The effects of distance, lairage duration and animal-related factors on pre-slaughter stress indicators, carcass characteristics, nanostructure and technological properties of beef

By

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Notes

This thesis is presented in the format prescribed by the Department of Livestock and Pasture Science at the University of Fort Hare. It is structured to form several chapters (Papers submitted to different journals) and is prefaced by an introduction chapter with the study objectives, followed by a literature review and concluded with a chapter containing the general discussion and recommendations.

Language, stye and referencing format used are in accordance with the requirements of the Department of Livestock and Pasture Science. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has therefore been unavoidable.

Results from the study have been presented at the following symposia:

1. **Chulayo Y. Amanda** and Muchenje Voster. *Activities of creatine kinase and lactate Dehydrogenase as welfare indicators in slaughter cattle.* Poster presented at the 59th ICoMST: International Congress of Meat Science and Technology in Izmir, Turkey, ***14th - 23rd August 2013*** (*www.ICoMST2013.org*).
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ii. **Chulayo A.Y.** and Muchenje V. The activities of some stress enzymes as slaughter cattle welfare and how they relate to physico-chemical characteristics of beef. Submitted to *Meat Science (Under review)*.

iii. **Chulayo A.Y**. and Muchenje V. Carcass characteristics and physico-chemical attributes of beef. Submitted to the *Journal of Animal Science (Under review)*

iv. **Chulayo A.Y.**, Bradley G. and Muchenje V. The effect of age, genotype and number of stunning shots on the expression of heat shock proteins in slaughtered bovine species. Submitted to *Meat Science* (*Under review*).

v. **Chulayo A.Y**. and Muchenje V. The effect of animal class, genotype and sex on the nanostructure of beef and meat quality. Submitted to the *South African Journal of Science (Under review)*

Declaration

I, **CHULAYO Amanda Yucca,** declare that this dissertation has not been submitted to any University and that it is my original work conducted under the supervision of Prof V Muchenje. The research was approved by the University of Fort Hare Research Ethics Committee (Certificate No.: MUC0121SCHU01). All assistance towards the production of this work and all the references contained herein have been duly credited.

CHULAYO Amanda Yucca ______________________________ **Date:** ____________________

(Signature)

Approved as to style and content by:

Prof Voster Muchenje ______________________________ **Date:** ____________________

(Promoter) *(Signature)*

Abstract

The effects of distance, lairage duration and animal-related factors on pre-slaughter stress indicators, carcass characteristics, nanostructure and technological properties of beef

By

CHULAYO Amanda Yucca

The main objective of the current study was to determine the effect of distance travelled (DT), lairage duration (LD_{hr}) and animal-related factors (animal class, breed, sex) on pre-slaughter stress indicators, carcass characteristics, nanostructure and technological properties of beef. The study was conducted in an Eastern Cape abattoir under the Buffalo City District Municipality. Six genotypes of cattle (Beefmaster, Bonsmara, Brahman, Holstein-Friesian, Non-descript and Nguni) brought for slaughter at the abattoir on different days were used in the study. Slaughter weight (SWT), warm and cold carcass weight (WCW and CCW), fatness and conformation of each animal were measured and recorded. Representative samples of blood plasma and meat quality from the *Muscularis thoracis et lumborum* (LTL) were collected at slaughter and 24 hours after slaughter, respectively. Heat shock proteins 70kDa (HSPA1A), glucose (GLU), cortisol (CORT), creatine kinase (CK) and lactate dehydrogenase (LDH) were determined from the blood plasma. The representative LTL muscle was used to determine ultimate pH (pH_u), lightness (L*), redness (a*), yellowness (b*), meat temperature (T_m), Warner Bratzler Shear Force (WBSF) and histological structure of beef. There were significant effects of distance on pH_u, T_m, a*, b*, TL%, CK and LDH. Pearson correlations were observed between distance travelled (DT), creatine kinase (CK), lactate dehydrogenase (LDH), ultimate pH (pH_u), colour coordinates (L*, a* and b*) and Warner Bratzler Shear Force (WBSF). Principal component analysis (PCA) revealed that the 1^{st} (pH_u), 2^{nd} (T_m) and 3^{rd} (L*) principal components were the critical meat quality attributes. Positive relationships between carcass weights and beef quality characteristics for bovine cattle where the cumulative percentage of the first six principal component`s (PC`s) (SWT, WCW, CCW, DP, pH_u and T_m contributed to 93.4 % of the total variance. Significant correlation coefficients between DT (r = 0.15), LD_{hr} (r = 0.29), T_a (r = -0.52), T_m (r = 0.26), L* (r = 0.33), a* (r = 0.32) and WBSF (r = -0.49) were observed. Meat from Brahman (BR), Non-descript (ND) and Nguni (NG) cows had visible skeletal muscle fibres which were thin and long indicating improved tenderness of beef. Exposing animals to longer hours of transportation did not only reduce glucose levels but also increased the expression of HSPA1A and levels of cortisol. The CK and LDH were not related to beef quality. Therefore, CK and LDH can be used as indicators of animal welfare in slaughter cattle but cannot be used to predict the quality of meat. Bulls were the heaviest, although heifers had the highest dressing percentage than cows. Furthermore, the nanostructure of beef indicated that heifers had the best muscle fibres, sarcomere length and visible intercalated discs. The observed nanostructures of Nguni genotype are an indication of tender meat. Beef from heifers of Nguni had visible and tender meat with traces of intramuscular fat while males had shorter sarcomere length and tougher meat.

Keywords: Eigenvalues, HSPA1A, glucose, meat quality, muscle fibres, nanostructure, pre-slaughter stress, road transportation, scanning electron microscope, stress biomarkers, ultimate pH

Dedication

I dedicate the thesis to the Lord God Almighty and my lovely and wonderful family for granting me the grace, strength, enablement, support and ideas to fulfil my dream of becoming a doctor for life.

"Uzuko kuThixo oSenyangweni ngezinto ezinkulu azenzileyo;

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To God be the Glory, great things He has done".

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List of Symbols

%	Percentage
[ ]	Concentration
µl	Microliters
^{0}C	Degrees Celsius
A	Amperes
G	Grams
H	Hours
h^2	Heritability
Hz	Hertz
Kg	Kilograms
Km	Kilometres
Mg	Milligrams
Mm	Micrometres
N	Number
Ng	Nanograms
Nm	Nanometres
nmol/L	Nanomoles per litre
S	Seconds
U/l	Units per litre
V	Volts

List of Abbreviations

a*	Redness
ATP	Adenosine triphosphate
b*	Yellowness
BH	Brahman
BR	Bonsmara
CCW	Cold carcass weight
CH	Charolaise
CK	Creatine kinase
CL%	Cooking loss percentage
CNS	Central nervous system
DA	Dopamine
DP	Dressing percentage
DT	Distance travelled
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
EPI	Epinephrine
GLU	Glucose
HF	Holstein-Friesian
HPA	Hypothalamic-pituitary-adrenocortical axis
HRP-avidin	Horseradish Peroxidase
HSF1	Heat Shock Transcription Factor
HSPA1A / Hsp70	Heat shock protein 70 kDa
HUE	HUE angle

L*	Lightness
LDH	Lactate dehydrogenase
LTL	*Muscularis longissimuss thoracic et lumborum*
ND	Non-descript
NE	Norepinephrine
NEFA	Non-esterified fatty acids
NG	Nguni
PCA	Principal Component Analysis
pH_u	Ultimate pH
SAS	Statistical Analysis System
sHsp	Small heat shock proteins
SNS	Sympathetic nervous system
SWT	Slaughter weight
T_a	Ambient temperature
TL%	Thawing loss percentage
T_m	Meat temperature
WBSF	Warner Bratzler Shear Force
WCW	Warm carcass weight

Chapter 1 General Introduction

1.1 Background and justification of the study

Animal welfare has become one of the most important aspects in meat production (Gregory, 2007). The increased public concern for animal welfare in the logistic chain requires more research to be done in order to safeguard animal and meat products on a large scale. The consideration of animal welfare status is expected to begin at the farm, during transportation and at the abattoir. Transportation and handling of slaughter animals is associated with a series of events that expose animals to stressful and unfavourable conditions, compromising their welfare and meat quality (Ljungberg *et al.,* 2006). Studies conducted revealed that transportation is the major stressor on animals (Huertas *et al.,* 2010; Gajana *et al.*, 2013; Vimiso and Muchenje, 2013). Prolonged exposure of cattle to different stressors disrupts the mobilization of energy and thus affecting the normal body functions of the animal and physiological reactions that are involved in stress response. These body functions include immunity, growth, reproduction and expression of normal behaviour (Kilgour *et al.,* 2012).

Stressful conditions prior to slaughter may also result in some changes in blood constituents such as the plasma concentration of creatine kinase (CK), lactate dehydrogenase (LDH), glucose, cortisol and catecholamines (Mota-Rojas *et al.*, 2011; Ekiz *et al.*, 2012a; Nakyinsige *et al.*, 2013). Creatine kinase and LDH are enzymes that are released into the bloodstream when muscles are bruised (Djordjevic *et al.,* 2010). Cortisol is a mammalian hormone that is released routinely by the hypothalamic-pituitary-adrenocortical axis, often due to response to stress and it has a number of constructive functions (Probst *et al.*, 2012). If homeostasis is not regained, there will be an increase in cortisol levels and a marked depletion of glycogen reserve. Depleting glycogen levels under various conditions promote stress and consequently will lead to poor meat quality (Lepschy *et al.,* 2008; Muchenje *et al.*, 2008, 2009b). The release of a hormone epinephrine at sympathetic nerve endings increases the rate and force of muscular contractions resulting in elevated glucose levels, skeletal glycogenolysis and catabolism of fats (Bristow and Holmes, 2007). Thus upon glycogen depletion, the production of lactic acid is lowered in the muscle resulting in abnormally high pH values (Mota-Rojas *et al.*, 2012; Vimiso and Muchenje, 2013).

The biological and physiological state of the animal can be measurable using some oxidative stress indicators (Muchenje *et al.*, 2009a; Sentandreu and Sentandreu, 2011; Ekiz *et al.*, 2012b; Mota-Rojas *et al.*, 2012; Falowo *et al.*, 2014). The pain and stress experienced by animals activates the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS) leading to an increased heart rate, blood pressure and stress hormone release (EFSA, 2004; Kataria *et al.*, 2010). A variety of stress indicators are normally used to determine the risk of contraction and the presence of disease (Aggarwal *et al.*, 2013) and they include glucocorticoids, non-esterified fatty acids (NEFA), glucose, cortisol, heat shock proteins and peroxidase. Besides being a response to stressful stimuli, expression of heat shock protein (Hsp) in animals may vary depending on certain physiological conditions such as pregnancy and novelty of the environment (Catalá, 2009). It has been reported that heat shock proteins may have ample effect on meat tenderness due to a rapid use of glycogen reserves during cell stress, resulting in increased pH_u and DFD beef. This therefore reduces the quality of meat (Pulford *et al.*, 2008, 2009; Xing *et al.*, 2014).

Increased pH_u results in tougher and darker meat, which is not always ideal for consumption (dalla Costa *et al.*, 2007). With animal welfare negatively affected, meat quality can be reduced, carcasses downgraded and sometimes condemned, thus leading to increased economic losses (Adzitey, 2011). The cause of downgraded meat begins at most during handling, transportation, loading and off-loading, lairage period and at the stunning box. In addition, slaughtering procedure and improper stunning may expose cattle to pain and distress which can adversely affect meat quality and food safety (Atkinson *et al.*, 2013). Animals' should be rendered unconscious just in a single shot of stunning. However, failure to do so will cause the nanostructure of meat to display quality defects such as bruises, DFD beef, less visible muscle fibres and shorter sarcomeres leading to tougher meat and economic losses (Muchenje *et al.* 2008; American Meat Institute, 2012; Atkinson *et al.*, 2013).

Several studies have been conducted to evaluate the response of animals to the pre-slaughter stress and meat quality of different genotypes from natural pastures (Muchenje *et al.*, 2008, 2009b; Huertas *et al.,* 2010; Miranda-de la Lama *et al.*, 2012). In addition, it has been reported that even the slaughtering procedure causes undesirable defects such as haemorrhages, bruising, broken bones, pale soft exudative (PSE) and dark firm dry (DFD) meat, short shelf life (Atkinson *et al.*, 2013). However, there is paucity of information on distance, lairage duration, and stunning procedure, repeated shots at stunning and animal-related factors on the pre-slaughter stress indicators (heat shock proteins, cortisol and glucose). Therefore, the research will focus on the effects of distance travelled, lairage duration and animal-related factors on biomarkers of stress, carcass characteristics and nano-technological structure of beef.

1.2 Objectives

The main objective was to determine the effects of distance, lairage duration and animal-related factors on pre-slaughter stress indicators, carcass characteristics, nanostructure and technological properties of beef.

Specific objectives of the study were to:

a) Determine the expression of heat shock proteins, cortisol and glucose and the quality of beef in slaughtered bovine species.
b) Determine the activities of stress enzymes in relation to carcass and physico-chemical characteristics of beef from cattle slaughtered under practical conditions.
c) Determine carcass characteristics and physico-chemical attributes of beef from cattle slaughtered under practical conditions.
d) Determine the effect of animal class, genotype and sex on the nanostructure of beef and meat quality.

1.3 Hypothesis

The null hypothesis tested was that there are no effects of distance, lairage duration and animal-related factors on pre-slaughter stress indicators, carcass characteristics, nanostructure and technological properties of beef.

Specific Hypothesis:

a) There is no expression of heat shock proteins, cortisol and glucose and the quality of beef in slaughtered bovine species.
b) There is no relationship between the activities of stress enzymes, carcass and physico-chemical characteristics of beef from cattle slaughtered under practical conditions.
c) There are no comparisons between carcass characteristics and physico-chemical attributes of beef from cattle slaughtered under practical conditions.
d) There are no effects of animal class, genotype and sex on the nanostructure of beef and meat quality.

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Chapter 2 Literature Review

A.Y. Chulayo and Muchenje V

(*Submited to the South African Journal of Animal Science*)

2.1 Introduction

Research on the sustainability of the South African livestock sector (Meissner *et al.*, 2013), impact of animal source products on human nutrition (Schönfeldt *et al.*, 2013), climate change and livestock production (Rust and Rust, 2013), production systems and management practices on the quality of meat products (Webb and Erasmus, 2013) has been conducted. Hemsworth *et al.* (2011), Chulayo *et al*. (2012), Gajana *et al*. (2013), Stockman *et al.* (2013) and Vimiso and Muchenje (2013) highlighted that animals experience problems during the period of transportation which negatively affect animal welfare and meat quality. Delgado (2003) and Hoffman and Cawthorn (2013) carried research that was aimed at finding ways of increasing protein sources that will meet all needs considering the fact that meat is an important constituent in the diet for many consumers.

There is an increased public concern about animal welfare in the production logistic chain. Therefore such concerns require more research attention in order to avoid violations of animal rights (Grandin, 2005; Boissy *et al.,* 2007; Fraser, 2008). Animal production processes include rounding up, kraaling, loading, transportation and off-loading in the lairages at the abattoir. There are increasing concern about use of methods which are pain and stress free when handling animals (Al-Fartosi *et al.*, 2010) in such production processes. For example, it has been noted that during transportation, certain events expose animals to stress in turn compromising their physiological and biochemical processes, meat quality and quantity subsequently resulting in economic losses (Hemsworth *et al.*, 2011). Of particular concern is the fact that, this happens against a background where there are numerous acts, regulations and codes of conduct which are in place to safeguard animals from stress and pain. Prior to slaughter, animals experience undue stress due to a sudden change of the environment which in turn increases the secretion of enzymes and hormones subsequently reducing the quality of meat and its products. In such instances the animal welfare properties are compromised resulting in reduced meat quality and carcass downgrading (Muchenje *et al.*, 2009a; Adzitey, 2011).

