatments 4, 7 and 3, which had numerically close values. This showed that the longer the stimulation, the lighter the meat, for carcass groups stimulated early pm. This is probably due to the heavy carcass weight and faster glycolysis (Warner et al., 2014a). It is suspected that there is reduced fluid retention, which leads to lighter meat owing to faster glycolysis and resultant pH

decline (Eikelenboom & Smulders, 1986). Roeber et al. (2000) also indicated that duration and voltage intensity affected meat colour. Generally, a shorter stimulation time was reported by many researchers to be effective (Strydom & Frylinck, 2014 (15 s); Pearce et al., 2006 (34 s)). In this study, 30 seconds or less duration would be recommended because of the low numerical difference in L* values between the 30 and 60 s duration. Application of ES for 60 s would obviously attract more electricity cost and would lead to PSE-like condition, as observed in this study with higher L*, and so, 30 s or less would be more ideal.

There were strong negative correlations of L* values with all pH readings (average r = -0.56) from 45 min to 24 h pm at 2 and 14 dpm. This indicates that as L* values increases, there were corresponding decreases in pH values and vice versa. This further affirms that faster glycolysis and pH decline at higher carcass temperature plays an important role in meat L* value (Abril et al., 2001; Eikelenboom & Smulders, 1986; Kim et al., 2014). There was also a moderate positive correlation (r = 0.35 and 0.30) of L* values at 2 dpm with initial temperatures (45 min and 3 h pm respectively) (Mareko, 2000), but at 14 dpm, the correlation became weaker (r = 0.27 and 0.24 respectively). L* values day 2 also showed strong positive correlations with drip loss at 2 and 14 dpm (average r = 0.54), likewise L* value at day 14 (average r = 0.60). A similar scenario goes for WHC at day 2 but the correlation was negative (r = -0.50 & r = -0.43 at 2 and 14 dpm respectively) and L* values at 14 days for WHC (r = -0.53 & r = -0.38 at 2 and 14 dpm respectively). This shows that as L* value increases, drip loss increases and WHC decreases. This is in accord with Scopes (1970) and Bendall and Swatland (1988), who reported a strong correlation between pH and myosin filament separation. They concluded that myosin filament separation and myofibrillar shrinkage brings about fluid extrusion, which is highly dependent on pH. L* day 2 pm values also showed a negative moderate correlation with redness (a*) at 14 days p.m (r = -0.31), likewise L* value day 14 pm correlated moderately and negatively with a* day 14 (r = -0.30). This shows that as lightness increases, redness decreases at storage time of 14 dpm. This indicates the accumulation of metmyoglobin which decreases redness and increases browning as meat ages (McKenna et al., 2005; Wu et al., 2015). L* values at 2 and 14 dpm also correlates strongly and positively with meat yellowness (b* value) (r = 0.58 at 2 dpm and r = 0.68 at 14 dpm respectively). This indicates that as L* values increases, there is a corresponding increase in b* values from 2 to 14 dpm. Wulf and Page (2000) reported a close positive relationship of L* and b* values when used as a non-invasive method to predict tenderness. They reported that the combination of L* and b* values gave them a better predictive value than marbling in their regression analysis to predict shear force and palatability. Hawrysh and Wolfe

(1983) also reported that Hunter L* and b* varied in a similar way for the treatment groups using LVES (50-100 V) on matured cow carcasses at 48 h and 7 dpm.

There was also a moderate positive correlation of L* value with chroma 2 dpm (r = 0.36 with L* 2 dpm and r = 0.45 with L* d14 pm). This is no surprise as chroma value is a derivative of a* and b* values ($C = a^{*2} + b^{*2})^{1/2}$ (AMSA, 1991). A similar scenario goes for hue angle which is also a derivative of a* and b* values ($H = \tan^{-1} b^*/a^*$) at 2 dpm (r = 0.65 with L* 2 dpm and r = 0.56 with L* 14 dpm) and at 14 dpm (r = 0.61 with L* 2dpm and r = 0.67 with L* 14 dpm). This implies that the positive correlation of chroma with L* is only temporal (at 2 dpm). At 14 dpm, there was a negative, low and insignificant correlation. This is in accord with Li et al. (2011) in which ES produced brighter red colour and colour saturation before 48 h pm but as the meat aged, discoloration sets in. Similarly, Wu et al. (2015) concluded that several proteins and their reduction and denaturation through glycolytic activity, energy metabolism and redox process post-mortem affected meat discolouration as meat aged. This shows the reason why L* value and H* values still correlated strongly and positively (average r = 0.64; P < 0.001) at 14 dpm. Discoloration is an ongoing process as the meat ages and metmyoglobin formation increases (Pearce et al., 2009).

L* values also showed moderate to strong correlations with energy metabolites. Lactate 1 h pm showed a strong correlation with L* values (L* 2 dpm, r = 0.58; L* 14 dpm, r = 0.48). Lactate 4 h pm also showed a moderate/strong correlation with L* values (L* 2 dpm, r = 0.50; L* 14 dpm, r = 0.48). At 24 h pm, the correlations were low and insignificant. This indicates that as muscle lactate increases early pm, L* values increases, even up to 14 dpm. Li et al. (2011) demonstrated that the effects of ES lasted longer for L* values (7 days) in meat compared with a* and b* values. This is in accord with the reports of Mancini and Hunt (2005) and Zapata, Reddish, Miller, Lilburn and Wick (2012), which showed the effect of glycolysis, which brings about the accumulation of lactic acid, which indirectly affects meat colour. It also affirms the work of Abril et al. (2001) and Eikelenboom and Smulders (1986), which indicated that decreasing pH due to faster glycolysis as a result of ES brought about lighter and paler meat (Hector et al., 1992). Also, in this study, L* values increased from 2 to 14 dpm and this could be attributed mainly to the altered oxygen consumption by endogenous enzymes and metmyoglobin formation (Mancini & Hunt, 2005). L* also correlates moderately with muscle glucose content. At 1 h pm, glucose value showed a moderate correlation with L* values (L*2 dpm, r = 0.36; L* 14 dpm, r = 0.34). At 4 h pm, there was a stronger correlation with glucose level (L* 2 dpm, r = 0.55; L* 14 dpm, r = 0.41) and at 24 h pm (L* 2 dpm, r = 0.46; L* 14 dpm, r = 0.36). This implies that as muscle glucose

level increases pm due to ES, meat lightness increases, even up to 14 dpm. Muscle glucose level is known to increase pm and at a faster rate due to ES application (Polidori et al., 1999; Frylinck et al., 2013).

The correlation analysis revealed negative moderate correlation of L* values with glycogen levels immediately pm (at 4 hr pm, L* day 2, r = - 0.30; at 24 h pm, L* day 2, r = - 0.29). The negative correlation implies that a decrease in glycogen concentration brought about a corresponding increase in meat lightness, but only at 2 dpm. Glycogen is known to decrease pm (Frylinck et al., 2013) but the rate of decrease due to ES is higher owing to faster glycolysis (Frylinck et al., 2013). Creatine phosphate (CP) 1 h pm also showed moderate negative correlations with L* values (L* 2 dpm, r = - 0.33; L*14 dpm, r = - 0.36). At 4 h pm, there was also a moderate negative correlation of CP with L* but only for the 2 dpm L* value (L* 2 dpm, r = - 0.36). This implies that as muscle CP level decreased at 1 h pm, there was a corresponding increase in L* value even at 14 dpm but at 4 h pm, only the L* day 2 measurement correlates with CP, indicating a shorter time effect. Similarly, ATP 4 h pm also correlated negatively with L* day 2 and day 14 pm (L* 2 dpm, r = - 0.45; L* 14 dpm, r = - 0.40). This implies a similar effect as for CP, as ATP decreases pm, L* value increases. Zapata et al. (2012) suggested that CP and ATP depletion in meat and various substrate from anaerobic metabolism could be further converted into lactic acid which indirectly affects meat colour (Mancini & Hunt, 2005). According Polidori et al. (1999), ES increases the re-synthesis of ATP through CP to fuel glycolysis, which brings about the production of lactic acid, which reduces meat pH (Gregory, 1998). Glucose-6-phosphate at 4 h pm showed positive moderate correlation with L* values (L* 2 dpm, r = 0.48; L* 14 dpm, r = 0.37). This implies that at 4 h pm, an increase of G-6-P in the muscle due to ES brought about an increased L* value at 2 and 14 dpm. Again, this bears down to increased glycolysis because of ES, which interconnects with all the energy metabolites (Tarrant et al., 1972; Frylinck et al., 2013). G-6-P is a function of glycolytic potential, which has been reported to affect meat lightness. Wulf et al. (2002) indicated that L* values increased as glycolytic potential increased.

Regarding the correlation of the proteolytic enzymes with L* values, there was moderate negative correlation between L* values and calpain-1 24 h pm (L* day 2, r = - 0.44; L* day 14, - 0.35). This implies that as micro calpain decreases post-mortem, meat L* values increase even up to 14 dpm. The ratio of calpastatin/calpain-1 at 1 h pm was also negatively and moderately correlated with L* day 14 measurement (L* day 14, r = -0.34). Again, this implies that as this ratio decreases, L* value increases for the 14-day measurement. Eelectrical stimulation is generally known to produce paler colour in meat through faster glycolysis, which brings about faster pH

decline (Hector et al., 1992; Ledward et al., 1986; Martin et al., 1983). The autolysis of calpain-1, which reduces ca^{2+} requirement for calpain activity (Li, Thompson & Goll, 2004) and associated with faster pH decline, is considered the peak for activation of calpain-1 in pm muscles. If one links the effects of ES on glycolysis to produce faster pH decline (Hwang et al., 2003) and its ability to activate calpain-1, one could conclude that an increase in L* value is imminent pm, with proteolytic enzyme activation. However, the application of ES leads to early depletion or exhaustion of calpain-1 owing to high temperature and low pH early pm. This same condition is what leads to increased tenderization (Hwang et al., 2003). This is in accord with the findings of the current study, in which early stimulation led to lighter meat colour and lower shear force compared with late stimulation and controls.

5.12 Effects of treatments on meat redness

A number of researchers have stated that redness (a*) is a more important colour attribute of meat compared with L* and b* values (Olivera, Bambicha, Laporte, Cardenas and Mestorino, 2013). In this study, analysis revealed low but significant variations among the treatment groups at 2 and 14 dpm. At 2 dpm ($R^2 = 0.22$, $P < 0.0001$) while at 14 dpm, $R^2 = 0.19$ ($P = 0.0007$). MANOVA test revealed a significant difference ($P < 0.0001$) between the two times of measurement (2 and 14-day pm). Of the 10 treatment groups, eight (1, 2, 3, 4, 5, 6, 9 and 10), including the controls increased in a* values from 2 to 14 dpm, while two treatment groups, namely heavy, early stimulated carcasses (7 and 8) had marginally lower a* values after ageing for 14 days. This is partially in contrast with the work of Wu et al. (2015) (without ES), who reported a general decrease in a* values when meat was aged for 15 days. Shaw et al. (2005) and Ledward et al. (1986) also reported higher a* values at processing, which was temporary, but as meat aged, there was a decrease in redness. This gives an indication that LVES could improve meat redness, even after 14 days of storage. A suspected important factor could be the MRA (metmyoglobin reductace activity), which plays a significant role in maintaining the bright red cherry colour of meat (Bekhit & Faustman, 2005). MRA has been reported to be stable for up to 10 dpm after which the value dropped to zero (Wu et al., 2015). McKenna et al. (2005) also reported that MRA maintained their initial level during the first three days of retail, after which the level started to reduce. MRA has been reported to be affected by lactate dehydrogenase (Kim, Keeton, Smith, Berghman and Savell, 2009). Some researchers also suggested a potential relationship between glycolytic metabolism and post-mortem discoloration (Dalle Zotte, Remignon & Ouhayoun, 2005). They speculated that the faster the glycolysis, the redder the meat.

Meat colour stability is usually known to decrease as post-mortem age increases (Mancini & Hunt, 2005; Suman, Hunt, Nair & Rentfrow, 2014; Chunbao Li et al., 2011; Strydom & Frylinck, 2014). This is because of metmyoglobin activities, which increases browning as meat ages (Ledward, 1992). Some researchers reported increased redness, hue and chroma for vacuum-packaged and wet-aged meat between 7 and 21dpm (Oliette et al., 2005; 2006). Vitale et al. (2014) reported that steaks stored for 0 to 8 days were more colour stable than those aged for 14 to 21 days. Another suspected factor that could have contributed to the redder colour at14 days of ageing for most treatments in this study could be the initial vaccum packaging for two days before the analysis of the samples (Oliette et al., 2005; 2006). However, from the results obtained in the current study, ES time, which shows a pattern or trend of reaction from the groups, seems to have the highest influence early post mortem (2 days pm), due to glycolytic metabolism, which is supported by Dalle et al. (2005). Furthermore, Joseph, Suman, Rentfrow, Li and Beach (2012) reported that superior colour stability of beef LL steaks could be due to over-abundance of certain antioxidant proteins such as carnosine and anserine, and glycolytic enzymes such as creatine-kinase and pyruvate dehydrogenase, which correlated positively strongly (r = 0.72 and 0.65, respectively) with the redness of meat in their study. These enzymes are generated at a faster rate due to ES (Gregory, 1998) and the times of stimulation in particular, which could influence the level of the release of these enzymes early pm.

Still on the time of stimulation, at day 2 pm, there was a clear pattern of reaction. Carcasses that were stimulated early pm (3, 4, 7 and 8) had the highest a* values, followed by the controls and carcasses stimulated late. At 14 dpm, there was no definite pattern of reaction from the treatment groups. Again, this points to the work of Shaw et al. (2005) which showed temporary improvement in a* values after processing owing to ES, compared with NES controls. However, the authors indicated that the effect was short-lived. The work of Toohey et al. (2008) recorded a similar result on lamb meat, where ES produced significantly higher a* value early pm (1 dpm) compared with NES, but at 5 dpm, there was no difference between the ES and NES a* values. From the results, the pattern of a* values could be attributed mainly to the faster glycolysis and accumulation of lactic acid owing to ES, in the groups that were stimulated early.

Regarding the duration of stimulation, there were mixed reactions from the treatment groups at 2 and 14 dpm. However, at 2 dpm, of the groups that were stimulated early, 30 s stimulation (Groups 3 and 7) (Table 4.19), produced the reddest meat compared with the 60 s stimulation in the early stimulation groups. On the contrary, Strydom and Frylinck (2014) reported that shorter duration of stimulation, produced relatively lower a* values and lower chroma values compared

with longer (45 s and 90 s) stimulation, which were attributed to lower penetration of oxygen and lesser denaturation of sarcoplasmic proteins.