The world human population is expected to grow to reach 9 billion by 2050 and these people will ultimately require animal products from associated processes from the farm to the slaughter floor (UN, 2011). Food producers are also mindful and aware of the challenges associated with feeding this ever-increasing population. In-spite of these challenges animal producers are eager to adopt and apply biotechnologies that will improve yields of crops that are utilised by both livestock and humans (Wang *et al.*, 2013) among other things. However, there are animal welfare challenges which continually exist within such systems. Therefore there are growing activist group campaigns that promote reduction in meat consumption and if consumers take heed of such calls there will be a significant environmental impact and ultimately a change in the whole agricultural sector (Capper, 2013).

Schönfeldt *et al.* (2013) indicated that human health and the quality of life can be improved by choosing animal food sources that have a high nutrient content. However, strategies that can be implemented to make food healthier from well-balanced animals require further research. Therefore, the objective of this review is to highlight the effects of pre-slaughter stress on animal welfare and meat quality and strategies that can be employed to improve meat quality and meat products.

2.2 Pre-slaughter stress, animal welfare and its implications on livestock production

2.2.1 Demand for animal-friendly products

Animal welfare interests have been on the rise in recent years because of poor treatment given to animals prior to slaughter. Handling affects the biochemical and physiological processes of an animal resulting in the reduction of meat quality. Animal welfare incorporates behaviour, feeling, ability of an animal to cope in different environmental conditions (whether they are conducive or not) and to control disease, management, and humane slaughter (Al-Fartosi *et al.*, 2010; OIE, 2010). At the farm, animals go through daily, monthly and one-life routines. Some of these routine husbandry practises include weighing, physical health examination, vaccination, dehorning, branding and castrations. These farm management practices are to a certain extent painful and stressful.

Animal welfare involves investigating the relationship between animals and their physical environment as well as to other organisms such as animal-human interaction. However, there have been debates around the term "welfare" because of the different interpretations and moral assessments between cultures, regions, time and individuals (Yeates, 2010; Ohl & van der Staay, 2012; Probst *et al.,* 2012; Zulkifli, 2013). Grandin (2013) also highlighted that there are increasing concerns about the methods that are used to handle and render the animal unconscious before throat cutting (slaughtering). It has therefore been suggested that when defining animal welfare; moral and ethical standards of the society should be considered because of the values that are laid by the society upon their animals (Webb, 2013). Some consumers still complain about the poor management of animals and suggest a need to improve the animal in its environment while also establishing a framework for integrations and uptake within the industries (Einsiedel, 2005; Croney and Millman, 2007; Verbeke, 2009).

In most cases, consumers' views about animal welfare issues are rarely considered in-spite of the fact that production must also be aimed at drawing their purchasing behaviour.

Consumers argue that farmers can claim to be taking care of their animals while they disregard the fact that product quality is the predictor of a welfare-friendly buying power (Croney and Millman, 2007). However, despite consumer concerns, there is still a need to conduct special programmes that will integrate what to consider during shopping and what actually happens at the farm. This is because of the misconception that exists between rearing of farm animals for improvement of meat and pets for companionship (Freedom Food Report, 2007; Verbeke, 2009). Animal welfare has become a thought-provoking topic nowadays because consumers are also interested in complete acquaintaince with the production methods such as where the animal was raised and slaughtered until it reaches the table (Velarde and Dalmau, 2012). Animal welfare is generally associated with producers, retailers and the industry forgetting that consumers are also interested to know where and how the animals has been raised.

The demand for welfare-friendly products has a potential of marketing and communicating activities that can trigger consumer attitudes towards purchasing the product. In addition, consumers' interests are crucial to consider when adopting improvement methods at the farm as this has a direct impact on their purchasing decisions (Troy and Kerry, 2010). This is associated with health, welfare and disease resistant animals (Einsiedel, 2005; King *et al.*, 2006; Freedom Food report, 2014). Therefore, in order to provide an assurance of good animal welfare and healthier foods to all participating supply chains, animal genetics has been selected to breed animals that can even survive well in novel environments (Koknaroglu and Akunal, 2013). Scholtz *et al.* (2013) also suggested that farmers must use genetic modification to breed animals that will adapt to the changing environment, less susceptible to heat stress, occurrence of drought and floods. This will also reduce the competition between humans and species for agricultural land.

2.2.2 Management of human-animal relationships

MacKay *et al.* (2013) indicated that there is a relationship between humans and animals which is closely related to welfare freedoms. Among the animal welfare freedoms, fear has been indicated as one of the important traits for cattle and this trait is known to alter their behavioural response to stimuli. Fear results in stress, decreased health and reduced productivity in farm animals. However by definition, animal welfare is the negative or positive quality of life observed in an animal and it depends on how they feel at a particular time and place (Carenzi and Verga, 2007; Green and Mellor, 2011; Veissier *et al.*, 2012). It is related to avoidance behaviour, animal physiology, cognitive, emotional state and animal biochemistry as they respond to their environmental stimuli (social or physico-chemical) (OIE, 2010; Hemsworth and Coleman, 2011; Wickham *et al.*, 2012). High levels of fear in animals reduce their growth and reproductive performance. It has been reported that, in fearful pigs, cortisol concentration is produced in higher levels as a result of longer hours of transportation with shorter lairage duration (MacKay *et al.*, 2013; Jama, 2014). In cows, it was observed that fear of humans resulted in reduced milk yield; protein and fat content (Breuer *et al.,* 2000).

Poor animal handling has been identified to have a substantial effect on the behaviour, physiology and the productivity of commercial farm animals (Williams *et al.,* 2012). Due to poor transportation which leads to physical stress in animals, even in a novel environment such as abattoir lairages, they become afraid to drink water. In a novel environment, fear results in economic losses by reducing carcass weight, increased bruises and high ultimate pH (pH_u), dark cutting beef (DFD) which are not conducive for packaging. Therefore, management practices during herding, regrouping, loading and transportation are crucial in order to reduce stress and fear response so as to improve animal and meat production (Werner *et al.*, 2013).

2.2.3 Welfare of animals during transportation

Transportation is an inevitable husbandry practise that is encountered by animals hence it becomes stressful to them (Nwe *et al.,* 1996; von Holleben, 2009; OIE, 2010). This is because of the processes and activities involved such as creation of stress and at times death, spread of diseases, bruise occurrence, high stocking density, noise, vibration, lack of exercise, prolonged standing and environmental temperature and humidity and feed deprivation (Vimiso and Muchenje, 2013). Long hours of transportation cause changes in the behaviour of animals, circulatory system and immune function which negatively affects the welfare of animals and meat quality. On the other hand, the genetic make-up of the animal regulates any handling stress especially when it comes to animal temperament (dalla Costa *et al.,* 2007; von Holleben *et al.,* 2010; Browning and Leite-Browning, 2013). Research in poultry indicated that the stress responses are considered to be essentially adaptive or protective because they are expected to minimise the detrimental effects of the stressor.

Animals are exposed to pain during the pre-slaughter environment especially under hot weather. The mortality of animals due to heat stress increases (von Keyserlingk *et al.,* 2009; Melesse *et al.,* 2011). In addition, heat stress has an impact on the metabolism and performance of animals because they become too sensitive to heat especially if there is no air circulation during transportation (dalla Costa *et al.,* 2007; Koknaroglu and Akunal, 2013;

Zeferino *et al.,* 2013). Stress is a physiological disturbance which is linked to mental states associated with threatening or harmful and painful situations (von Holleben *et al.,* 2010; Zulkifli, 2013). As environmental temperature rises beyond the animal's body temperature, the rate at which heat is dissipated by the animal decreases, causing a rapid increase in the respiratory rate of the animal (Caulfield *et al.,* 2014). Heat is initially dissipated primarily by passive mechanisms which include radiation and convection. This scenario is prevalent in live animals whereby metabolic heat and heat from the environment increases the animal's body temperature. Heat stress is an indication of poor animal welfare and it results in increases in chances of mortality.

Heat stress results in increased restlessness, sweating, respiration and heart rate, rectal temperature and a decrease in the reproductive traits in animals. This leads to a rapid reduction of glycogen levels required for *post-mortem* conversion of muscles to meat, and dark firm dry (DFD) beef meat develops. In cattle (pH > 5.9) and in poultry and pork, a rapid fall of pH (pH < 5.3) results in pale soft and exudative (PSE) meat (Young *et al.,* 2004; Gregory, 2010; Gajana *et al.,* 2013). Elevated environmental temperatures affect poultry performance, physiology and their immune system (Star *et al.,* 2008; Melesse *et al.,* 2011), reduced feed intake, slaughter weight, myoglobin content and meat sensory properties in rabbits (Zeferino *et al.,* 2013). Pale Soft and Exudative (PSE) pork and increased bruising score is associated with cold ambient temperatures (dalla Costa *et al.,* 2007). There are few reports on the effect of heat stress in sheep and cattle although Gregory (2010) indicated that occurrence of DoA (Dead on Arrival) cattle and some incidences of DFD beef were observed.

Animals show some signs of avoidance behaviour which leads to delayed movement (Mota-Rojas *et al.,* 2012; Strappini *et al.,* 2012). Marenčić *et al.* (2009) reported that when bulls are in the same lairage there is undue stress and detrimental effects to the quality of the beef. Animals squash each other, fall or fight while others will need a prod in order to move them forward. These may lead to the occurrence of bruises that negatively affect the quality and quantity of meat. Bruises are an indication of welfare problems during the road transport (Miranda-de la Lama *et al.,* 2012). Bruised tissues may store historical information about the harmful situations that the animal underwent prior to slaughter. The occurrences of bruises have been found to have a negative impact on both animal welfare and carcass characteristics and as well as the quality of beef (dalla Costa *et al.,* 2007; Strappini *et al.,* 2012). It therefore becomes imperative to reduce stress effects in slaughter animals, mixing of different species and sexes especially during transportation and at the lairage because of its adverse effects on the animal and its products.

2.3 Biochemical effects of pre-slaughter stress

2.3.1 Homoeostatic imbalance associated with oxidative damage

Livestock animals experience both extrinsic and intrinsic stress that leads to homeostatic imbalance. This is because animals undergo an intense selection for specific traits which have some negative implication on their behaviour, physiology and immunological state (Adenkola and Ayo, 2010). On the other hand, animals become more sensitive when they are exposed to novel environmental conditions (Kristensen *et al.*, 2004). The balance of homeostasis is maintained by the operation of several overlapping and complex antioxidant systems which is a contributory factor of animals to disease susceptibility (Caron *et al.,* 2000). Animals exposed to pathogens that reduce immunity have been observed to be determined by the intraction of the animal and its environment. Under those environmental changes, the homeostatic processes are regulated by reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Falowo *et al.*, 2014). The reaction of ROS such as superoxide anion (O_2^-) and hydroxyl radical (OH) damage cellular proteins, lipids, carbohydrates, nucleotides and fats that leads to tissue damage which leads to a large number of diseases (Qwele *et al.*, 2013; Yang *et al*., 2013; Zulkifli, 2013). The ROS can also initiate lipid peroxidation and DNA damage leading to mutagenesis and cell death (Bulut *et al.,* 2013; Durgut *et al.*, 2013; Yang *et al.,* 2013). Reactive oxygen species are chemically reactive molecules that contain oxygen which are important in cell signalling and homeostasis especially when an animal experiences environmental stress (Panday and Rizvi, 2010; Falowo *et al.,* 2014). When an animal is stressed by an unusual environment, these ROS increase leading to oxidative stress. Physiological stress and distress is mostly associated with higher levels of oxidative damage (Aschbacher *et al.,* 2013).

2.3.2 Heat shock proteins

Stress experienced by animals in the novel environment results in the synthesis of proteins which are called heat shock proteins. In a heat shocked cell, the proteins begin to unfold and denature resulting in production of heat shock proteins. During this process, heat shock proteins (Hsp`s) may bind to heat-sensitive proteins and protect them from degradation (Al-Aqil and Zulkifli, 2009). Exposure of both prokaryotic and eukaryotic cells to unfavourable conditions increases the synthesis of stress proteins. According to Ellen *et al.* (2002), under normal growth, heat shock proteins maintain homeostasis by regulating the folding quality control of proteins. Heat shock proteins are a subgroup of molecular chaperones which are classified into five families. The name ‘chaperone’ is normally used to highlight the functions of these proteins. It includes both stressed and non-stressed proteins which accompany unfolded polypeptides (Kopeček *et al.,* 2001).

Chaperones have an essential role in assisting the folding, assembly and transportation of newly synthesized polypeptides and in measuring the conformational status of pre-existent proteins (Ellen *et al.,* 2002; Calloni *et al.,* 2012). They prevent the irreversible aggregation of polypeptides with other proteins that are found in the cell. Chaperones are found at various stages in protein biogenesis by regulating their structure and function under normal physiological conditions, as well as during stress resulting in protein unfolding and misfolding (Morimoto, 2008; Shahein *et al.*, 2010; Verghese *et al.*, 2012). Under stressful conditions, heat-shock proteins prevent inappropriate inter and intramolecular interactions, which may lead to protein misfolding or aggregation. It is important to bear in mind that the Hsp70 in prokaryotes are expressed in 3 forms; Dnak, HscA and HscC. However, the DnaK (major bacterial Hsp70) is one of the most abundant constitutively expressed and stress inducible chaperones in the *Escherichia coli* cytosol. However, under non-stress conditions such as intermediate temperatures, it is not indispensable (Schrönder *et al.,* 1993; Sharma and Masison, 2009; Calloni *et al.,* 2012).

The molecular chaperones such as Hsp90, Hsp70 and Hsp23 regulate the steroid hormone aporeceptors and kinase activities. They also function with other protein folding activities that include protein disulphide isomerases and peptidylprolyl isomerases (immunophilins). The classification depends on their molecular weight such as Hsp100, Hsp90, Hsp70, Hsp60 and as well as the family of small Hsp's (Kristensen *et al.,* 2004; Calloni *et al.,* 2012). They are associated with signalling molecules and steroid receptors through the establishment of Hsp70 and Hsp90 (Ellen *et al.,* 2002). They function as molecular chaperones as they assist in refolding, protecting cellular systems against damage, solubilising aggregates, sequestering overloaded and damaged proteins into larger aggregates and also targeting damaged proteins into the degradation machinery (Schrönder *et al.,* 1993; Aggarwal *et al.,* 2013).

2.3.3 Catecholamines, cortisol and glucocorticoids

Certain stress hormones such as epinephrine, norepinephrine and dopamine (catecholamines) are related to blood splash (Foury *et al.,* 2005; Fernstrom and Fernstrom, 2007). Catecholamines are neurotransmitters and hormones that can cause weight loss through a number of mechanisms such as lipolysis, secretion of insulin inhibition, increased blood glucose and thermogenesis. They have a core structure of catechol and an amine group which include dopamine (DA), norepinephrine (NE) and epinephrine (EPI) (Fernstrom and Fernstrom, 2007; Goldstein *et al.,* 2009). They are found in the central nervous system (CNS), peripheral nervous systems (PNS) and adrenal glands also release and inactivate the organisation and functioning of dopaminergic and noradrenergic systems (Muchenje *et al.,* 2009a). Catecholamines are associated with an increase in the activity of tyrosine hydroxylase (TH). They are synthesised from the adrenal medullary cells that are derived from the embryonic neural crest of the kidneys (Yanagihara *et al.,* 2005).

Tyrosine hydroxylase is regulated at times by either allosteric mechanism or by enzyme induction (Candace *et al.,* 1977). The medullary cells have been found to provide a convenient model for studying the inhibition of TH in the neurons like that of the sympathetic neurons (Blanchard *et al.,* 2001). It has been reported that the function of the transported norepinephrine in the adrenal medullary cells is inhibited by estrogenic pollutants (Alyea and Watson, 2009). The rate limiting enzyme in catecholamine synthesis which is tyrosine hydroxylase (TH) indicates a selective increase in TH mRNA (mitochondria Ribomsomal Nuclea Atom). This is normally accompanied by a correspondence response in immune reactive TH protein due to different stressors in the nearby environment (Blanchard *et al.,* 2001; Sobek *et al.,* 2013; Janska and Kwasniak, 2014).