Regarding the effect of carcass weight on a* values, at 2 dpm, there was no definite pattern of reaction from the treatment groups. However, the early stimulated heavier carcasses (7 and8) (Table 4.19) had the highest a* values. At 14 dpm, the lighter carcasses (6, 5, 4 and 3) had the highest a* values, save Treatment 10. The heavy carcass groups (2, 7, 8 and 9) had the lowest a* values at 14 dpm. Currently, there is not much literature to support these findings, but Sammel et al. (2002b) reported that traditional chilling of large beef carcasses could be damaging to colour stability because protein denaturation caused by faster pH decline at higher muscle temperature. Generally, carcasses stimulated early exhibits faster pH decline, which brings about redder meat colour at the early pm stage (Eikelenboom et al., 1985) but heavier carcasses exhibit even faster pH decline which particularly increases redness but exhibits lesser colour stability at the display period (Agbeniga & Webb, 2018). This could alert us to the reason the heavy carcasses had less a* values at 14 dpm. Nevertheless, some authors have observed contrary results. The study of Calnan, Jacob, Pethick and Gardner (2014) on lamb meat showed that compared with lighter weight, increased carcass weight produced higher meat reflectance (R630/R580) (oxymyoglobin/metmyoglobin ratio; which is used to represent the ratio of redness to browness of meat surface) (Hunt et al. (2001)). In their study, increased muscle weight resulted in lamb meat retaining its red colour for an extended period of display. Selection for increased muscle weight was also shown to increase the expression of type IIX glycolytic myofibres (Greenwood et al., 2006; Wegner et al., 2000). This could affect meat colour by changing the meat myofibre. Reduced maturity and increased growth rate, which brings about heavier carcasses have also been shown to reduce muscle oxidative capacity, which could influence the R630/R580 of their meat during display (Calnan et al. (2014).

At 2 dpm, there was moderate negative and significant correlation of a* values with all the pH readings (r = -0.40, -0.41, -0.45, -0.46 & -0.42 at 45 min, 3, 6, 12 and 24 h, respectively). This implies that as pH values decreases early pm, meat redness increases. Swatland (1989) reported that pm glycolysis decreased muscle pH, making it brighter and superficially wetter. This is in accord with the work of many researchers (Toohey et al., 2008; Dalle et al., 2005; Oliete et al., 2005; 2006; Strydom & Frylinck, 2014), which shows the improving effect of ES on meat redness early pm due to faster glycolytic metabolism and accumulation of lactic acid. At 14 dpm, there were no significant correlations between a* values and pH values. This is in accord with the work of Shaw et al. (2005), Wu et al. (2015) and Li et al. (2011), who indicated lesser effects of

glycolytic metabolism on a* values as the meat ages. There were also moderate negative correlations between a* day 2 pm and SF values at 2 and 14 dpm (SF 2 dpm = -0.43 and SF 14 dpm = -0.37). This implies that as redness value increased early pm, pH decreased. The reports of Goni, Beriain, Indurain and Insausti (2007) observed that Chroma value of young bulls accurately predicted the texture index (TI) of meat at 45 min pm. Researchers such as Canell et al. (2002), Wulf et al. (1997) and Wulf and Page (2000) have also used colour to classify carcass texture. Several authors also classified meat colour into groups as a function of pH, which correlates well with tenderness in this study (Abril et al., 2001; Korkeala, Maki-Petays, Alanko and Servettula, 1986; Neagueruela et al., 1992). These studies are in accord with the current findings. Abril et al. (2001) reported that each colour attribute (L*, a*, b*, C*, h*) was affected by ultimate pH. The initial a* value (2 dpm) also correlates moderately and positively with drip loss (percentage DL 2 dpm = 0.31 and percentage DL 14 dpm = 0.30). This implies that as the drip loss increases early pm, a* value also increases or vice versa. This effect also lasted on drip loss up to 14 dpm. Again, this has a bearing on the effect of ES, especially early post-mortem, which exerts a profound effect on proteolysis and glycolytic metabolism by lowering the pH at a faster rate and causing ultrastructural changes in muscle (Sleper et al., 1983). This shows that the effect of ES and faster glycolysis/metabolism on meat, cuts across the major meat quality attributes (colour, texture, drip loss and WHC) (Hwang et al., 2003; Stiffler et a., 1984; Schafer et al., 2002; Toohey et al., 2008; Li et al., 2011) and these effects are interrelated. At 14 dpm, a* value did not exert any effect on drip loss and there was no correlation. Meat redness (a* value) 2 dpm showed a negative moderate correlation with WHC 3 dpm. This was expected because drip loss is inversely proportional to WHC and the effect of ES, that brings about accelerated rigor mortis is binding on WHC as well (Bekhit et al., 2007). There was a negative moderate correlation of a* value with L* value at 14 dpm (r = - 0.30). This implies that as L* value increases during storage, a* value had a corresponding decrease. However, this increase/decrease is marginal. As stated earlier, the value of ES on a* value is more temporal than L* value (Li et al., 2011).

The researchers recorded moderate negative and positive correlations between a* values 2 dpm and some of the energy metabolites (glucose 4 h (r = 0.33); glucose 24 h (r = 0.29); CP 1 h (r = - 0.29); ATP 4 h (r = - 0.29); G-6-P 4 h (r = 0.31)). This implies that as ES brought about accelerated glycolysis and rigor development, lower pH, and an increase in muscle glucose at 4 and 24 h pm, a* value increased. Similarly, an increase in muscle G-6-P 4 h pm corresponds with increased a* value. Creatine phosphate (CP) 1 h pm and ATP 4 h pm, which are known to decrease pm (Bowling et al., 1978; Gregory, 1998) also corresponds with an increase in a* value.

As stated earlier, the effects of ES on glycolytic metabolism and energy metabolite on meat quality attribute are interconnected (Bowling et al., 1978; Hwang et al., 2003, Li et al., 2011, Sleper et al., 1983). A moderate negative correlation was observed between a* day 2 pm and micro calpain at 24 h pm (r = - 0.36). This implies that as redness increased early pm, there was a corresponding decrease in calpain-1 activity. The autolysis of calpain-1 is often associated with pH decline and is also considered the hallmark of calpain-1 activation in pm muscle (Li, Thompson & Goll, 2004). The accelerated decline of pH in ES carcasses has been associated with micro calpain autolysis in LD muscle (Melody et al., 2004, Rowe, Maddock, Lonergan and Huff-Lonergan, 2004) and subsequent rapid tenderization. In this study, carcasses with rapid pH decline and most tender meat were shown to have the highest a* value early pm (2 days) and this is in accord with the work of these authors (above). It is also in accord with the work of Janz et al. (2001), Eikelenboom et al. (1985) and Aalhus et al. (1994) which shows that LVES and HVES brought about more intense cherry red colour (chroma and hue), even up to six dpm.

5.13 Effects of treatments on meat yellowness

Analysis revealed a significant and moderate variation among the treatment groups regarding b* value (yellowness). At 2 dpm, $R^2 = 0.31$($P < 0.0001$) while at 14 dpm, there was a much lower but significant variation, $R^2 = 0.12$ ($P = 0.0349$). Early stimulated carcasses, especially the heavier ones (7 and 8), had significantly ($P < 0.05$) higher b* at 2 dpm. Like a* values, there was a convergence of values and reduced numerical differences among the groups at 14 dpm. There was also a significant difference ($P < 0.0001$) between the 2 and 14 dpm measurements. Most of the treatment groups showed marginal increase in b* value from 2 to 14 dpm, except Treatments 7 and 8, which showed marginal decrease. Similar result was obtained for a* value in this trial and there were strong correlations between a* and b* values at 2 and 14 dpm (values given below). Toohey et al. (2008) also showed an increase in b* value on lamb loins from 1 to 5 dpm but the difference was not significant. This is contrary to some reports that showed general decrease in b* value as meat aged (Wu et al., 2015). These authors showed that b* values generally decreased from 0 to 15 dpm in beef *semitendinosus*. The work of Abril et al. (2001) on beef *longissimus thoracis* showed an almost linear relationship between a* and b* values up to 9 days of ageing. In their report, a* and b* values increased up to 6 dpm but at 9 dpm, the values decreased slightly. The current result showed a strong correlation between b* and a* values at 2 and 14 dpm (r = 0.71 and 0.65, respectively). This is because of a similar increase in all carcasses, except in heavy early stimulated carcasses (7 and 8), which had a corresponding decrease in a* and b* values at 14 dpm.