Catecholamines, cytokines, energy metabolism and leukocytes including cortisol are useful components of the metabolic and immune function. Any changes in the circulating of these components may be a good indicator of stress (Browning and Leite-Browning, 2013). The release of catecholamines is activated through the direct pathway where neurons terminate on target organs in response to fear. Through an indirect pathway in the adrenal medullary axis, norepinephrine and epinephrine are released into the blood stream. They stimulate respiration; promote the activity of enzymes that break down glycogen in the liver and muscles causing glycogenesis thereby increasing glucose concentrations in the bloodstream (Morale *et al.,* 2001; Baker *et al.,* 2013). In the muscles, glycogen levels are depleted as a result of availability of epinephrine.

Epinephrine has been recorded to help in the breaking down of glycogen into glucose which is converted into energy for the muscles in the presence of oxygen. High amounts of epinephrine in the muscles can cause muscle tremors which is also associated with flight response and the element will lose control. In most cases, release of these hormones into the blood stream is determined by the type of stressor such as food deprivation, dehydration, physical exertion and fear / arousal. When an animal has exhausted its energy levels due to rough handling prior to slaughter, glucose is converted to energy resulting in lactic acid and water which later increases the pH_u of meat (O'Neill *el at.,* 2006). The actions of epinephrine do not facilitate the stress response alone but cortisol is also at play.

Blood and cortisol concentration are produced in higher levels as a result of the stimulation of hypothalamo-pituitary-adrenal (HPA) axis (Figure 2.1). An active response to stress is said to be associated with the activation of the adrenal medulla and the sympathetic nervous system (von Holleben *et al.,* 2010).

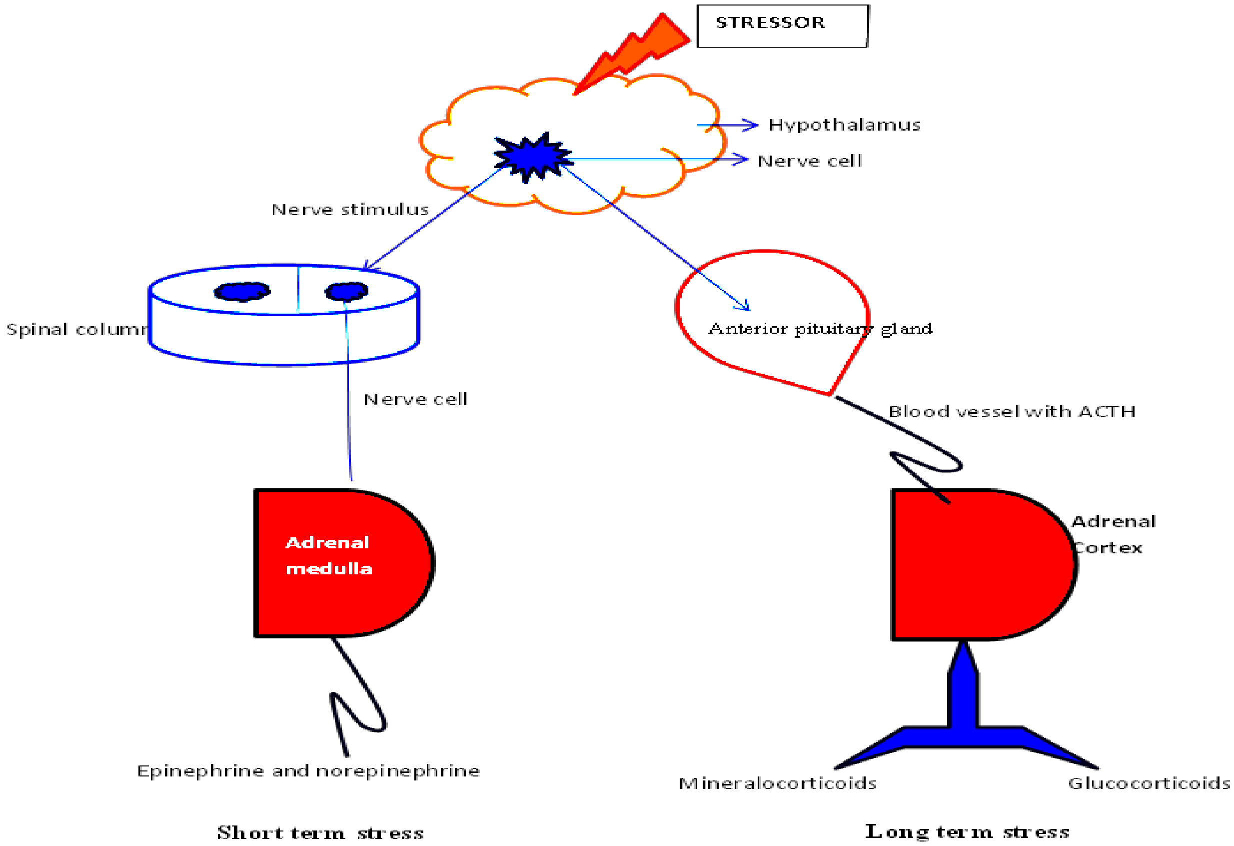

Figure 2.1 A schematic representations of the kinds of reactions displayed by the hypothalamus, the internal (adrenal medulla) and external (adrenal cortex) of the adrenal gland at moments of stress.

ACTH – Adrenocorticotropic hormone

The passive response is linked to the stimulation of the pituitary-adreno-cortical system (Nwe *et al.,* 1996; Foury *et al.,* 2005; Baker *et al.,* 2013). The biochemical synthesis of catecholamines is directly modified by the brain concentrations of amino acids such as tryptophan (5HT), phenylalanine (Phe) and tyrosine (Tyr) only if they are available in the blood. They are firstly hydrolysed to dihydroxyphenylalanine (DOPA) by the catalysitic enzyme; TH. This DOPA is immediately decarboxylated to DA by the action of aromatic L-amino acid decarboxylase (Figure 2.2). The neurons that use DA as a transmitter inhibit the modification of enzymes. However, neurons that use NE contain an additional enzyme (DA-β-hydroxylase) which converts DA to NE and it is further converted to EPI by neurons that contain phenylethanolamine-*N*-methyl transferase (Candace *et al.,* 1977; Fernstrom, 2000; Fernstrom and Fernstrom, 2007).

Cortisol is a primary blood constituent that is normally used to quantify stress in animals (Browning and Leite-Browning, 2013; Jama, 2014). It is a major component of stress response in conjunction with catecholamines which can modulate immune functions. Stressful conditions lead to elevated plasma cortisol concentrations which may also be related to pale, soft and exudative (PSE) meat especially in pigs (Chai *et al.,* 2010). In calves, temperament and excitability was assessed and it was reported that cortisol and catecholamines are produced when calves are experiencing acute stress during weaning (Burdick *et al.*, 2009; O'Neill *et al.* 2009).

In humans, cortisol levels were reported to have increased due to related abnormal behaviour (Haller *et al.,* 2005) whereas in animals, it is related to aggressive and social behaviour (Reisnet *et al.,* 2007). The glucocorticol hormone shows an important mechanism that links chronic stress with accelerated aging.

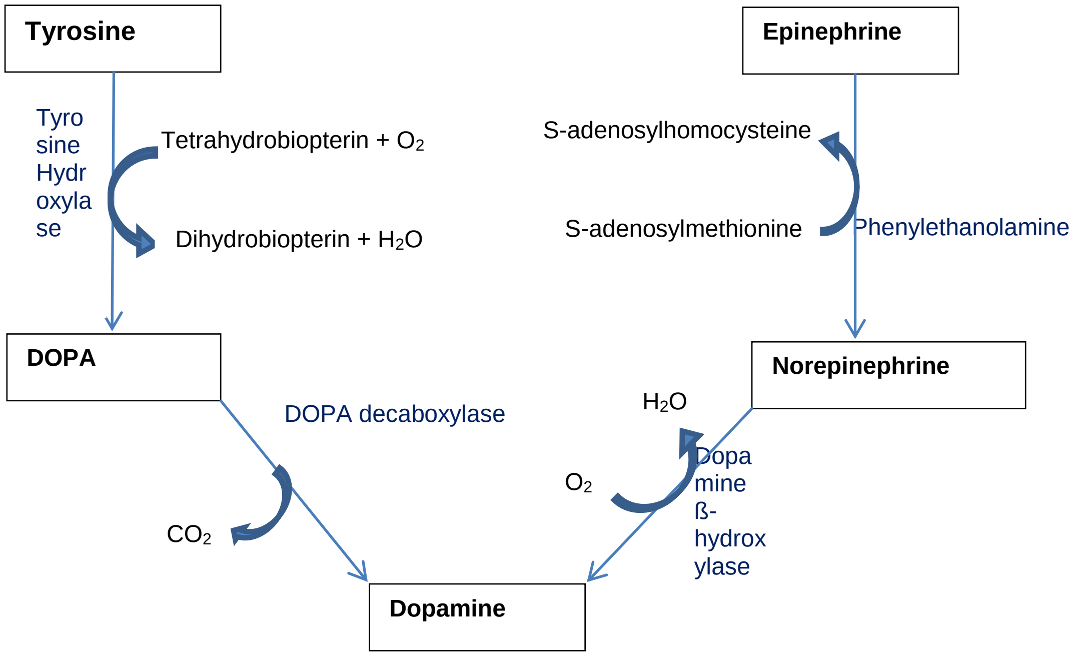

Figure 2.2 The biochemical synthesis of catecholamines by brain amino acids

Adapted from Candace *et al.* (1977).

Glucocorticoids are the effectors of the hypothalamic-pituitary-adrenal (HPA) during stress. They also modulate the immune response through gene expression, transcription, translation, post-translational processing, protein secretion and cell progenitor proliferation and differentiation (O'Connor *et al.,* 2000). During aging, cortisol is said to be reduced when observed by a human eye although there are increased levels when detected microscopically or electronically (Aschbacher *et al.,* 2013).

A measurement of plasma cortisol is normally used to study stress reaction in animals exposed to novel environments (Trevisi *et al.,* 2005; Forslund *et al.,* 2010). However, the exploitation of cortisol as an indicator of stress may require caution. This is because cortisol levels may change due to several factors such as type and severity of stressors, circadian rhythms, blood sampling method and as well as non-aversive events (Trevisi *et al.,* 2005; Higashiyama *et al.,* 2005; Watts *et al.,* 2012; Grandin, 2013; Seshoka *et al.,* 2013). Tadich *et al.* (2009) reported an increased cortisol concentration in lambs after transportation as a result of unloading, handling and bleeding procedure, more than to transportaion itself although the measurements were taken at intervals of 30 minutes. Therefore, the interpretation of cortisol levels remains a puzzling issue especially in slaughter cattle.

2.4 Non-invasive assessment methods of stress

Stress and its biological mediators are increasingly recognised as important factors that influence the rate of biological aging in humans (Aschbacher *et al.,* 2013). However, in animals, stress indicators are released due to aversive handling and stunning methods used prior to slaughter. These stress indicators are normally used to determine the effect of poor welfare in animal production. Animal welfare has to do with the strong absence of negative feelings (suffering) and presence of positive feelings (pleasure). Aversive handling such as electric prod (goad), missed stuns, slipping in the stunning box and excessive pressure from the restraining device results in 99% of all vocalisations in cattle which is an indication of physiological stress (Grandin 2001; 2013). Abattoir managers are increasingly monitoring the workers in order to improve animal handling and reduce the sources of stress. However, there is a need to observe the behaviour of animals and or by collecting saliva, blood and urine samples to determine the hormonal-enzymatic and heat shock protein expression (O'Connor *et al.,* 2000; Ndou *et al.,* 2011; Seshoka *et al.,* 2013) in order to examine whether the precautionary measures are efficient. There are different methods that were established and used to assess the welfare of animals using non-invasive assessment methods (Boissy *et al.,* 2007; Geiger, 2013). These methods are said to gauge their body language, interpret health, depression, diseases, abnormality and welfare distinctively. The assessment methods that have been used so far include physiological and biochemical methods being applied either by direct animal-based and indirect animal based alterations (Duncan, 2005; Sejian *et al.,* 2011).

To measure the physiological aspect of an animal, its behaviour is observed at a distance to provide information of any impaired function such as fear and distress, hunger and thirst, injury and temperament, high parasite loads, lack of sophisticated machinery in drought power animals (Moyo and Masika, 2009; Bourguet *et al.,* 2011; Ndou *et al.,* 2011; Martins *et al.,* 2012; Minka and Ayo, 2013). Poor environmental conditions may lead to stress due to pain, fear, discomfort, thirst and hunger resulting in abnormal avoidance behaviours (Dodzi and Muchenje, 2011). Biochemical assessment for physiological response to stress entails the estimation of enzymes, hormones, glycolytic potential, proteins, lipid peroxidation, oxidative stress and tissue damage (Khan *et al.,* 2013; Piccione *et al.,* 2013; Choudhary and Devi, 2014; Falowo *et al.,* 2014).

Muchenje *et al.* (2009a) reported that, in cattle raised on natural pastures, the concentration of hormones in response to stress may not be similar due to breed differences. Fearfulness is a personal trait that drives animal behaviour and is mostly repeatable and consistent across time and situations (Njisane and Muchenje, 2013a). This is to a certain extent as a result of a novel arena and a novel object which can be expressions of neophobia. Neophobia is expressed by hesitation, avoidance or caution which therefore indicates the animal's internal state of risk as well as the degree of boldness (fear of novelty) (Mettler and Shivik, 2007; MacKay *et al.*, 2013).

According to Mintline *et al.* (2013), calves normally exhibit play behaviour when they are satisfied. As shown in Table 2.1, animals display different behaviours during transportation and at the abattoir depending on the type of stimuli. The assessment of animal behaviour is still a highly debated issue in domestic animals as is the question of behavioural indicators in response to fear or pain during pre-slaughter (Boissy *et al.*, 2005; MacKay *et al.*, 2013). In wintering birds, a form of aggressive behaviour was shown due to food conflicts in hungry individuals (Lange and Leimar, 2001). This could simply mean that there are different behaviours that animals exhibit to express their feelings (anxiety, temperament, aggressiveness, excitement and boldness). However, fear is the key to the behaviours shown by animals in certain instances and during cattle handling by humans (Harris and Knowlton, 2001; Sant'Anna and Paranhos da Costa, 2013). Discomfort experienced by animals during lairage period and the movement of animals to the point of stunning prior to death may lead to avoidance behaviours (Grandin, 2006; von Keyserlingk *et al.*, 2009; Njisane and Muchenje, 2013a and b).

Table 2.1 Different avoidance behaviours shown by livestock prior to slaughter

Species	Pre-slaughter stress	Behaviour	Positive or negative	References
Birds	Hunger	Aggressive	Negative	Lange and Leimar (2001)
Calves	Touching	Play	Positive	Cloutier *et al.* (2012); Mintline *et al.* (2013)
Pigs	Transportation and at the lairages	Fear	Negative	Hemsworth *et al.* (1996); Ali *et al.* (2006); Hemsworth *et al.* (2011); Murphy *et al.* (2014)
All species	Lairages, stunning box	Stress	Negative	Reefman *et al.* (2009); Hultgren *et al.* (2014); Njisane and Muchenje (2013)
Cattle	Handling	Fear	Negative	Breuer *et al.* (2000); Harris and Knowlton (2001); von Keyserlingk *et al.* (2009); Hultgren *et al.* (2014); Sant'Anna and Paranhos da Costa (2013)
Cattle	Handling and equipment problems	Vocalisation	Negative	Grandin (2001); (2013); Dodzi and Muchenje (2011)
Cattle	Gentle touching	Reduces fear	Positive	von Holleben *et al.* (2010); Voslářová *et al.* (2010); Ohl and van der Stay (2012); Probst *et al.* (2012)
Cattle, sheep and pigs	Housing, voyages	Reduces heat loss, mortality	Negative	Caulfield *et al.* (2014)
Dairy	Claw trimmers	Lameness	Negative	Becker *et al.* (2014)

The main causes associated with animal welfare problems at slaughter are categorised into five (Wilkins *et al.*, 2005; Grandin, 2013);

a. poor condition of the animal,

b. lack of employee training in principles of animal behaviour and methods of humane handling,

c. physical distractions that impede movement of animals as they are handled,

d. stressful equipment methods and

e. poor maintenance of equipment (captive bolt for stunning and slippery flooring).

A wide behavioural variability has been shown by animals of the same species; same genotype and those that are reared under the same conditions (Miranda-de la Lama *et al.*, 2011). The behavioural observations have been used only in a general way without quantifications such as signs of distress (jumping, bellowing, trembling, moving backwards and frequent urination).
It has been reported that animals waiting for slaughter can be stressed by restraint, handling and novelty of the pre-slaughter environment (i.e. abattoir set-up), adverse weather conditions, hunger, thirst and fatigue (Muchenje *et al.*, 2009a; Bourguet *et al.*, 2011; Njisane and Muchenje, 20013).

A combination of both behavioural and physiological measures has been used to assess animal welfare (Broom, 2000; Ndou *et al.*, 2011). Some studies have shown that cattle and sheep react to stressful settings (e.g. at the abattoir) with increased concentrations of catecholamines and creatine kinase activity (Vojtic, 2000; von Holleben *et al.*, 2009; Muchenje *et al.*, 2009a). Release of these hormones and enzymes has an effect on the meat quality due to rapid glycolysis and increased lactate production, resulting in elevated blood lactate (Lewis *et al.,* 2006). Stress has been found to cause changes in the immune system because of physiological changes that occur (Ekiz *et al.,* 2012). These changes include increased heart rate, respiratory rate and temperature. Stress in pigs activates the hypothalamic pituitary-adrenal axis and releases glucocorticoids into the blood stream (O'Connor *et al.*, 2000; Lewis *et al.*, 2006).

Cattle that are excited have increased stress responsiveness to handling and produce more cortisol concentrations than calmer animals. This is because cattle with excitable temperament are less easy to handle when exposed to novel surroundings (Boissy *et al.,* 2005). In general, an increase in physiological stress or physical activity in farm animals during pre-slaughter handling leads to depletion of muscle glycogen reserves before slaughter. This has an ample effect on pH_u, water holding capacity (WHC), dark cutting beef and reduced tenderness of meat (Muchenje *et al.,* 2009b; Ekiz *et al.,* 2012). However, if animals get accustomed to humans at an early stage in life fear towards humans and stress-related behaviours at the abattoir are reduced (Probst *et al.,* 2013).

2.5 Nanotechnology and beef quality

2.5.1 Nanostructures of meat

Nanotechnology makes use of the self-assembling of molecules of structures of protein into lower and higher concentrations (Busseron *et al.*, 2013). The proteins in meat are to be structured in order to produce an attracting appearance, aroma and taste. The challenge lies in the translation of concepts from the supramolecular to colloidal, microscopic and up to the level that the consumer can directly perceive, touch and consume the product (Jansson-Boyd, 2011;

Mandal *et al.*, 2014). However, the complex structure of meat makes it unique such that any alterations prior to consumption lead to less visible muscle structure. The precise position of the arrangements of muscle fibres, bundles and fascular-like structure are determined by making use of nanotechnology. The adoption of nanotechnology leads to a new era, making it possible to control the physical interactions between a cell and its surroundings. Understanding the mechanics of a cell is critical because it plays a role in organising the functioning ability of a cell for the improvement of meat (Addae-Mensah and Wikswo, 2008; Han and Sniadecki, 2011)

Muscle characteristics determine the quality of beef. This is because, fibres are the essential units of all muscles; they are long, narrow, multinucleated cells (Dubost *et al.*, 2013). The cells may stretch from one end of the muscle until they attain a length of 34 cm even though they have a diameter of 10-100 µm (Fukuda *et al.*, 2008; Han and Sniadecki, 2011). However, the diameter varies between muscles, species, genotypes and sexes, plane of nutrition, age and pre-slaughter stress. The small parallel myofibrils where muscle fibres develop may be similar irrespective of their size or development (Khoshoii *et al.*, 2013). Under the highest magnification of electron microscopy, the varying size and shape of individual fibres is evident viewing even the smaller units called myofibrils, Z-lines, A-band and the H-zone. Between two adjacent Z-lines, there is a sarcomere which is the functional unit of a myofibril. The myofibril consists of thin (actin) and thick (myosin) filaments that are arranged parallel to each other (Huxley, 1985; Tonino *et al.*, 2010). Observations of these sarcomere lengths revealed that there are so-called “gaps filaments” that appears when the muscle is over-strength. However, the gap filaments may have a significant effect on meat quality due to its elastin protein; which is present throughout the sarcomere of skeletal muscle. The major protein of the gap filaments is titin with a molecular weight of 2.8 mDa. Titin; being the first myofibrilar protein to develop in the skeletal muscle, has a length of about 7.8 µm which is approximately to the maximum extension of beef muscles (Fukuda *et al.*, 2008). The other proteins to develop which regulate the contractile machinery of the myofibril are myosin, actin and nebulin. As the myofibril develops further, these proteins may also promote the extracellular and intracellular cell adhesion (Huxley, 1985; Lodish *et al.*, 2000; McElhinny *et al.*, 2005; Tonino *et al.*, 2010). However, during muscle fatigue, there is a rapid increase in the size of muscle fibres, increase in the diameter by elaboration of new myofibrils and sarcoplasm leading to tougher meat (Albrecht *et al.* 2013; Dubost *et al.*, 2013).

2.5.2 Structural changes in meat

Meat quality is a term used by consumers, retailers, abattoir managers, farmers and the industry at large. This is because the end product of any type of animal production is meat (Northen, 2011; Gebresenbet *et al.*, 2011). However, animals may not produce the same levels of carcass and intramuscular fat, colour, pH and tenderness due to the type of genotype, age, sex, type of production systems, pre-slaughter conditions and *post-mortem* changes during meat processing and storage (Muchenje *et al.,* 2008; Sentandreu and Sentandreu, 2011). The slaughter environments and processing of meat have an impact on the quality of meat. Meat with increased pH_u results in tougher and darker meat which is not always ideal for human consumption (dalla Costa *et al.,* 2007; Dubost *et al.*, 2013). These attributes are also as a result of the occurrence of bruises before animal slaughter and during slaughter. Therefore, it is important to understand the effects of pre-slaughter stress and avoidance behaviour of cattle and how they relate to beef quality. Meat is expected to be safe in terms of its composition and appearance with more flavour (Hoffman *et al.,* 2007).

Meat has a valuable contribution to the diet of humans as it provides concentrated proteins, iron, zinc and vitamins A and B. The reduction of quality of meat and meat products are affected such that discolouration in meat colour, flavour, nutritional value and safety occurs (FAO, 2014b). The consumer demands for meat quality are determined by the palatability, appearance, ease of preparation and mastication (Tschirhart-Hoelscher *et al.,* 2006; Mohrhauser *et al.,* 2014). Meat quality attributes that are normally used by consumers to judge and when purchasing meat include colour, juiciness and tenderness. A desirable colour or appearance is one expected to be uniform throughout the entire cut (Muchenje *et al.*, 2009b; Peña *et al*., 2009; Mapiye *et al.,* 2010). The critical consumer quality requirements that are altered while processing and preparing meat include muscle fibre, connective tissue, tenderness, juiciness, colour and flavour. The quantity of collagen in a muscle determines the amount of connective tissues and tenderness. In addition, intramuscular lipid content plays an important role concerning flavour and indirectly juiciness and tenderness. The percentage marbling of meat also impacts on its juiciness, flavour and tenderness (Hoffman *et al.,* 2007; Dubost *et al.,* 2013).

Table 2.2 summarises some of the structural changes that occur in meat as a result of animal related- factors (Age, genotype, class and sex), *ante*- and *post-mortem* period. Animals that are stressed prior to slaughter have a rapid release of glycogen into the blood stream. This in turn becomes broken down into lactic acid especially in warm carcasses. Lactic acid accumulation damages the structure of the muscle leading to loss of water, pale soft and exudative (PSE) meat (Grandin, 2000; Young *et al*., 2004). One of the quality parameters of raw meat is its ability to retain moisture.

Table 2.2 Some structural changes that occur in meat during *pre-* and *post-mortem* treatment

Treatment	**Response variable**	**Changes**	**References**
Animal-related factors (Age, gender, genotype, slaughter weight)	Fatty acids, pH, colour, cooking loss	↑	Yang *et al.* (2002); Dhanda *et al.* (2003); Simela *et al.* (2004); Peña *et al.* (2009)
Pre-slaughter handling (farm, transportation, truck size, stocking density, lairages)	Intensified physical activity, bruises, pH, DFD beef, PSE pork non- esterified fatty acids (NEFA),	↑	O'Neill *et al.* (2006); dalla Costa *et al.* (2007); von Holleben *et al* (2009); Marenčić *et al.* (2009); Strappini *et al.* (2012); Gajana *et al.* (2013); Koknaroglu and Akunal, (2013)
Post-mortem (Storage, Cooking method, Salting and curing)	Cholesterol and saturated fat, tenderness, flavour	↓	Tateo *et al.* (2008); Muchenje *et al.* (2009c)
	Protein content, metmyoglobin content	↑	Ahhmed *et al.* (2013); Dubost *et al.* (2013)

DFD – Dark Firm and Dry
PSE – Pale Soft and Exudative

The muscle stores the majority of its water in the sarcolemma which is reduced due to several factors during meat handling and processing (Huff-Lonergan and Lonergan, 2005).

The amount of collagen content and the stability of cross-ridges increase as the animal advances in age causing reduced tenderness (Petracci and Cavani, 2012). Collagen is a protein that contains a large amount of proline and hydroxproline which are directly related to the thermal stability of the triple helix. The triple helix structure is important for the eating quality of meat. The difficulty in breaking down collagen is due to the formation of nonreducible links that are formed as a result of three or more chains (3-dimensional network) (Saha *et al.*, 2013). Short fibres, affects the sarcomere length hence meat becomes tough. Tougher meat is observed in dark firm dry (DFD) meat especially in animals that were starved for too long.

During starvation, there is a rapid use of glycogen in the muscle such that lactic acid production is reduced leading to (DFD) meat instead of red firm and non-exudative (RFN) meat (Gajana *et al.*, 2013). Dark firm dry meat has a reduced shelf-life because low acidity in meat favours bacterial growth. Both PSE and DFD meat are difficult to process due to low water binding capacity and an increase in rigor mortis. Rigor mortis is as a result of contraction in muscle fibres because of the cross ridges that form between thick and thin filaments. Tougher meat resulting from older animals can be resolved by cooking meat for long under low temperatures to hydrolyse the connective tissue even though some nutrients might be affected. Tenderness is also one of the most important meat quality characteristics that influence consumers' perceptions.

It is considered to be a complex multi-factorial trait that has been described according to actomyosin effect of myofibrillar proteins, background effect of connective tissue and the bulk density or lubricating effect of fat (Hugo *et al.*, 2009; Naveena *et al.*, 2011; Joo *et al.*, 2013). This is because as the animal advances in age, the collagen in connective tissues becomes more complex and stronger.

Several factors that include enzymes, diet, animal-related factors (age, genotype and sex), transportation and the amount of fat cover available on the muscle influences the colour of meat. It has been indicated that colour is related to the level of the protein and myoglobin pigment present in the muscle (Muchenje *et al.*, 2009b; Frylinck *et al.*, 2013). There is now considerable emphasis on modification of the fatty acid composition of animal tissues in order to produce “healthier” foods. Food is termed healthy if it can be digested immediately after it has been consumed. However, in some, there are changes that occur in the meat processing which alters the quality. During cooking, meat shrinks and some denaturing of protein in fibres occurs which leads to hardened tissues. This then reduces the intake of protein as some of it becomes undigested when cooked at high temperatures (Kandeepan *et al.*, 2013; Kaur *et al.*, 2014). The water soluble compounds (amino acids, peptides and glycoproteins), lipids (polyunsaturated fatty acids of phospholipids) and maillard reactions (heterocyclic compounds) are at play during cooking to improve flavour (Dubost *et al.*, 2013; Zuckerman *et al.*, 2013). However, due to the formation of lipid oxidation with carbonyl compounds, abnormal flavour is produced.

Oxidation is one of the major causes of quality deterioration in meat due to high concentration of unsaturated lipids, heme pigments, metal catalysts and a wide range of oxidizing agents in the muscle (Falowo *et al.,* 2014). It is associated with an increase in the production of oxidising agents and or decrease in the effectiveness of antioxidant defences such as glutathione (Lykkesfeldt and Svendsen, 2007; Zhong and Zhou, 2013). Ahhmed *et al.* (2013) also indicated that due to the series of processes and treatments of meat, the muscle structure and proteins undergo various physico-chemical changes. Proteins and fats are hydrolysed due to the presence of endogenous enzymes which alter flavour of meat. Lipids and proteins are susceptible to oxidative damages as a result of rapid depletion of endogenous antioxidants after slaughter. Oxidative stress is explained as the imbalance between oxidant and antioxidant levels in an animal. Therefore, lipid peroxidation has been suggested as a reliable marker or mechanism to evaluate the severity of oxidative stress which is cellular injury (Taysi *et al.,* 2006; Guo *et al.*, 2013).

2.6 Opportunities for animal improvement

The welfare of animals begins at the farm, during transportation and at the abattoir in order to bring about stress free meat from animals. It is said that productivity can be improved through advances in different facets of production systems from conception to consumption through employment of new technologies (Webb, 2013). Zulkifli (2013) highlighted that proper selection and training of farm personnel can reduce fear of humans, reduces avoidance behaviour, enhance health of animals and also improve productivity. Improving animal welfare could mean the assessment of animal-based measures (Boissy *et al.,* 2007) which is a way forward in the perfection of animal welfare, quality of life and good quality meat that is expected by consumers. The use of production systems for good and hygienic farming practices provides optimal animal welfare and increase resistance of animals to infections especially pigs. In addition, mitigating the old developments performed in the field and existing knowledge to come-up with new methods of evaluating welfare will appropriate tools for good animal welfare (EFSA, 2007; Sejian *et al.,* 2011). One of the problems that require a thoughtful and solemn intervention in farm animals is lameness; especially in dairy production systems. This is because; lameness affects animal welfare, reduces productivity and also affects the quality of the product after slaughter. Examining lameness in farm animals require farmers, claw trimmers, scientists, universities, veterinary technicians and veterinarians to collaborate in order to adequately manage and get rid of causative agents of pain (Becker *et al.,* 2014).

Miranda-de la Lama (2013) indicated that the farmers need to manage weight, number of animals that are suitable for transportation and also work together with the hauler in order to bring about live and fit animals for slaughter. To improve this scenario, farmers together with the industry may need to apply biotechnologies and genetic selection to assist in the alleviation of hunger and poverty by breeding animals and crops that will adapt well in the changing climate (Mitchell, 2007; Wheeler, 2013; FAO, 2014b). It is well documented that environment and type of breed affects some of the meat quality attributes. Scholtz (2007) suggested that there is a need to select *Bos Indicus* genes for the slaughter population to solve the problems faced by the industry. Even though there were questions regarding different traits of meat quality attributes that can be improved according to market requirements, Pimentel and König (2012) suggested that genomic selection will improve birth weight of the targeted beef breeds, beef cuts, carcass ratios, intramuscular fat and muscularity score (Table 2.3).