At 2 dpm, there was a clear pattern of reaction from the treatment groups with regards to the effect of time of stimulation on b* value. This pattern is like for a* values, which showed that all the carcasses stimulated before evisceration (8, 7, 4 and 3) had the highest b* values, followed by the control groups (1and 2) and the late stimulated groups (10, 6, 5 and 9). There were also significant differences between carcasses that were stimulated early pm and the ones stimulated late pm, and the controls ($P < 0.05$). As stated, there was a strong positive correlation between a* and b* values at 2 and 14 dpm. This implies that as a* increases, b* also increases or vice versa. It would therefore make sense to infer that the same faster pH fall that played a significant role in affecting a* value responsible for the b* values. Li et al. (2011) showed that a* and b* values increased or varied alike, which accords with the current result. At 14 dpm, there were mixed reactions and most of treatment groups were not significantly different from one another. At this time, most of the early stimulated groups had marginally higher b* compared with the late stimulated carcasses (except Treatment 6). This suggest that the effect of the rapid pH decline and glycolytic activity (Dalle et al., 2005) in the carcasses stimulated before evisceration still lingered up to 14 dpm, in terms of b* value. Most of the literature on b* values is contrary to this result, in the sense that they reported a shorter time of stability in terms of meat yellowness in ES muscles (Mancini & Hunt., 2005; Suman et al., 2014; Strydom & Frylinck, 2014, Li et al., 2005; Wu et al., 2015). In terms of duration of stimulation and carcass weight, there were no definite patterns or reactions from the treatment groups. This suggests little or no influence of duration of stimulation and carcass weight on b* value.

From the correlations (Table 4.41), b* value correlates negatively and strongly with all pH readings from 45 min to 24 h pm at 2 dpm but at 14 dpm, it only correlated negatively and weakly to 3 h pm readings ($r = - 0.30$). Other pH correlations were much weaker and insignificant. Again this shows a strong effect of faster glycolysis, which brought about a more rapid accumulation of lactic acid on b* values immediately pm. Although, most of the reports from previous research showed a declining trend in b* value, after a shorter time (Shaw et al., 2005; Wu et al., 2015). The current study recorded marginally higher b* values in most of the groups treated with ES at 14 dpm. Similar reactions were displayed by a* values in this research. Another significant correlation with b* value at 14 dpm is the SF at 14 dpm ($r = -0.35$). This implies that as b* value increased, shear force decreased for the 14-day ageing period. Many researchers have used colour attributes to classify carcass texture and also as a function of pH (Abril et al., 2001; Korkeala et al., 1986). Goni et al. (2007) used chroma, which is a derivative of a* and b* values to predict meat texture. This is in accord with the current findings as a* and b* correlates with meat shear

force in this study. There was also a strong positive correlation of b* at 2 days pm to drip loss at days 3 and 14 pm (day 2, r = 0.58; day 14, r = 0.56). Water holding capacity was also strongly correlated (r = - 0.57) with b* value day 2 pm. This is like the correlation of a* value to the aforementioned attributes, but in the case of b* values, they were more strongly correlated compared with a* values. Again, this comes down to the influence of faster pH decline due to accelerated glycolysis on these attributes which accords with the report of Hwang et al. (2003), Stiffler et al. (1984), Toohey et al. (2008), and Li et al. (2011). All the influences of pH decline are interrelated on meat quality attributes according to these authors.

Another important and significant relationship is the strong correlation of b* value day 2 pm with L* values day 2 and 14 pm (r = 0.58 and 0.68 respectively). At day 14 pm, the correlation of b* value was weak, but still significant (r = 0.22 and 0.29 respectively) with L* day 2 and 14 pm. This implies that as b* value increased early pm, L* values also increased accordingly, early pm and at the ageing time of 14 dpm. This accords with the work of Abril et al. (2001) and the review of Mancini and Hunt (2005) and Li et al. (2011) which indicated that L*, a* and b* values were affected by pH decline and ultimate pH and that is why there were strong correlations of these colour attributes with all the pH readings. As stated, there was a strong correlation between b* and a* value at 2 and 14 dpm. This implies that the same factors that influenced a* values are most likely to influence b* values. This indicates faster glycolytic metabolism (Dalle et al., 2005) and the likelihood of MRA which are also known to be affected by lactate dehydrogenase (Kim et al., 2009), which also has a strong correlation (r = 0.79) with a* value (Wu et al., 2015). Strong positive correlations were recorded between b* value 2 and 14 dpm and chroma (C*) (r = 0.91 at day 2; r = 0.88 at day 14. respectively). This is no surprise as C* is derived from b* and a* values. However, weak correlations were recorded between b* values day 2 and hue angle (H*) (r = 0.15 with H* day 2 and r = 0.31 with H* day 14). At 14 dpm, b* values had very weak, negative and insignificant correlations with H*. This shows that as meat ages, discoloration is imminent as demonstrated by Strydom and Frylinck (2014) and as we know, b* is a function of hue angle.

Like a* value which correlates with most of the energy metabolites at 2 dpm, some significant moderate correlations of b* value at 2 dpm with some of the energy metabolites (lactate 1 h pm, r = 0.38; lactate 4 h pm, r = 0.45; glucose 1 h pm, r = 0.37; glucose 4 h pm, r = 0.50; glucose 24 h pm, r = 0.47; CP 1 h pm, r = -0.36; ATP 1 h pm, r = -0.31; ATP 4 h pm, r = -0.49; G-6-P 4 h pm, r = 0.45). As lactate value increased early pm, there was a corresponding increase in b* value. Likewise, as glucose value increased pm, there was a corresponding increase in b* value from 1 to 24 h pm. This shows a stronger relationship of b* and glucose. Creatine phosphate (CP)

showed a negative moderate correlation, and as it decreased early pm in the muscle, b* value increased. Similarly, as ATP decreased early pm, there was a corresponding increase in b* value. Glucose-6-phosphate also showed a moderate positive correlation with b* value early pm. All these increases and decreases in the energy metabolites are in accord with the reports of Gregory (1998) and Tarrant et al. (1972). The current result is in accord with the work of Li et al. (2011), who reported an increase in b* and a* values early pm, using LVES. This effect can be explained by acceleration of rigor mortis and subsequent ultrastructural changes in muscle (Sleper et al., 1983; Young et al., 1999). Wulf et al. (2002) reported a curvilinear relationship between glycolytic potential and colour attributes in beef LD muscle. Some aurthors have classified meat according to its pH decline and ultimate pH (Abril et al., 2001). This gives an indication that the faster rate of glycolysis brought about by early stimulation and the heavy carcass weight in this study has an effect on the later colour stability.