Table 2.3 Use of genetic selection and modification for the improvement of carcass and meat quality characteristics

Parameters		References
Carcass characteristics	Birth weight, growth performance and slaughter weight	Scholtz (2007); Smith *et al.* (2007); Mortimer *et al*. (2010); Pimentel and König (2012); Freedom Food Report (2014)
	Backfat thickness in pigs, meat yield, intramuscular fat in cattle	Utrera and Van Vleck (2004); Vote *et al.* 2009; Liu *et al.* (2013); Burrow (2014); Miar *et al.* (2014)
	Muscle - bone ratios, *Longissimus* muscle, breast, leg and yield grades	Utrera and Van Vleck (2004); Pimentel and König (2012)
Meat quality characteristics	Muscle temperature, Ultimate pH, Colour (*CIE* L*, a* and b*)	Commission International De I' Eclairage (1976); Abril *et al.* (2001); Gaya *et al.* (2011); Hope *et al.* (2013); Lui *et al.* (2013); Mortimer *et al.* (2014)
	Fatty acid profiles, lean carcass growth	Karamichou *et al.* (2006); Smith *et al.* (2007); Ardiyanti *et al.* (2009); Muchenje *et al.* (2009c)
	Tenderness (Warner Bratzler Shear Force)	Warner (1928); Bratzler (1932); Hopkins and Fogarty (1998); Muchenje *et al.* (2008); Smith *et al.* (2007); Hopkins *et al.* (2010); Bagatoli *et al.* (2013); Allais *et al*. (2014)

CIE - Commission International De I' Eclairage

Measures that include genetic selection, breeding programmes, stress response and physiological markers of positive emotions are still investigated since they have a possibility of adding to the betterment of welfare, carcass and meat quality in slaughter animals (Boissy *et al.,* 2005; Pimentel and König, 2012; Grandin, 2013). However, genetics are not easily understood especially when considering the *B. indicus* cattle in response to handling facilities. This is because; animals behave and respond differently to stress as a result of their genetic characteristics which aids in the animals being able to resist and cope under heat stress (Ali *et al.,* 2006; Allais *et al.,* 2014). Stress is a non-specific phenomenon that represents the consequences of the behavioural, physiological and emotional status of an animal in response to a wide variety of environmental stimuli (Voslářová, 2010). These include ambient temperature, humidity, stocking density, transport and lairage duration and sometimes management both at the farm and at the abattoir (Wilkins *et al.*, 2005; Chai *et al*., 2010; Melesse *et al.*, 2011; Chen *et al.*, 2013; Miranda-de la Lama, 2013).

2.7 Summary

Improvement of animal welfare and meat products for consumer satisfaction is on the rise worldwide. This is because of the increasing consumer demands for healthy food from healthier animals. Due to the processes that are involved in animal production, transportation, slaughter procedures and processing of meat, consumers are concerned with the quality of meat they consume. The change in climate has led to modification in the genetic make-up of animals and application of biotechnology to produce animals that will provide hea lthy food. However, there is an increase in the scrutiny of animal welfare and animal food source which led to the growing understanding in the issues regarding the assessment of properties of meat and meat products in relation to how animals are treated. In addition, consumers require more programmes to be conducted that will make them acquainted with the production supply chain since they are the prime targets for production. Therefore, there is a need to refine and adopt the proposed biotechnologies and genetic modification in animals that will adapt in the changing climate to reduce the fear of consuming animal protein.

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Chapter 3 The expression of heat shock proteins, glucose and cortisol in slaughtered bovine species and the quality of beef

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(Submitted to Meat Science)

Abstract

The objective of the study was to determine the relationship between the number of stunning shots, the expression of heat shock proteins, cortisol and glucose levels and meat quality of bovine species. Blood samples were collected during exsanguinations from the jugular vein for the determination of plasma heat shock proteins (HSPA1A), glucose and cortisol. Representative samples from the *Muscularis thoracis et lumborum* (LTL) were collected 48 hours after slaughter to measure meat quality. A negative correlation was observed between heat shock proteins 70 kDa (HSPA1A) and other parameters with the exception of (L*), glucose (GLU) and cortisol (CORT). Warner Bratzler Shear Force (WBSF) was negatively correlated with L*, redness (a*), yellowness (b*) HUE (HUE angle), HSPA1A, GLU and CORT positively correlated with ultimate pH (pH_u) and meat temperature (T_m). There was no significant effect of animal class on the concentration of CORT, GLU and HSPA1A. However, glucose levels were higher in heifers (5.82 ± 2.860 nmol/L) than in bulls (5.62 ± 2.650 nmol/L) and cows (5.53 ± 4.486 nmol/L) although bulls had the highest expression of HSPA1A (15.13 ± 2.650). Animals that travelled between 200 < 400 km had lower HSPA1A (10.05 ± 3.149 ng/ml) than those that were transported for 400 < 800 km (12.11 ± 1.889 ng/ml) and < 200 km (15.13 ± 2.362 ng/ml). Animals that travelled for < 200 km had higher glucose (6.10 ± 3.149 nmol/L) and lower cortisol concentration (88.87 ± 3.149 nmol/L) while cattle that travelled for 400 < 800 km had lower levels of glucose (5.50 ± 1.889 noml/L) and increased concentration of cortisol (103.56 ± 1.889 noml/L). Lairage duration had a significant effect on the expression of Hsp ($P < 0.001$), GLU ($P < 0.05$) and CORT ($P < 0.001$) levels. Animals that were shot more than once had the highest expression of HSPA1A (15.09 ± 1.734ng/ml) and CORT (101.16 ± 1.734 nmol/L). It was concluded that long hours of transportation with fewer lairage duration increased the expression of HSPA1A and CORT while glucose levels are reduced. In addition, HSPA1A, CORT and GLU were related with beef quality. Stunning animals more than once should be avoided because it increases the levels of CORT and HSPA1A in the blood.

Keywords: Distance travelled, glycogen, HSPA1A, lairage duration, proteins, ultimate pH

3.1 Introduction

Transportation of live animals to auctions and abattoirs for slaughter has been reported to cause stress in animals. The energy reserves of the animal get reduced as they experience changes due to novel environment (Kadim *et al.*, 2009; Mota-Rojas *et al.*, 2012; Ake *et al.*, 2013). During this period, animals devise means to cope with the environmental changes that end-up affecting even their physiology. In most prokaryotic and eukaryotic organisms, tissues and in individual cells, there are proteins that play a role in these physiological changes. These proteins were found to be expressed during heat shock hence the name heat shock proteins (Hsp) (Mormede *et al.*, 2011; Storey and Storey, 2011). When animals are exposed to elevated ambient temperatures, stress is induced leading to the gene expression for a small group of polypeptides of Hsp. Stress increases cell death as a result of the accumulation of damaged proteins and denaturation of enzymes in all organisms (Wang and Edens, 2008). Small heat shock proteins (sHsps) exist in essentially all organisms, especially in plants. The Hsp70 work together with the adenosine triphosphate (ATP) dependent proteins so as to fold as soon as stress returns to normal (Åhrman *et al.*, 2007).

The reduction of ATP prior to animal slaughter is as a result of exposure of animals to a novel environment leading to the depletion of glucose and inversely increases cortisol production. The pre-slaughter stressors include feed withdrawal during long hours of transportation and at the lairages, change of environment from the farm, loading, transport conditions (mixing of animals, limited space, long hours of standing, no grass or feed), off-loading and noise (Foury *et al.*, 2007; Škrlep *et al.*, 2009; Mota-Rojas *et al.*, 2012). The use of energy and protein imbalance during this critical stage of production is influenced by the hypothalamic-pituitary-adrenocortical (HPA) axis leading to the secretion of cortisol. Cortisol is a hormone found in the axis of HPA which is released by the adrenal cortex in response to stress leading to darker meat as a result of reduced glycogen levels (Foury *et al.,* 2007; Ake *et al.*, 2013). Due to different sources of stress, it becomes challenging to identify exactly the main causes of stress in animals. In pigs and poultry incidences of higher mortality, poor welfare and meat quality resulting in economic losses have been reported (Mota-Rojas *et al.*, 2012; Zhang *et al.,* 2014). Welfare requirements dictate that animals should be insensitive to noxious and potentially harmful stimuli prior and post slaughter. Animals are stunned to render them insensible to pain during throat cutting to reduce stress (Sazili *et al.,* 2013). However, improper use of the captive bolt machine (increased number of shots) may lead to an increase in the production of stress proteins, hormones and reduce muscle glycogen which is responsible for lactic acid production (Warriss *et al.,* 1995; Warriss and Brown, 2000).

Research has been done on body temperature, blood pressure, heart rate, and vocalisation, different markers of stress such as cortisol and hormones and heat shock proteins (Muchenje *et al.,* 2009; Yu *et al.*, 2009; Grandin, 2013; Zhang *et al.*, 2014). There is evidence of Hsp as affected by antibodies in fertilisation, embryo and mammalian development and proteome preservation (Matwee *et al.,* 2001; Christians *et al.*, 2003; Storey and Storey, 2011; Zhang *et al.*, 2011); diseases and inflammatory response (Velazquez *et al.,* 2010; Grundtman *et al.*, 2011) and their expression in lactating cows and by *Helicobacter pylori* (Eitam *et al.*, 2009; Wendy *et al.*, 2009). Much research has been done on the involvement of steroid hormones, association of stress markers, stress neuroendocrine profiles in pigs (Foury *et al.*, 2007; Wang and Edens, 2008; Škrlep *et al.*, 2009). However, in the area of transportation of cattle, lairage duration at the abattoir, animals and genotype effects on the expression of heat shock proteins, glucose and cortisol levels and meat quality is limited. Hence, the objective of the study was to determine the expression of heat shock proteins, glucose and cortisol in slaughtered bovine species and their correlations with the quality of beef. The null hypothesis tested was that there is no expression of heat shock proteins 70 kDa, cortisol and glucose and they are not correlated with beef quality

3.2 Materials and Methods

3.2.1 Study site

The study was conducted at a high-throughput commercial abattoir in the Buffalo City local municipality of the Eastern Cape Province, South Africa. The municipality is bounded to south-east by the long coastline along the Indian Ocean. The abattoir is located at 120km South East of the University of Fort Hare, Alice campus at latitudes and longitudes of 32.97^0S and 27.87^0E and 542m above sea level. The climate of the area is mild with an average rainfall of 850mm. The temperatures during the study period were ranging from 14^0C -18^0C. Permission to conduct the study was approved by the Research Ethics Committee of the University of Fort Hare, South Africa (UFH/UREC, MUC012 1SCHU01).

3.2.2 Animal management and sampling

One-hundred (100) cattle (39 Bonsmara, 28 Nguni and 33 Non-descript) genotypes of different classes (heifers, bulls and cows) were identified on arrival at the abattoir (Figure 3.1). At the lairages for about five minutes, animal behaviours were observed in the holding pens.

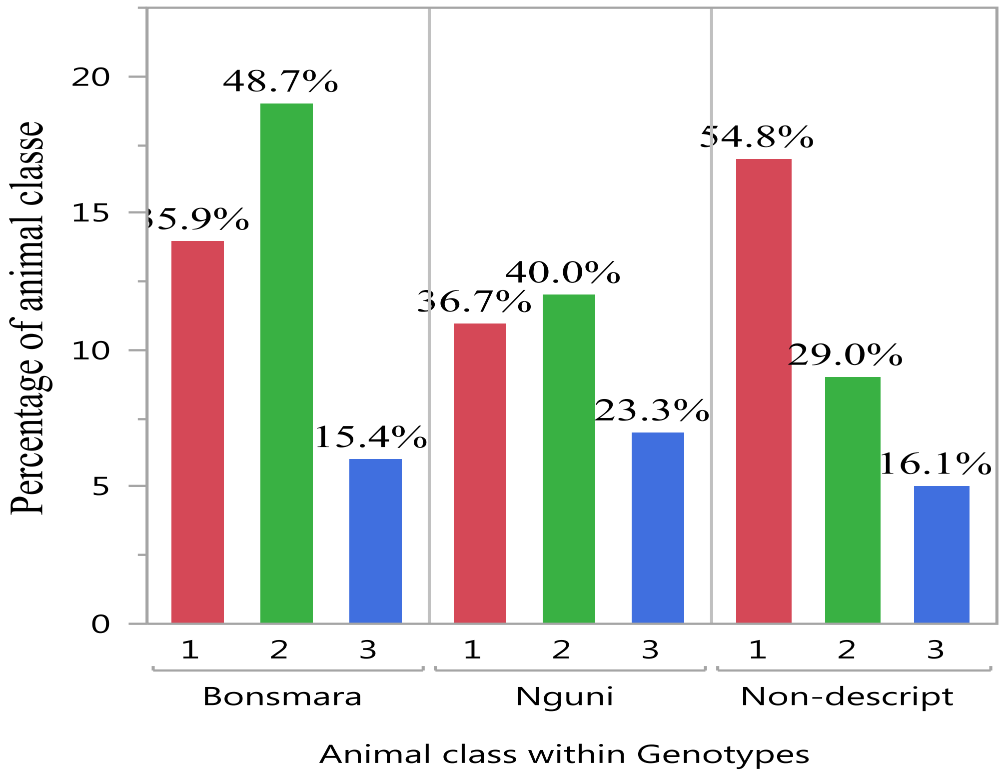

Figure 3.1 Percentage of animal classes within genotypes

1 – Heifers, 2 – Bulls and 3 – Cows

At the lairages, the animals were given *ad-libitum* access to water, although feed was not provided to avoid carcass contamination at slaughter. At the stunning box, the number of times an animal was shot was recorded. Ambient temperatures at the abattoir were obtained and recorded accordingly. Cattle were then slaughtered following the usual commercial abattoir slaughter procedures (Meat Safety Act, 2000: Act No. 4). The captive bolt method of stunning was used. Some of the carcasses depending on their animal classes (Heifers, Cows or Bulls) were electrically stimulated at 300 V, 50 Hz and 5 A over 40-45 s at 12 pulse/s, to minimise the likelihood of cold shortening due to the rapid chilling regime used. This is the normal procedure followed by the abattoir workers.

3.2.3 Blood sample collection and storage

Blood samples were collected at slaughter during cattle exsanguinations using the yellow top tubes for easy separation of plasma. The blood tubes were then kept in a cooler box with ice until they were centrifuged. All the samples were centrifuged at 1000 g for 15 min at 2-8 ^{0}C within 30 minutes of collection. The plasma samples were stored at -20 ^{0}C until the assay to avoid loss of bioactivity and contamination.

3.2.4 Determination of Heat Shock proteins

The analysis of heat shock proteins (Hsp) was performed using the enzyme-linked immunosorbent assay to determine the concentration of heat shock protein 70 (HSPA1A) in plasma. The kit (CUSABIO CSC-EL010821BO) contained the following reagents: standard (freeze dried) x2 and sample diluent 50 ml, Biotin-antibody (1x) 120 µl, Biotin-antibody diluent 15 ml, HRP-avidin (Horseradish Peroxidase) (1x) 120 µl, HRP-avidin Diluent 120 µl, wash buffer 25x 20 ml, TMB substrate 10ml, stop solution 10ml and an adhesive strip for 96 wells x4. The reagents and samples were brought to room temperature (18-25 ^{0}C) and centrifuged again after thawing. The assay of the standards and samples was done in duplicates. The minimum detection range of HSPA1A was 3.12 ng/ml to 200 ng/ml and the intra- and inter-assay coefficients of precision were < 8 % and < 10 % respectively. The standard, biotin-antibody and HRP-avidin were prepared by adding 1000 μl of sample diluent into the standard, 100 μl of biotin-antibody into a 9900 μl of biotin diluent and by adding 100 μl HRP-avidin to 9900 μl HRP-avidin Diluent.

The standards and samples were pipetted into the wells and any present HSPA1A was bound by the immobilised antibody within the 2 hour incubation. After removing any unbound substances by aspiration and wash for 3 times, the biotin-conjugated specific for HSPA1A was added into wells and incubated for an hour. After incubation and washing, avidin conjugated HRP was added into the wells and incubated for an hour. Any unbound avidin-enzyme reagent was washed and removed and a substrate solution was added to the wells. It was then incubated for 20 minutes and in observations, a blue colour developed in proportion to the amount of HSPA1A bound in the initial step. The colour development was stopped using a stop solution and the intensity of each well was read in a micro plate reader set to 540 nm (SynergyMx BioTeK, model SN 236255, Made in USA). The minimum detectable dose of bovine Hsp70 was less than 0.78 ng/ml. The Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. The results were calculated to make a standard curve and the standards and samples were averaged per duplicate readings to subtract the zero standard optical density.

3.2.5 Determination of blood cortisol and glucose levels

The determination of blood cortisol was carried through a competitive assay (EIA, RADIM, Pomezia, Italy). During the first incubation of the sample, the plasmatic cortisol competed with the cortisol merged with enzyme Horseradish peroxidase (HRP) for the specific places of the antiserum that covers the cup walls. After incubation, all the material which was not stuck in the walls was removed by vacuuming and washing. The enzyme activity which was bound into the solid phase was inversely proportional to the cortisol concentration in the standards and samples, which were evident by incubating the walls of the chromogenic solution in a substrate buffer. Colorimetric readings were carried out using a spectrophotometer at 450 and 540 nm. The assay was performed in ng/ml and then the concentrations of cortisol were determined.

Glucose was determined by taking 50 µl of blood serum and the samples were added to the measuring cells for dilution (dilution after injection 1:40). Glucose concentrations in blood serum samples were determined by using the calibration curve in nmol/L. A part of the linear range of the calibration curve up to 1.5 mM glucose in the model solution was used which corresponded with up to 60 mM glucose in the undiluted samples. Reference measurements of the blood serum glucose concentration were conducted by the classical colorimetric method using an enzymatic kit (Diaglue from L'viv Plant of Bacterial preparation, 1994 and the well-known biosensor analyser, Eksan-G from Panevezhis Plant of precision mechanics, 1990).

3.2.6 Beef quality measurements

3.2.6.1 Determination of ultimate pH, meat temperature and colour

The carcasses were split, weighed and chilled at 0-3 °C before being processed at 48hours after slaughter. Representative samples (100 mm thick) of the *muscularis longissimuss thoracis et. lumborum* (LTL) were sampled from the 10^{th} rib to the third lumbar vertebra at 48 hours after slaughter . The measurements of pH_u and colour coordinates (L*, a* and b*) were carried out at 48 hours after slaughter.

A portable fibre-optic pH and T_m meter probe with a sharp metal sheath to prevent damage from raw meat (CRISON pH 25 Instruments S.A., Alella, Spain) was used to measure the ultimate pH and temperature of the carcasses 48 hours *post mortem.* The pH meter was calibrated before taking measurements using pH 4, pH 7 and pH 9 standard solutions (CRISON Instruments, SA, Spain). Both pH_u and T_m were recorded accordingly.

The colour of meat (L* = lightness, a* = redness and b* = yellowness) was determined at 48 hours after slaughter using a Minolta colour-guide 45/0 BYK-Gardener GmbH machine with a 20 mm diameter measurements area and illuminant D65-day light, 10^0 standard observer. The machine was calibrated each day before taking measurements using the green, black and white colour standard samples provided for this purpose. The readings were taken by rotating the Colour Guide 90^0 between measurements so as to obtain the average value for the colour. The colour values were recorded and compared with pH_u of the same meat samples.

3.2.6.2 Warner Bratzler Shear Force

The *Muscularis longissimuss thoracis et. lumborum* samples were weighed 1 week after refrigeration, thawed for 10 hours and weighed again. They were placed in a plastic bag and cooked using a water bath at 85 °C for 45 minutes. The results were expressed as the thawing and cooking loss. Thawing and cooking losses were calculated as described below:

$$\text{Thawing (thaw)loss \%} = \left[\frac{(\text{weight from freezer} - \text{weight after thawing})}{\text{weight from freezer}}\right] * 100\%$$

$$\text{Cooking Loss (CL) \%} = \left[\frac{\text{weight before cooking} - \text{weight after cooking}}{\text{weight before cooking}}\right] * 100\%$$

Tenderness of beef was determined using the Instron - Warner-Bratzler Shear Force (WBSF) machine. Following cooking, sub-samples of specified core diameter will be cored parallel to the grain of the meat. The samples were sheared perpendicular to the fibre direction using a Warner Bratzler (WB) shear device mounted on an Instron 3344 Universal Testing (cross head speed at 400mm/min, one shear in the centre of each core. The mean maximum load recorded for the three cores will represent the average of the peak force in Newtons (N) for each sample.

3.2.7 Statistical Analysis

The principal component analysis was performed to determine the correlations between HSPA1A, GLU, CORT and beef quality using JMP 9.0 (SAS, 2010). The mixed model procedure (PROC MIXED) of SAS (2003) was used to determine the effect of distance and lairage categories, number of shots at stunning and animal class on the expression of heat shock protein 70kDa, cortisol and glucose. Genotype was used as a random variable.

3.3 Results and Discussion

3.3.1 Heat shock proteins, cortisol glucose and meat quality

Figure 3.2 and 3.3 indicate the relationship between meat quality, heat shock proteins (HSPA1A), glucose (GLU) and cortisol (CORT) of bovine species. A negative correlation was observed between, HSPA1A and other parameters with the exception of lightness (L*), GLU and CORT. The negative relationship between Hsp70 and ultimate pH is as a result of the binding that occurs between Hsp70 and adenosine triphosphate (ATP) during the period of stress. This leads to the depletion of muscle glycogen and consequently causes increased pH_u. According to Scheffler *et al.* (2013), low glycogen levels also limit the process of glycolysis and causes darker meat. The relationship between cortisol and fatness is not a new finding, in humans; the increase in cortisol was observed to be caused by a significant increase in fatness (Mormede *et al.*, 2001). Similar results were reported by Foury *et al.* (2007) where an increase in cortisol was observed in pigs with higher carcass fat. This implies that cortisol plays a role in the metabolic processes, carcass characteristics and meat quality.

Warner Bratzler Shear Force (WBSF) was negatively correlated with L*, a*, b*, HUE, Hsp70, GLU and CORT and positively correlated with pH_u and T_m. There was a positive correlation observed between the HUE angle, ultimate pH (pH_u), meat temperature (T_m), redness (a*), yellowness (b*) and CORT. Yellowness was also positively correlated with pH_u, T_m, L* and a*. The relationship is based on the fact that pH_u can improve the bright red colour and improve tenderness because of the amount of intramuscular fat.

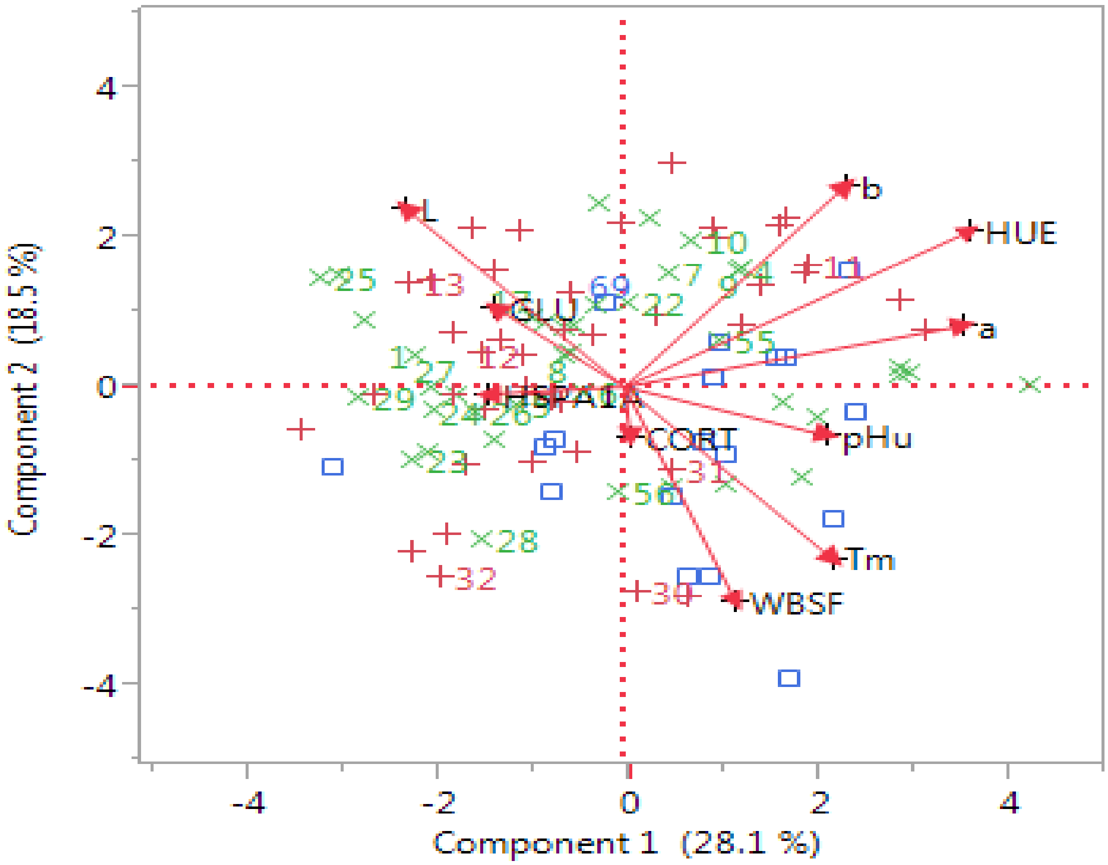

Figure 3.2 The relationship between the expression of heat shock proteins, glucose cortisol and beef quality

T_m - Meat temperature, pH_u - Ultimate pH, L* - Lightness, a* - Redness, b* - Yellowness, HUE - HUE angle, WBSF - Warner Bratzler Shear Force, HSPA1A - Heat shock proteins 70 kDa, GLU - Glucose, CORT - Cortisol

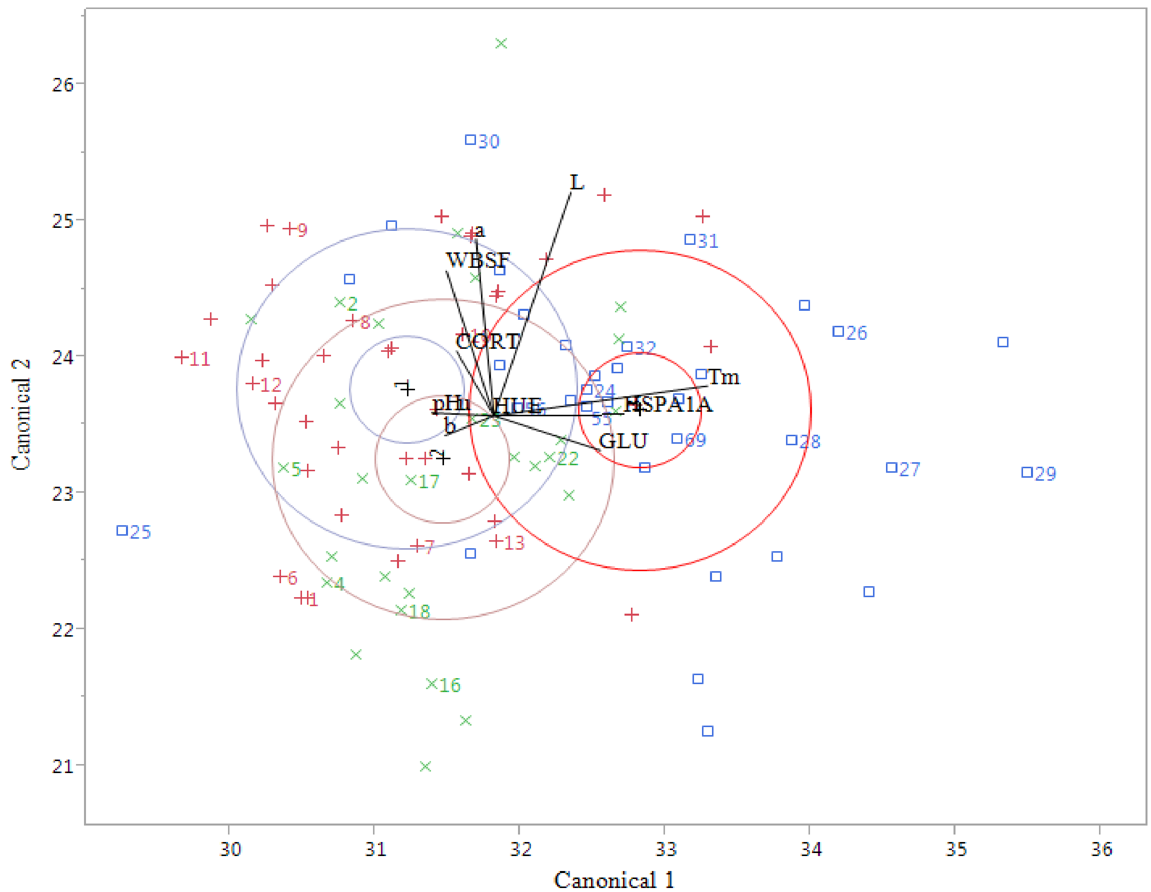

Figure 3.3 Canonical presentation of the relationship between cortisol, glucose and heat shock proteins and beef quality

T_m - Meat temperature, pH_u - Ultimate pH, L* - Lightness, a* - redness, b* - Yellowness, HUE - HUE angle, WBSF - Warner Bratzler Shear Force, HSPA1A - Heat shock proteins 70 kDa, GLU - Glucose, CORT - Cortisol

The rate at which pH declines determines the quality of meat especially colour, water holding capacity and tenderness of meat (Cecchinato *et al.*, 2009; Frimpong *et al.*, 2014). Meat tenderness is associated with normal ultimate pH, however, during a stressful situations, there is low glycogen levels which increases pH_u, dark firm dry (DFD) and less red beef (Terlouw, 2005; Muchenje *et al.*, 2009; Mapiye *et al.*, 2011; Hopkins and Mortimer, 2014). Mortimer *et al.* (2014) highlighted that selection of more traits in lamb may result in discoloured and less red meat during display at the markets. This is becasue of genetic selection for intramuscular fat and tender meat.

Similar correlations of pH_u with the other meat quality attributes in beef were reported by Bidner *et al.* (2004), Wiklund *et al.* (2009) and Wu *et al.* (2014). In addition, muscle pH has been reported to play a critical role even in the degradation of muscle proteins during meat storage (Hanzelková *et al.*, 2011; Wu *et al.*, 2014). However, there is also a background influence of the extent of pH decline in red muscles during the periods of anoxia (Hudson, 2012; Hopkins *et al.*, 2014). In addition, pH_u variation is mostly associated with *post-mortem* muscle glycogen content and metabolism, which is affected by many factors including psychological stress, animal diet, season, transport, and lairage times, thus affecting initial meat shear force values and rate of tenderization (Bourguet *et al.*, 2011).

3.3.2 Cortisol, glucose and heat shock protein levels by animal class

Table 3.1 shows the production of cortisol, glucose and HSPA1A as affected by animal class (heifers, bulls and cows).

Table 3.1 Parameter estimates (± SE) of cortisol, glucose and heat shock proteins by animal class

Parameter	Animal Class			Significance level
	Heifers (*n* = 39)	**Bulls** (*n* = 28)	**Cows** (*n* = 33)	
Cortisol	$98.02^{a} \pm 2.860$	$99.26^{a} \pm 2.650$	$96.87^{a} \pm 4.486$	ns
Glucose	$5.82^{b} \pm 2.860$	$5.65^{ab} \pm 2.650$	$5.53^{a} \pm 4.486$	**
HSPA1A	$10.02^{a} \pm 2.860$	$15.13^{c} \pm 2.650$	$-10.05^{b} \pm 4.486$	***

^{a,b,c}Means with different superscripts within the same row are significantly different at *$P < 0.05$
Significance level: **$P < 0.01$, ***$P < 0.001$, ns – not significant at $P > 0.05$
HSPA1A – Heat shock proteins 70 kDa

There were no significant effects ($P > 0.05$) of animal class on cortisol levels. Animal class had significant effects on the expression of heat shock proteins ($P < 0.001$) and glucose levels ($P < 0.01$). Bulls had the highest expression of HSPA1A (15.13 ± 2.650 ng/ml) while cows had the lowest expression of HSPA1A (-10.05 ± 4.486) and heifers had 10.02 ± 2.860 ng/ml. This confirms that the bulls were stressed, hungry and exhausted from the exposure to new environment. Previously, the presence of Hsp70 in the bovine oocytes significantly reduced the tight binding of spermatozoa (Matwee *et al.,* 2001). In older animals (Warriss *et al.,* 1995), in horses (Stull and Rodiek, 2002) and bulls (Thompson, 2004), cortisol levels were elevated due habituation, long hours of transportation, starvation and mixing of bulls in the same pen.