Some proteolytic enzymes also showed significant correlations with b* value 2 dpm in this study (calpain-1 24 h pm, r = -0.43; calpastatin 24 h pm, r = -0.35; calpastatin/micro calpain 1 h pm, r = 0.30). At 14 dpm, b* value also showed a moderate negative correlation with calpastatin 24 h pm (r = -0.37). This implies that as calpain-1 and calpastatin decreased due to autolysis at 24 h pm, b* value increased. This effect was also conspicuous on b* value at 14 dpm. Calpastatin is known to serve as a calpain-specific endogenous inhibitor and they are both activated together pm (Wendt, Thompson & Goll, 2004). At lower pH and higher temperature due to ES, there is an accelerated decrease in both calpain-1 and calpastatin activities, according to Hwang and Thompson (2001). The pm decrease in calpain-1 and calpastatin coincided with increased b* value at 24 h pm in this study. Currently, there are no clear reports of any influence of proteolytic enzymes on b* value, but a common ground on this effect still points to the accelerated pH decline in the carcasses that were stimulated early post-mortem. Meadus and Maclnnis (2000) reported that increased glycolytic potential promotes acidity, paleness and yellowness. The same effect is what brought about the early activation and early exhaustion of proteolytic enzymes especially when ES is involved as mentioned earlier. The report of Goni et al. (2007) indicated that b* value and chroma yielded the most information on texture of meat and this also accords with the work of Wulf et al. (1997). As we know that proteolytic enzymes have been implicated in many researches as a significant factor when it comes to tenderness, it therefore makes sense to relate b* value to the activities of calpains and calpastatin.

5.14 Effects of treatments on chroma

As mentioned earlier, chroma value (saturation index) is a derivative of redness (a*) and yellowness (b*) values. It is the intensity of the red colour or redness and it is calculated as $(a^{*2} + b^{*2})^{1/2}$ according to MacDougall (1977). As obtained in the result, the chroma values depicts the results obtained for a* and b* values and there were strong correlations between a* and b* values at 2 and 14 dpm (r = 0.71 and 0.65, respectively). There was also a strong correlation between C* and a* values at 2 and 14 dpm (r = 0.92 and 0. 93, respectively). Likewise, C* and b* values at 2 and 14 dpm (r = 0.91 and 0.88, respectively). This was expected because chroma is derived from a* and b* values. As seen in the results, carcasses stimulated before evisceration (3, 4, 7 and 8) especially the heavy ones (7 and 8) had the highest C* ($P < 0.05$) value at 2 dpm while at 14 dpm, there were mixed reactions but most of the early stimulated carcasses still had the highest C* values even though the differences were marginal. Like a* and b* values, there were marginal increases in C* value from 2 to 14 dpm in all carcass groups except for Groups 7 and 8, which had marginal decline in C* values. This could be attributed to higher rigor temperature which caused greater denaturation of protein in the heavier carcasses (7 and 8) that were stimulated early pm (Kim et al., 2014). Also, C* values correlated moderately and strongly with all pH, SF, DL and WHC especially at 2 dpm (Table 4.41b), which was also observed for a* and b* values. To buttress the report of Kim et al. (2014), from the regressions (Table 4.42), C* 2 dpm could be predicted by pH3 h pm, drip loss and L* values early pm. C* 14 dpm could also be predicted by H* values at 2 dpm. This pattern of reaction was mainly due to pH decline and the time of stimulation which exerted the greatest influences on SF and WHC (Hwang et al., 2003). Similar to a* and b* values, C* also recorded moderate correlations with most of the energy metabolites, especially at 2 dpm. These were also expected (Ferguson & Gerrard, 2014; Simmons et al., 1996). Overall, it was observed that LVES favoured C* value at 2 dpm especially for the carcasses stimulated early and produced marginal increase in C* values after 14 days of ageing. Furthermore, C* 2 dpm showed moderate negative correlations with 24 h calpains and calpastatin, as did a* and b* values (r = -0.41, calpain-1; r = -0.30, calpain-2; r = -0.30, calpastatin). This implies that as muscle calpain and calpastatin decreased early pm, C* values increased. Dransfield (1994) predicted that calpain activity would be six times greater in rapidly glycolysing muscles compared with more standard rates of glycolysis under standard cooling conditions. This is in accord with the review of Kim et al. (2014) which indicated that early pm meat colour is enhanced by rapid glycolysis. However, at 14 dpm, there were no correlations between C* and proteolytic enzymes.

5.15 Effects of treatments on hue angle

Hue angle (H*), also known as discoloration, is a derivative of a* and b* values, calculated as $\tan^{-1}$ (b*/a*) (Young, Priolo, Simmons and West, 1999). Longer ES (60 s) Groups 4 and 8 (early stimulation), had the highest discoloration (H*) at 2 dpm. At 14 dpm, group 8 also had the highest ($P < 0.05$) discoloration compared with other groups. H* correlated strongly with the L* values at 2 dpm ($r = 0.65$) and 14 dpm ($r = 0.67$). These carcass groups (4 and 8) also had the highest L* values at 2 and 14 dpm. This shows that as L* value increased pm, discoloration also increased. This was supported by the regression analysis (Table 4.42) in which H* 14 dpm could be predicted by L* 2 dpm and DL 3 dpm. This was caused by reduced rate of oxygen consumption to form oxymyoglobin as meat aged (Ledward et al., 1986) and myofibrillar lattice shrank (Offer & Trinick, 1983; Swatland, 1993). This theory is supported by Wu et al. (2015), who concluded that several proteins and their reduction and denaturation through glycolytic activity, energy metabolism and redox process pm affects meat discoloration as meat ages. Buts et al. (1986) also suggested that ES brought about increased lightness due to accelerated denaturation of protein, which accords with the results of George et al. (1980), who found irregular bands of denatured sarcoplasmic proteins deposited in fibres of stimulated muscles, analogous to PSE in pig muscles. Li et al. (2011) reported that ES increased L* values of bovine *longissimus* muscle as the meat aged. Roeber et al. (2000) also indicated that duration and voltage intensity affect colour. Longer duration of stimulation and higher voltage results into lighter meat, with higher H* values. Hue angle showed moderate positive correlations with drip loss at 3 dpm ($r = 0.36$) and 14 dpm ($r = 0.37$). This was also reflected in the L* values and the pH_u ($r = -0.32$) at 14 dpm. It implies that the higher the H* values, the more the drip loss as seen in the early stimulated carcasses, with faster pH decline at high initial carcass temperature (Bendall & Swatland, 1988). At 14 dpm, most of the carcasses stimulated early (Groups 4, 7 and 8) had the highest H* value, although there were no statistically significant differences except for Group 8, which had the highest values ($P < 0.05$). In this case, the combination of the heavy carcass weight, 60 s stimulation and early stimulation played out in the high H* value for this group. The numerical differences were low among the other treatment groups at 2 and 14 dpm.

5.16 Effects of treatments on energy metabolites

5.16.1 Lactate

Early stimulation (groups 3, 4, 7 and 8) produced the highest ($P < 0.05$) amount of muscle lactate (μmol/g) at 1 and 4 h pm, but at 24 h pm, the differences in all carcass groups were not significant, although, the early stimulated carcasses still had numerically higher values (Table

4.27). This agrees with the report of Polidori et al. (1999). Of the early stimulated carcasses, longer duration of stimulation (60 s) (Groups 4 and 8) produced the highest muscle lactate at 1 and 4 h pm. This shows the effects of faster glycolysis and faster pH decline because of ES on the accumulation of lactate in early and longer stimulated carcasses, especially at the early pm period. This was reflected in the drip loss and WHC and agrees with the report of Hwang and Thompson (2000) who reported that the magnitude of pH decline in ES carcass was mainly a function of the time of application, which affected drip loss significantly. Lactate is known to act as an anionic chaotrope, which impairs the interaction of water and protein, resulting in reduced WHC and increased drip loss (Puolanne & Halonem, 2010). Strydom and Frylinck (2014) and Roeber et al. (2000) also confirm that longer duration of ES is not so beneficial to drip loss owing to a faster pH decline rate compared with shorter duration. Early lactate accumulation owing to early LVES was reflected in meat tenderness. This agrees with the work of Fabianson and Laser Reutersward (1985), in which LVES was also used. The authors found a significant correlation between muscle lactate content and the rate of pH decline within 6 h of stimulation, which also correlates significantly with meat tenderness improvement. Regarding carcass weight, there were mixed reactions. However, the heavy, early stimulated carcasses (8 and 7) were among treatments with the highest amount of muscle lactate at 1, 4 and 24 h pm. This agrees with the recent report of Warner et al. (2014) which indicated that heavier carcasses from feedlot cattle exhibited faster pH decline and higher rigor temperature, which implies faster lactate accumulation.

5.16.2 Glucose

Electrically stimulated carcasses generally had higher glucose content compared with the NES carcasses at 1, 4 and 24 h pm. Time of stimulation produced a clear pattern of reaction among the treatment groups at 1 and 4 h pm (Table 4.28). The early stimulated carcasses had the highest ($P < 0.05$) muscle glucose at 1 and 4 h pm, but at 24 h pm, there were mixed reactions among the carcass groups. This agrees with the report of Fabianson and Laser-Ruterswaard (1985), who indicated that early application of LVES produced a significant increase in muscle glucose, which coincided with faster pH decline compared with NES carcasses. In this study, the NES and the late stimulated carcasses (Table 4.28) had close numerical values of glucose at 1 and 4 h pm, which were significantly lower than the early stimulated carcasses. This shows the significance of LVES on muscle glucose, which also correlates with reduced SF, decreased WHC and meat colour attributes (L*, C* and H* values) (Table 4.41). The result for glucose also accords with the findings of Frylinck et al. (2013) who found negative correlations of increased muscle glucose with faster pH decline which also correlates with increased drip loss, lower WHC but improved

colour attributes. In this study, there were mixed reactions on the effects of carcass weight on glucose. However, heavy early stimulated carcasses had the highest amount of muscle glucose, save Treatment 4, at 1 and 4 h pm (Table 4.28). In terms of duration of stimulation, there were mixed reactions from the treatment groups.

5.16.3 Glycogen

Result showed a general decrease in muscle glycogen content with larger decline in the early stimulated carcass groups, especially between 1 and 4 h pm (Table 4.29). This is in accord with many of the previous studies (Hamm et al., 1973; Dalrymple & Hamm 1975). Early application of LVES produced the lowest amount of muscle glycogen relative to late stimulated and NES carcass groups. This agrees with the findings of Fabianson and Laser Reutaswaard (1985), who reported a ten-fold increase in energy consumption during LVES and a two-fold increase following early stimulation, which was reflected in tenderness improvement. According to the same authors, glycogen breakdown was the best indicator for predicting tenderness improvement in their work. Calkins et al. (1983) also showed a significant association between tenderness and pm metabolic rate. The higher drip loss and lower WHC obtained in this trial also points to a correlation of muscle glycogen depletion with increased drip loss, reduced WHC and improved colour parameters early pm, which agrees with the findings of Frylinck et al. (2013). However, there was no direct correlation of muscle glycogen with any of these meat quality attributes in this study. Of the late stimulated carcasses, the carcass groups stimulated for 60 s (longer duration-Groups 10 and 6) had the highest muscle glycogen. This shows that late stimulation and longer duration resulted in less glycogen depletion for LVES. There were mixed reactions to the effects of carcass weight on glycogen depletion, which implied that carcass weight had no effect on glycogen consumption pm. Glycogen concentration is regarded as central to pm pH decline. However, some authors have reported that muscle glycogen alone is not responsible for pH decline pm. Other factors such as genetics, feeding and productions system, sex, age, and carcass pm temperature and treatments should also be considered as discussed in the review of Ferguson and Gerrard (2014).

5.16.4 Creatine phosphate

There was no very clear pattern of reaction at 1 h pm, but the early stimulated carcasses had the lowest amount of creatine phosphate (CP) at 1 and 4 h pm while the late stimulated carcasses and the NES (control) groups had the highest muscle CP (Table 4.30). However, at 4 h pm the pattern of reaction was clearer and all the early stimulated carcasses (3, 4, 7 and 8), had the lowest, but

not significantly different muscle CP. This accords with the report of Fabianson and Laser Reuterswaard (1985), in which LVES caused a significant decline in muscle CP content at 6 h pm, compared with the NES counterparts. A strong correlation was found between 1 and 4 h pm, but the 24 h CP content did not correlate with the initial content, which implied that the effects of ES was for a short period on CP content in the muscle. This is in line with the findings of Fabianson and Laser Reuterswaard (1985). According to Ferguson et al. (2001), the faster glycolysis in ES muscle usually last for about 4 to 5 h before it stabilizes. There were moderate correlations between CP 4 h pm and SF (14 dpm), drip loss at 14 dpm and L* 2 dpm. Also, CP 1 and 4 h pm correlated strongly with drip loss at 14 dpm (Table 4.41). Likewise, CP 1 h pm correlated moderately with C* 2 dpm, L* 2 and 14 dpm. The correlation of CP 1 and 4 h pm and pH (1 to 24 h pm) were moderate to strong but at 24 h pm, the correlation was weak. This implies that early application of LVES directly affected pH decline and meat quality attributes which implies high energy turnover early pm as indicated by Fabianson and Laser Reuterswaard (1985). Bendall (1980) hypothesized that ES affected the ability of sarcoplasmic reticulum to retain ca^{2+} which led to faster pump activity with more ca^{2+} in the cytosol. This triggers the ATPase activity to convert more ADP to ATP from store of CP and hence, more ATP is being used up at a faster rate, leading to faster glycolysis (Ferguson, 2003). Another important factor in terms of the rate of CP decline is that, the late stimulated carcasses and controls had higher values of CP at the initial hours pm compared with the early stimulated carcasses, especially the heavy ones (Table 4.30). This implies that the early stimulated carcasses had a more depleted amount of CP, even before the samples were collected at 1 h pm. This accounted for the lower decline in the early stimulated carcasses from 1 to 24 h pm (Table 4.30). The time of stimulation (Hwang & Thompson, 2001) and the high initial muscle temperature, because of early ES (Ferguson and Gerrard, 2014) played major roles in the manipulation of CP.

5.16.5 Adenosine tri-phosphate

Early stimulation played a major role in the decline of muscle ATP (Table 4.31). At 1 h pm, the reaction pattern was not so clear but the heavier, early stimulated carcasses (7 and 8) had the lowest ($P < 0.05$) muscle ATP. At 4 h pm, the pattern of reaction was more definitive, as all the early stimulated carcasses had the lowest ($P < 0.05$) ATP. At 24 h pm, the differences among all carcass groups were not significant. This implies that more ATP was used up during early pm glycolysis at a faster rate in the early stimulated carcasses (1 to 4 h pm). The result was reflected in the moderate correlation of ATP and CP at 1 and 4 h pm. This agrees with the work of Scopes (1970) who indicated that ATP activity is moderated by the ability of CP store to re-synthesize

ATP from ADP. He further stated that glycolysis pm is greatly impacted by the disappearance of ATP. The moderate to strong correlation of ATP 1 and 4 h pm to pH values from 1 to 24 h pm confirms the findings of Robergs (2001), who postulated that ATP is a major contributor to H^+ concentration, which also buffers pH decline in muscles. The author indicated that lactate formation cannot be used solely as a measure of the extent of glycolysis and pH decline in muscles. Regarding the rate of ATP decline pm, the early stimulated carcasses had a combined average decline of 32.3%, followed by the late stimulated carcasses (21.9%) and the NES (10.1%) from 1 to 4 h pm. This is in accord with the report of Hwang and Thompson (2001), which indicated faster glycolysis in early stimulated carcasses, which led to faster pH decline. The impact of carcass weight on ATP was small, but heavy carcasses from the early stimulated carcass groups had the lowest ATP at 1 and 4 h pm. This could be deduced from the faster response of heavier carcasses to ES and faster rate of glycolysis in heavier muscles, as reported by Thompson (2002) and Kim et al. (2014) and probably more muscle glycogen composition as reported by Pethick et al. (1999).