Glucose levels in cows were lower (5.53 ± 4.486) than in bulls (5.65 ± 2.650) and heifers (5.82 ± 2.860). The low level of glucose for cows is not clear considering the fact that they were not stressed. However, it can be explained that dairy cows are mostly slaughtered when they are old, or with mastitis and or lameness. In this case, it can be observed that these cows were slaughtered due to age hence low energy reserves. If it was starvation or diseases, heat shock proteins were going to be higher (Velazquez *et al.*, 2010). In addition, cortisol levels were expected to be very low as reported by Zirmeman *et al.* (2011) who indicated that starvation or fasting in goats led to lower cortisol levels. In dairy cows, low glucose levels were associated with poor fertility (Miettinen, 1991). In ruminants, glucose is regulated by cortisol from amino acids. Cortisol is an indicator of pain and stress and it has been shown to increase even in cows with inflamed foot lesions (Almeida *et al.*, 2008) and in cows stressed by recumbency (Forslund *et al.*, 2010).

3.3.4 The levels of heat shock proteins, glucose and cortisol as affected by pre-slaughter stress

Figure 3.4 to 3.5 shows the standard concentration curve of HSPA1A at different absorbance. For the accuracy of results, Figure 3.4 was used to calculate the absorbance of HSPA1A from bovine plasma. The animals with values greater or equal to 0.05 ng/ml were considered stressed as the presence of Hsp indicates stress prior to slaughter.

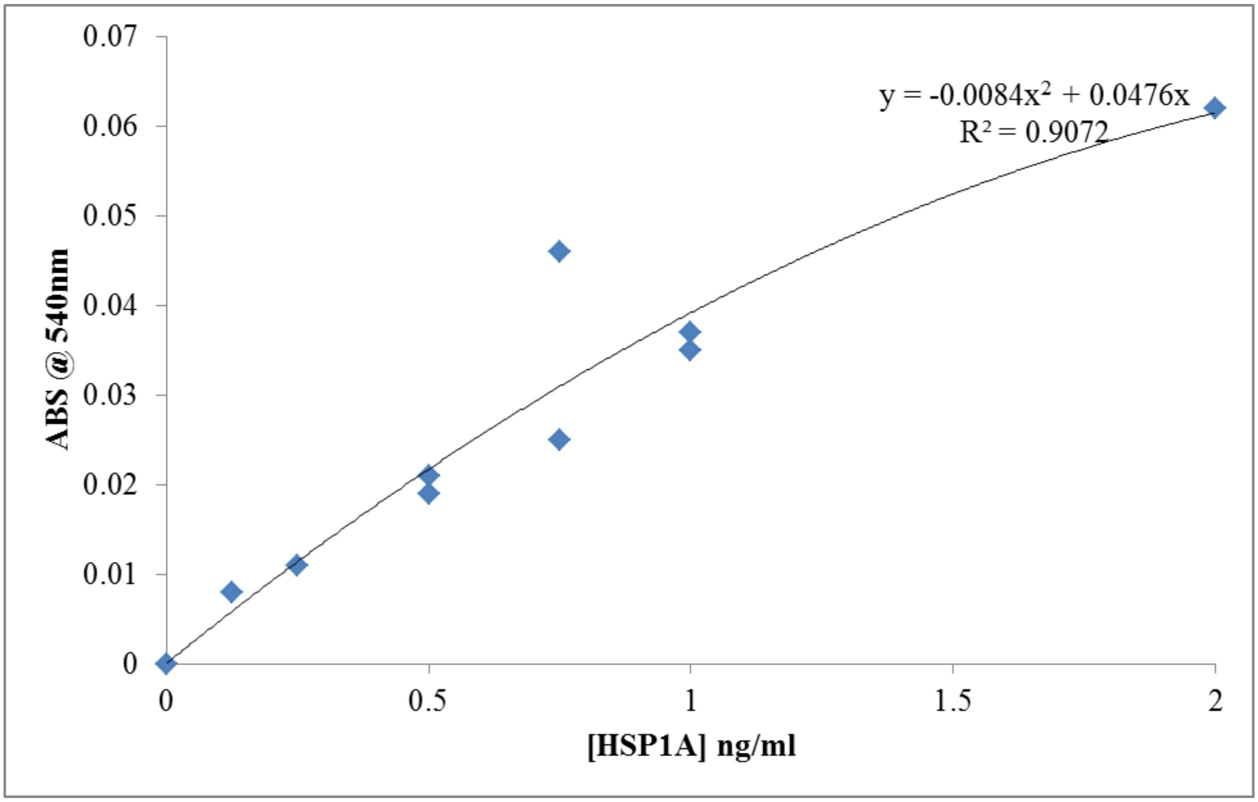

Figure 3.4 The standard curve of the ELISA heat shock protein 70 kDa at 450 nm

HSPA1A - Heat shock protein 70 kDa

ABS – Absorbance

ELISA – Enzyme Linked Immunosorbent Assay

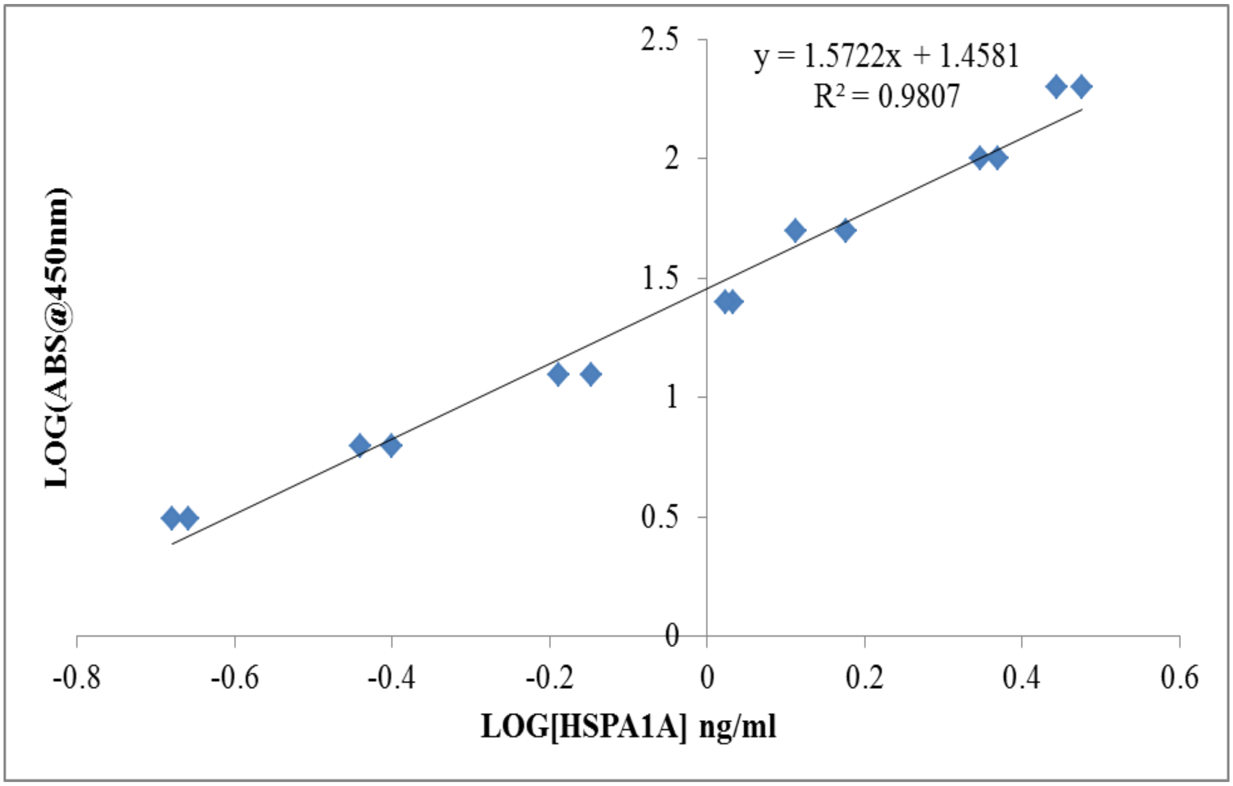

Figure 3.5 The log concentration standard curve of bovine heat shock protein 70kDa at 450 nm

HSPA1A - Heat shock protein 70 kDa

ABS - Absorbance

Log – Logarithm

As shown in Table 3.2, distance duration (km) ($P < 0.001$), lairage duration (hours) ($P < 0.001$) and number of shots at stunnning ($P < 0.01$) had significant effects on the expression of plasma heat shock proteins and levels of plasma glucose and cortisol. Animals that travelled for 200 < 400 km had lower heat shock proteins (10.05 ± 3.149 ng/ml) as compared to those that were transported for 400 < 800 km (12.11 ± 1.889 ng/ml) and < 200 km (15.13 ± 2.362 ng/ml). During transportation stress, physiological and biochemical activities occur including the exhange of gases such as carbon dioxide (CO_2) and oxygen (O_2) (Mota-Rojas *et al.*, 2012). In agreement with the effects of stress on the expression of Hsp70, Varlos *et al.* (2013) highlighted that when animals are stressed, there is a higher production of intestinal heat shock protein which is also linked to carcass weights. Heat shock proteins are also part of the stress hormones and enzymes reducing the ATP in the muscles (Åhrman *et al.*, 2007; Foury *et al.*, 2007).

Glucose and cortisol concentration of animals that travelled for < 200 km was higher (6.10 ± 6.297 nmol/L) than those that travelled between 400 < 800 km (5.50 ± 1.889 nmol/L) while cortisol was vise versa (88.87 ± 3.149 nmol/L and 103.56 ± 1.889 nmol/L, respectively). During the initial stage of transportation, the glucose levels in animals is still high and cortisol is low (less stress). However, as they get to the abattoir after 800 km of transportation, truck movements and exhaustion from the environmental stress, glucose gets reduced while cortiol is at its peak. This agrees with the reports by (Averos *et al.*, 2007) who indicated that as transport duration increases, cortisol is also produced in higher levels.

Table 3.2 Parameter estimates and standard errors of heat shock proteins, glucose and cortisol as affected by pre-slaughter conditions

Variables		*n*	Heat shock proteins (ng/ml)	Glucose nmol/L	Cortisol nmol/L
			Response variables		
DT	< 200 km	32	$15.13^{b} \pm 2.362$	$5.81^{a} \pm 2.362$	$95.31^{a} \pm 2.362$
	200 < 400 km	18	$10.05^{a} \pm 3.149$	$6.10^{b} \pm 3.149$	$88.87^{a} \pm 3.149$
	400 < 800km	50	$12.11^{b} \pm 1.889$	$5.50^{a} \pm 1.889$	$103.56^{b} \pm 1.889$
Significance level			**	***	***
LD_{hr}	18	32	$15.09^{c} \pm 2.401$	$5.42^{a} \pm 2.401$	$105.77^{c} \pm 2.401$
	20	18	$-10.01^{a} \pm 4.069$	$5.89^{b} \pm 4.069$	$91.32^{a} \pm 4.069$
	24	50	$14.03^{b} \pm 3.507$	$5.67^{b} \pm 3.507$	$97.05^{b} \pm 3.507$
Significance level			***	*	***
Number of shots at stunning	1	78	$-10.01^{a} \pm 4.338$	$5.78^{a} \pm 4.338$	$94.93^{a} \pm 4.338$
	> 1	22	$15.09^{b} \pm 1.734$	$5.55^{a} \pm 1.734$	$101.16^{b} \pm 1.734$
Significance level			**	*ns*	***

[a,b,c]Means with different superscripts within the same column are significantly different
DT - Distance travelled, LD_{hr} - Lairage duration in hours
Significance level: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns - not significant at P > 0.05

Moreover, Zimerman *et al.* (2011) and Tateo *et al.* (2012) also reported that exercise (in kids) and long journeys (horses) prior to slaughter has an ample effect on cortisol levels which inversely affect ultimate pH. Kadim *et al.* (2009) also reported that, goats that were traported for 3 hours had higher cortisol levels at the begining of transport than non-transported goats. Contrastingly, Valros *et al.* (2013) reported that inducing stress in pigs led to lower cortisol response after stunning. Therefore, from the available literaturee, it is evident that cortisol is released in higher levels as the animal copes with adverse pre-slaughter stress be it starvation, increased ambient temperature or long hours of transportation.

Lairage duration had a significant effect on the expression of Hsp ($P < 0.001$), GLU ($P < 0.05$) and CORT ($P < 0.001$) levels as observed in Table 3.2. Animals that rested for 18 hours at the lairages had higher levels of HSPA1A and CORT levels (15.09 ± 2.401 ng/ml and 105.77 ± 2.401 nmol/L, respectively) while a 24 hour period of rest resulted in lower levels of HPSA1A and CORT levels (14.03 ± 3.507 ng/ml and 97.05 ± 3.507 nmol/L, respectively). The low levels of Hsp during 24 hours of rest are an indication that cattle were not exhausted, not in pain and were not stressed. The expression of Hsp is induced by the amount of heat shock transcription factor (HSF1). The HSF1 is the primary factor that is funcitional during heat stress (Pawar *et al.*, 2002). This could be the other reason for low expression of Hsp in cattle that rested for 24 hours because there are shades at the lairages preventing animals from suffering from heat stress.

In the lairages, animals are expected to re-gain the energy lost during transportation; however, the rate at which energy is gained depends on how stressful transportation was and the conditions of the lairages at the abattoir. Glucose levels were still low (5.42 ± 2.401 nmo/L) after an 18 h rest while longer resting (24 h) allowed the animals to regain their energy levels by 0.25 nmol/L (5.67 ± 3.507 nmol/L). Contrasting results were reported by Pérez *et al.* (2002) where pigs rested for 0 h, 3 h and 9 h had decreasing levels of glucose although cortisol levels increased with longer hours of resting. According to (Thompson (2004), management of animals at the lairage addresses the criteria of reducing stress incurred during transportation thus minimising the depletion of muscle glycogen reserves prior to slaughter. Gispert *et al.* (2000) highlighted that when animals are kept in the lairages for too long, energy reserves are reduced rapidly

The highest expression of HSPA1A and CORT (15.09 ± 1.734 ng/ml and 101.16 ± 1.734 nmol/L, respectively) were also observed in cattle that were stunned more than once than cattle stunned only once (-10.01 ± 4.388 ng/ml and 94.93 ± 4.388 nmol/L). On the other hand, the glucose levels were reduced in animals that were stunned more than once (5.55 ± 1.734). The acclimation of the proteins and cortisol has been reported to be due to the aversiveness of the stimulus which interrupts the proper functioning of the brain. In addition, the last 15-20 minutes prior to slaughter tend to exert detrimental effects on the physiology of the animal (Smith and Dobson, 2002; Thompson, 2004). van der Wal *et al.* (1999) reported that pigs that were stressed before stunnig had lower pH and high temperatures. This is because; stress causes a reduction in the energy levels which are responsible for improvement of beef quality. However, due to paucity of information regarding the number of shots at stunning on stress indicators of cattle, there is still a need to conduct further research on the biochemical status of cattle at slaughter.

3.4 Conclusions

Longer transport duration causes undue stress in cattle leading to the expression of heat shock proteins, reduced levels of glucose and higher plasma cortisol. Heat shock proteins were negatively correlated with other parameters with the exception of lightness, glucose and cortisol. The effects of transport duration, lairage duration and animal-related factors led to a negative correlation of heat shock proteins, glucose and cortisol with ultimate pH which is a widely used indicator of meat quality. The stunning procedure and shots of stunning led to an increase of HSPA1A and cortisol. Therefore, further research is needed so as to identify the activities of some stress enzymes in slaughter cattle since transportation has a negative impact on the indicators of stress.