5.16.6 Glucose-6-phosphate

There were small numerical differences among the treatment groups at 1 h pm (Table 4.32). This implies little or no effects of ES on G-6-P, early pm. However, at 4 h pm there was a clearer pattern of reaction from the treatment groups. There was a general increase in all groups (Frylinck et al., 2013) and early stimulated carcasses had the highest, but not significantly different muscle G-6-P content, followed by the late stimulated carcasses and controls. At 24 h pm there was no significant difference among groups, which implies lack of ES effect on muscle G-6-P. G-6-P is known to be produced from glucose residues released from glycogen (Ferguson & Gerrard, 2014). It is converted to G-6-P by an enzyme (glycogen phosphorylase), which repositions the phosphate on the glucose molecule (Lehninger et al., 1993). At 4 h pm, G-6-P correlated moderately with lactate, 1 and 4 h pm, glucose, 1 and 4 h pm, CP, 1 and 4 h pm, ATP 1 and 4 h pm, and also with pH at 1 to 24 h pm, but there was no correlation with muscle glycogen (Table 4.41). This is in contrasts the findings of many researchers, as indicated by the review of Ferguson and Gerrard (2014). Most of the muscle energy metabolites are inter-dependent and are affected by one another during glycolysis (Ferguson & Gerrard, 2014). Many authors have also indicated that glycogen concentration alone should not be used as a measure of pH decline (Scheffler & Gerrard, 2007; Scheffler et al., 2011; Sellier & Monin, 1994; Ferguson et al., 2008). This implies that other factors may be involved in the process of glycolysis as related to ES.

5.16.7 Glycolytic potential

Normal pH_u (pH 5.5-5.6) is only affected when pre-slaugher level of glycogen falls below the critical threshold of 45-55 μmol/g (Monin, 1981: Wulf et al., 2002). In this study, pH_u in all the treatment groups were not far from range (minimum 5.46 and maximum 5.71) (Table 4.1) and weas expected to be normal or close to normal for most of the carcasses. Although the pre-slaughter glycogen levels were not determined, the first glycogen readings were taken at 1 h pm when glycolysis had already commenced. Most of the early stimulated carcasses had the highest GP at 1 and 4 h pm. However, Treatment 10 which was stimulated late had the highest GP at 1, 2 and 24 h pm. This was because of the high ($P < 0.05$) initial muscle glycogen content (Figure 4.18), which agrees with Monin and Sellier (1985), and Thompson (2002). Longer duration of stimulation (Treatments10, 8 and 6) also produced the highest GP early pm and this was reflected in the SF, drip loss and WHC of the resultant meat. Groups with the highest glycolytic potential because of early ES (8, 7 and 4, save Treatment 10) had the lowest SF at 3 and 14 dpm. This agrees with the findings of Fabianson and Laser Reuterwaard (1985), who found glycogen metabolism and fast energy metabolism owing to ES as the best predictors of tenderness development. Also, in their work, early stimulation induced a huge difference at 6 h pm between the stimulated and the NES carcasses. Similar results were obtained in this study in which early stimulation induced a large difference in pH and its decline at 6 h pm between the early and late stimulated and NES carcasses (Table 4.1). However, the ultimate pH values were not significantly different among carcass groups. The higher glycolytic potential of the early stimulated carcasses in this study, however, led to reduced WHC and this was largely due to the faster pH decline and faster lactate accumulation in the muscles (Frylinck et al., 2013). Longer duration of stimulation also resulted into higher GP and lower WHC and higher drip loss early pm, which agrees with the report of Strydom and Frylinck (2014). The initial carcass temperature in early stimulated carcasses also played a part in the higher drip loss (Puolanne & Halonem, 2010). Heavy carcasses exuded more water as drip loss and had lower WHC compared with lighter carcasses. Heavy carcasses have higher myofibrillar protein density, which reduces intra-myofibrillar water content and increases inter-myofibrillar water content (Bertram et al., 2007). This inter-myofibrillar water can easily be flushed out in the form of drip as lactate accumulates owing to faster glycolysis and faster pH decline at higher initial carcass temperature and as myosin heads denatures, thereby losing the ability to bind water. Generally, carcass weight and fatness are known to influence the cooling rate, which potentially influences glycolytic rate (Jacob & Hopkins, 2014). This agrees in part with the result obtained in this study, in that most of the heavy carcasses (7, 8 and 10) (Table

4.33) had the highest GP at 1, 4 and 24 h pm. Although, the differences were not statistically significant, there was a high tendency for a significant difference (P = 0.077) at 4 h pm (Table 4.35) between the heavy and lighter carcasses. The interaction of heavy carcass weight and early stimulation also produced a significantly higher GP at 24 h pm. Likewise, the interaction of early stimulation and longer duration of stimulation produced significantly higher GP at 1 and 4 h pm (Table 4.35). This is in accord with the report of Hwang and Thompson (2001) and the review of Ferguson and Gerrard (2014).

5.17. Effects of treatments on proteolytic enzymes

5.17.1 Calpain-1

The effects of ES and pH-temperature interaction on proteolytic activities have been widely documented. According to Simmons, Singh, Dobbie and Devine (1996), rigor or hot/heat shortening is due to a combination of high temperature and low pH causing early exhaustion of proteolytic activities. Hwang and Thompson (2001b) reported that rapid pH decline, due to early application of ES (3 min pm) exhausted the calpain-1 activity and increased calpastatin activity leading to reduced ageing potential and heat (rigor) shortened meat. They reported optimum tenderization when carcass temperature at pH 6 was between 29 and 30 °C. In this study, average of 36.2% decline in calpain-1 from 1 to 24 h pm in early stimulated carcasses, 29.7% decline in the late stimulated carcasses and 12.3% decline in the NES carcasses were recorded. This is much lower compared with what Morton et al. (1999) reported (80% decline in calpain-1), probably because of the HVES (900 V) that was used in their study. Morton et al. (1999) reported a strong correlation between the rate of pH decline and calpain and calcium activation and the rate of tenderization. Also, LVES may not be able to activate enough calcium ions depending on the other settings of the stimulator and this will affect calpain activation (Simmons et al., 2008). This might have led to lack of significant differences in the concentration of calpain-1 in most of the electrically stimulated groups in this study. It also suggested that more calpains would be available for extended ageing in most of the treatment groups. The current results imply slightly higher autolysis in the calpain-1 content in the early stimulated carcasses owing to faster pH decline at higher carcass temperature. This was reflected in the SF of the meat from these carcasses, as they had the lowest SF, followed by the late stimulated carcasses and controls at 3 and 14 dpm (Table 4.4). Although the early stimulated carcasses had the lowest SF at 3 and 14 dpm, it did not reflect in their MFL's as expected (Koohmaraie et al., 1987; Robson et al., 1991). The late stimulated carcasses had the shortest MFL's which suggested greater myofibrillar degradation. The physiological activity and significance of calpain autolysis is still unclear and

has been a controversial subject for many years, especially in-vivo (Goll et al., 2003). Also, Kim et al. (2014) stated that a knowledge gap still exists in understanding the underlying mechanisms that affects proteolytic enzymes and other metabolic processes as influenced by high temperature rigor, especially in intact carcasses from feedlot cattle. Authors such as Devine et al. (1999) have reported that MFL alone is not a complete indicator of the extent of tenderization during proteolytic ageing. For example, Huff-Lonegan et al. (1996) suggested that the degradation of structural proteins such as titin, nebulin, desmin and troponin-T which are incubated like calpain enzymes, could contribute to meat tenderness. This was corroborated by Li et al. (2011) who did not find a significant difference in MFL owing to ES in tenderness using LVES on *longissimus dorsi* muscles. Li et al. (2011) indicated that sarcoplasmic protein concentration increased slightly in the ES samples 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 tenderness. This agrees with the suggestion of Jacob and Hopkins (2014) who noted that carcass weight and fatness influences cooling rate which potentially affects glycolytic rate and proteolytic enzyme activity (Table 4.4). Similarly, Oddy, Harper, Greenwood and McDonagh (2001) reviewed the possible mechanism by which growth rate could affect tenderness because of structure and cross linking of collagen matrix and proteolytic activity and glycolytic rate of the myofibre component of muscles. Treatments 7 and 8 had the lowest shear force at 14 dpm. This implied that heavier carcass weight in relation with faster pH decline at higher temperature early pm has an influence on the lower shear force that was obtained. However, despite the mixed results in calpain-1 activity, the lower mean shear force obtained from the early stimulated carcasses especially at 14 dpm reflects the higher calpain-1 decline in the early ES carcasses (mean 36.2%) compared with the late stimulated carcasses (mean 29.7%).

5.17.2 Calpain-2

There was no significant decline in calpain-2 in all the treatment groups from 1 to 24 h pm (Table 4.23). Numerical differences between groups were also low or negligible. However, there were clear pattern of reactions in that the early stimulated carcasses had the lowest calpain-2 values at 1 and 24 h pm (even though the differences were not significant) followed by late stimulated and the NES carcasses. This was also illustrated in Figure 4.24. From all indications, calpain-2 was least activated by Ca^{2+} in the meat samples examined in this study. Calcium concentration in pm muscles is less than that required by calpain-2 for activation (Boehm, Kendall, Thompson & Goll, 1998). Also, calpain-2 persists longer than the less stable calpain-1 in ageing muscles, which suggest that they are not so activated early pm (Sensky, Parr, Bardsley & Buttery, 1996). Morton

et al. (1999), using HVES (900 V) on beef carcasses also reported very little activation of calpian-2 even after 48 h pm. It was concluded that calpain-2 played very little part in proteolysis due to lack of autolysis (Veiseth et al., 2001; 2004). Duration of stimulation and carcass weight did not exert any effect on calpain-2 in this study.

5.17.3 Calpastatin

Like calpain-1, there was a declining trend of calpastatin from 1 to 24 h pm (Table 4.24 and Figure 4.25). However, the early stimulated and the late stimulated carcasses had similar rates of decline (mean 24%), but higher than NES (15%). This suggests that the effect Zilmax on muscle calpastatin, which brings about tougher meat (Dunshea et al., 2005), remains the same, despite the timing of ES and the difference in pH decline between the early and late stimulated carcasses. Calpastatin is known to inhibit calpain-1 and calpain-2 in a process that requires calcium concentration close to or below those levels that are required to activate calpains (Goll et al., 2003). This implies that similar amounts of calcium were activated by LVES, regardless of the time of stimulation. Similar results were reported by Hwang and Thompson (2001) in which LVES and HVES applied 3 and 40 min pm resulted in similar levels of calpastatin, which were lower than controls at 24 h pm. However, calpain-1, which is the main proteolytic enzyme responsible for tenderization (Kemp et al. 2010), was more activated (36% decline at 24 h pm) in the early stimulated carcasses compared with 29% decline in the late stimulated carcasses, which resulted in the lower SF of the early stimulated carcasses. Also, carcasses with the most rapid pH decline produced the highest loss of calpain-1 at 24 h pm and had the most tender meat (Morton et a., 1999). Although, the effect of carcass weight was not conspicuous on calpastatin activity, a factor that is noteworthy is the high initial carcass temperature for the early stimulated carcasses. This might have favoured more proteolytic enzyme activity after the 24 h measurement that was made in this study. Zamora et al. (2005) found a correlation between peptidase inhibitor and meat toughness after 6 dpm. These authors concluded that inhibitors of peptidases had a better predictive value of meat quality than the enzyme directly involved in proteolysis, such as the inhibitory action of calpastatin on calpain. Regarding the influence of duration of stimulation on calpastatin, there were mixed reactions, which implied, lack of influence of duration of stimulation on muscle calpastatin content at 1 and 24 h pm. As there were no significant differences in most of the treatment groups, a factor that could be considered is the strength of LVES in activating calcium ions that in turn activates calpastatin. It is possible that LVES did not activate enough calcium ions that were required to activate calpastatin (Simmons et al., 2008). This is coupled with the use of Zilmax which is known to increase calpastatin activity which

reduces pm ageing. However, Hope Jones et al. (2010) concluded that ES reduced the negative effects of Zilmax on tenderness, but could not completely eradicate its effect on ageing, which was also observed in this study.

CONCLUSIONS AND RECOMMENDATIONS

From the results obtained in this study, it was concluded that heavier carcasses from commercial feedlot systems, in South Africa, treated with early application of LVES, favour some of the key meat quality attributes. Heavier carcasses exhibited the lowest shear force in the butchery (3 dpm) and display (14 dpm) periods. This was attributed mainly to faster pH decline at higher carcass temperature, which brought about higher proteolytic activity. The colour attribute of chroma (C*) in the early stimulated heavy carcasses was also significantly enhanced in the butchery period, but at the display period, the advantage diminished as ageing progressed. LVES brought about marginal increase in C*, even at 14 dpm, in all carcasses except the early stimulated, heavy carcasses. On the other hand, the advantages of early application of LVES on shear force and C* was met with marginal increase in drip loss and reduced water holding capacity, especially in the heavier carcasses. Early application of LVES generally brought about lower shear force, higher C* (3 dpm), higher H* (14 dpm), higher calpain-1 decline, faster glycolysis and higher glycolytic potential (GP) (early pm). As well as longer MFL (at 14 dpm), marginally higher drip loss and reduced water holding capacity, irrespective of carcass weight.

It was also concluded that shorter duration of LVES is preferable, especially when applied early pm on heavier carcasses. This was demonstrated in the significantly lower shear force values obtained by the interaction of heavy carcass weight and shorter duration of ES at 3 dpm. The detrimental effects of longer duration were evident on meat quality attributes such as higher drip loss, lower WHC, higher L*, b* and H* values at both 2 and 14 dpm as well as higher muscle lactate values, compared with shorter duration (30 s) of LVES. Longer duration of ES also exerted its effect on GP by generally producing the highest GP, especially in the early stimulated carcasses. This reaserch presents current data on meat quality from intact carcasses, of recently produced feedlot cattle with heavier carcasses, using low voltage electrical stimulation. It also showed that LVES produced meat of acceptable tenderness, despite the use of beta-agonist (Zilmax) and tropical breed, which were used in this study.

It is therefore recommended that the production of heavier carcasses be encouraged, and LVES should be applied early pm for its benefits on lower shear force and better meat colour early post-mortem and even after ageing. However, more research should be carried out on ways of mitigating the detrimental effects of higher drip loss, lower WHC and poorer meat colour, especially in the heavier and early stimulated carcasses, after longer ageing. The production of heavier carcasses should also be encouraged because heavier carcass favour slaughter house

pricing in many countries including South Africa. The study showed that heavier carcasses can conveniently be produced alongside lighter ones, with properly managed, low voltage electrical stimulation without significant detrimental effects to meat quality. Moreover, the production of heavier carcasses through improved nutrition or the use of feed additives such as zilpaterol hydrochloride (beta-agonist) provides an opportunity to produce more beef with fewer cattle, which implies less waste, and less use of land, water and energy.

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