3.5 References

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Chapter 4 The activities of some stress enzymes as slaughter cattle welfare and how they relate to physico-chemical characteristics of beef

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(Submitted to Meat Science)

Abstract

The study determined the activities of creatine kinase (CK) and lactate dehydrogenase (LDH) and how they relate with beef quality. Two-hundred and nineteen (*n* = 219) (85 Beefmaster, 48 Charolaise, 32 Holstein-Friesian and 54 Non-descript) brought to the abattoir for slaughter were used in the study. Disposable vacutainer tubes with anticoagulant (Ethylenediaminetetraacetic acid; EDTA) were used to collect 4 ml of blood samples to determine the activities of CK and LDH. The measurements of pH_u and colour coordinates (L*, a* and b*) were carried out at 48 hours after slaughter on the representative samples of *muscularis longissimuss thoracis et. lumborum* (LTL). Animals that travelled for 366, 877 and 1012 km had higher CK (> 3000 - 5000) whereas those that travelled for 1263 km had lower CK activities (1000). The activities of LDH were observed in cattle that travelled for 366 and 1012 km (7000) and for those that travelled for 877 and 922 km (6000). Results of the PCA showed that the first three PCs explained about 53% of the total variability. The first PC was correlated with meat quality attributes (pH_u, T_m, a* and b* values). The activities of CK and LDH were related and useful to define the second PC. However, CK and LDH were not related to beef quality. Therefore, CK and LDH can be used as indicators of animal welfare in slaughter cattle but cannot be used to predict the quality of meat.

Key words: Animal welfare; creatine kinase; lairage duration; meat quality; Principal Component Analysis; tenderness

4.1 Introduction

There is an on-going debate, discussion and re-framing of the concept of animal welfare in order to understand what is good, acceptable and/or poor as far as welfare is concerned (Buller and Roe, 2013). However, transportation has been identified as one of the prime factors which compromise animal welfare and meat quality (Gregory, 2007; Ndou *et al.*, 2011; Chapter 3). Transportation of animals to the abattoir is one of the topical issues in animal welfare because it involves a complex series of operations like handling, loading and off-loading and exposure of animals to unfamiliar environments. In some cases, transportation involves animal isolation, social disruption, confinement, loss of balance, fluctuations in environmental temperature and humidity, exposure to pollutants (e.g. truck exhaust), feed and water deprivation, and other stress inducing factors. These factors causes stress which leads to decreased health and productivity in farm animals (Probst *et al.*, 2012). Gregory (1998) indicated that road transport becomes difficult to monitor and validate under practical conditions because of different speed limits, terrain of the road and stoppages within the process.

As reported earlier (Melesse *et al.*, 2011; Chapter 3), transportation stimulates body responses that are aimed at re-establishing homeostatic conditions. When animals experience changes in the environment such as climate, temperature and nutrition, they become stressed and this results in physiological changes such as increases in the activity of enzymes, stress hormones, body temperature and glycolysis (Mader, 2003; Melesse *et al.*, 2011). Examples of enzymatic secretions which are released into the serum through leakage arising from altered membrane permeability include alkaline phosphate (ALP), acid phosphate (ACP), aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (Sattler and Fürll, 2004). Creatine kinase is one of the enzymes that are used in clinical biochemistry to perform differential diagnosis. In some cases, lactate, glucose and plasma CK are produced when an animal`s physiological system is formulating ways through which it can cope with the environment that might lead to poor welfare (Terlouw, 2005).

The increased levels of CK in the blood are an indication of how stressful the handling facilities were before the animal was slaughtered and the extent of muscular damage during handling (Kannan *et al.*, 2007; Edwards *et al.*, 2010). Moreover, the presence of this enzyme in the blood is due to breed temperament, excitability and fighting. This enzyme is mostly located in different tissues and its presence in the blood plasma serves as an indication of muscle damage (Tackett *et al.*, 2008). Creatine kinase (CK) and lactate dehydrogenase (LDH) are enzymes that are released into the blood when there is muscle damage such as bruising and when there is a vigorous exercise which has a negative effect on the quality of meat (Wickham *et al.*, 2012). Meat quality is affected by several factors that include animal-related factors such as sex, age, genotype and weight. Consumers of beef prefer to purchase meat that is compensated by good quality parameters despite its high price. The good quality parameters they consider include colour, freshness and tenderness, considerable fat content which will contribute to flavour and aroma during cooking.

Reducing stress in slaughter animals helps to improve animal welfare and meat quality. Pre-slaughter conditions are of great importance for final meat quality, because short-term and long-term stressors affect fresh meat colour, water holding capacity, shelf-life and technological yield (Dalmau *et al.*, 2009).

Consequently, as transportation and slaughter of farm animals is the final stages of the meat production chain (Ekiz *et al.*, 2012), any inappropriate handling procedures during this period will have a negative impact on the welfare of the animal, quality and quantity of the product. Therefore, the objective of this study was to determine the activities of creatine kinase and lactate dehydrogenase in slaughter cattle as welfare indicators and their subsequent relationships with beef quality. The null hypothesis being tested was that CK and LDH cannot be used as the indicators of animal welfare in slaughter cattle and they are not related to beef quality cattle.

4.2 Materials and Methods

4.2.1 Description of the study site

The study was conducted at a high-throughput commercial abattoir in the Eastern Cape Province of South Africa. The abattoir is located 120 km South East of the University of Fort Hare, Alice campus at latitudes and longitudes of 32.97^0S and 27.87^0E and 542 m above sea level. The climate of the area is mild with an average rainfall of 850 mm. The temperatures during the study period ranged from 14°C -18°C. Permission to conduct the study was reviewed and approved by the Research Ethics Committee of the University of Fort Hare, South Africa (UFH/UREC, MUC012 1SCHU01).

4.2.2 Animal Management / Experimental design

Two-hundred and nineteen (n = 219) cattle composed of 4 different genotypes (85 Beefmaster, 48 Charolaise, 32 Holstein-Friesian and 54 Non-descript) of different age and sex brought to the abattoir for slaughter were used in the study.

The animals were sourced from different farms with varying distances (366 – 1263 km) from the abattoir. Upon arrival at the abattoir, distance travelled by the animals was obtained from the driver. Ambient temperatures (Low and high temperatures) at the abattoir were ranging between 14.5 – 16.5 °C during the period of data collection. Animals were allowed to rest at the lairages while having *ad-libitum* access to water for 24 hours (overnight).

4.2.3 *Blood collection and plasma separation*

The captive bolt method of stunning was used to stun the animals. The animals were slaughtered following the commercial abattoir slaughter procedures. During exsanguinations, disposable vacutainer tubes with anticoagulant an (Ethylenediaminetetraacetic acid; EDTA) were used to collect 4 ml of blood samples. Each blood sample was kept in ice until plasma was separated within 2 h after collection. Blood tubes were centrifuged using a Model 5403 Centrifuge (Gatenbay Eppendorf, GmbH, Engelsdorp, Germany) at 21°C for 10 min at 3550 rpm and were then stored at -20°C.

4.2.3.1 Determination of creatine kinase and lactate dehydrogenase

The samples of stored plasma were analysed for CK and LDH using a Model DXC 600 machine (Beckman Coulter, Ireland) with reactive ingredients for SYCHRON Systems (CK 2 x 200 and LD-P 2 x 200). The activity of plasma CK was analysed by adding reactive ingredients which included 2 x 61ML CK Reagent, 1 Preparation insert and the reactive ingredients included creatine phosphate 53 mmol/L, disodium salt 461 mmol/L, nadide 30:0 mmol/L, adenosine-5-diphosphate 2.9 mmol/L, monopotassium salt, Dihydrate 36.0 mmol/L, glucose 18 mmol/L, glucose-6-

phosphate dehydrogenase >3.8 KIU/L and hexokinase >11 kU/L. The activity of LDH was analysed by adding the following reagents; 1.4 mmol/L of pyruvate and 0.23 mmol/L of NADH. All the ingredients added for quantitative determination of CK and LDH activities of units per litre (U/L) in plasma.

4.2.4 Beef quality measurements

4.2.4.1 Determination of ultimate pH, meat temperature and colour

The determination of ultimate pH, meat temperature, colour and Warner Bratzler Shear Force (WBSF) are similar to the method described in Chapter 3, Section 3.2.6.

4.2.5 Statistical analysis

The mixed model procedure (PROC MIXED) of SAS (2003) was used to determine any relationship between distance travelled, pH_u, T_m, L*, a*, b*, WBSF, CK and LDH where animal was used as a random variable. The following model was used:

$Y_{ij} = \beta o + ß_1X_1 + ß_2X_2 + \varepsilon_{ij}$

Y_{ij} = Dependent variables (pH_u, L*, a*, b*, WBSF, CK and LDH),

β_o – Overall Intercept,

$ß_1$ and $ß_2$ – Multiple regressions associated with distance travelled and lairage duration

X_1 and X_2 – Random effects of distance travelled, lairage duration and ambient temperature.

ε_{ij} – Random error term

Principal Component Analysis (PCA) of JMP 9.0 (SAS, 2010) was also performed to determine the relationship between between pH_u, L*, a*, b* and WBSF, CK and LDH.

4.3 Results and Discussion

4.3.1 The effect of distance on creatine kinase and lactate dehydrogenase

Figure 4.1 shows the activities of CK and LDH from cattle that travelled different distances to the abattoir. The distance is expressed in km whereas the activities of CK and LDH are expressed in U/l. Animals that travelled for 366, 877 and 1012 km had higher CK (> 3000 - 5000) whereas those that travelled for 1263 km had lower CK activities (1000). The activities of LDH were observed in cattle that travelled for 366 and 1012 km (7000) and for those that travelled for 877 and 922 km (6000). Both CK and LDH increased at the beginning of transportation but then reduced as the kilometres increased. This is an indication of muscle damage from disruption in muscle cell membrane which is the sarcolemma function and permeability during transportation. The activities of CK in the blood are an indication of how stressful the handling facilities and transport distance were before the animal was slaughtered and the extent of muscular damage during handling (Kannan *et al.*, 2007; Edwards *et al.*, 2010). These enzymes have been used to assess animal welfare in pigs and it was observed that CK and LDH increased ($P < 0.001$) with increase (1 – 2 h) in transportation time (Yu *et al.*, 2009).

Increased levels of CK and LDH are not only affected by transportation but also by feed deprivation, lairage duration and adverse environmental conditions (O'Neill *et al.*, 2006); although the feeding regimes of cattle at the farm were not part of the current study. This is because adverse conditions cause changes in the physiology of the animal which reduces the immunity of the animal and the muscles subsequently makes them susceptible especially after long journeys (Kannan *et al.*, 2007).

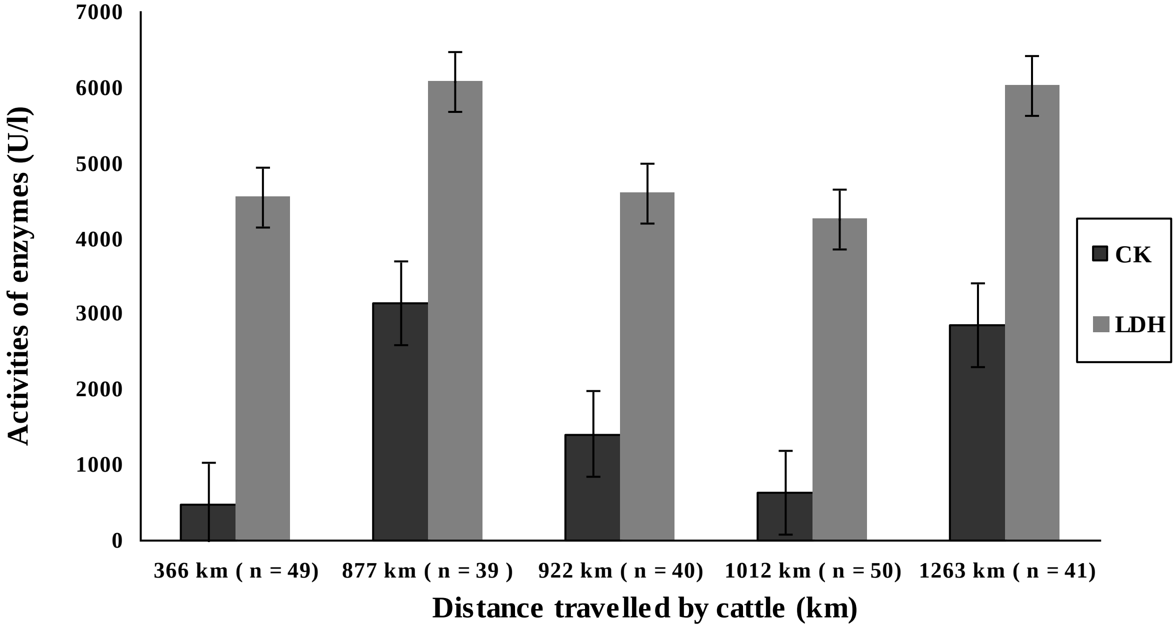

Figure 4.1 The relationship between distance travelled and the activities of creatine kinase and lactate dehydrogenase at P < 0.05

CK – Creatine kinase, LDH – Lactate dehydrogenase, U/l– Units per litre, km – kilometres

n – Number of cattle

The tendency of reducing the number of abattoirs and specialisation on certain species results in longer transportation between the farm and the abattoir which negatively affects animal welfare and meat quality. Hence animals produce higher levels of enzymes in order to endure the adverse transportation period. In addition, glucose levels also become affected after long hours of transportation in response to stress (Kannan *et al.*, 2000) even though they were not measured in the current study.

4.3.2 Distance travelled on meat quality characteristics

The relationships between distances travelled by cattle and beef quality characteristics are presented in Tables 4.1. Distance travelled (DT) had a significant effect on pH_u ($P < 0.05$), L* ($P < 0.001$), a* ($P < 0.05$) and WBSF ($P < 0.001$). The pH_u of animals that travelled for 877 and 1263 km was higher (> 5.9) leading to darker meat (L* = 29.7) and tougher meat (WBSF = 45.3). This is because the energy reserves are drained which in turn led to lactic acid production. A study conducted by Aradom (2012) in pigs showed that an increase in transport duration led to an undesirable pH_u of 6.2. Yu *et al.* (2009) highlighted that when there are lower energy reserves, there is a rapid activation of an AMP-activated protein kinase which increases glycolysis resulting to dark firm dry (DFD) meat. An increase in pH_u leads to darker meat which is susceptible to bacterial spoilage and reduced flavour which makes it less acceptable to consumers (Marino *et al.*, 2014). The quality of beef (pH_u, L* and WBSF) is affected by long journeys because animals suffer due to fasting and exhaustion (Van der Water *et al.*, 2003; Williams *et al.*, 2012).

Table 4.1 Parameter estimates of beef quality characteristics as affected by distance travelled by cattle

Parameter estimates	Variables	Equation	Relationship
Ultimate pH	Distance travelled (km)	$Y = 5.7^{ns}\ x_1 + 6.0^{*}\ x_2 + 5.7^{ns}\ x_3 + 5.7^{ns}\ x_4 + 6.3^{**}\ x_5$	Multiple
	Lairage duration (hours)	$Y = 5.8^{ns}\ b_1 + 5.7^{ns}\ b_2 + 5.7^{ns}\ b_3$	ns
Meat temperature	Distance travelled (km)	$Y = 16.2^{ns}\ x_1 + 14.9^{ns}\ x_2 + 15.9^{ns}\ x_3 + 15.0^{ns}\ x_4 + 16.2^{ns}\ x_5$	ns
	Lairage duration (hours)	$Y = 15.5^{ns}\ b_1 + 15.7^{ns}\ b_2 + 15.6^{ns}\ b_3$	ns
Lightness	Distance travelled (km)	$Y = 33.9^{**}\ x_1 + 29.7^{***}\ x_2 + 29.7^{***}\ x_3 + 34.2^{**}\ x_4 + 29.7^{**}\ x_5$	Multiple
	Lairage duration (hours)	$Y = 34.3^{***}\ b_1 + 35.9^{***}\ b_2 + 39.5^{***}\ b_3$	Multiple
Redness	Distance travelled (km)	$Y = 16.4^{*}\ x_1 + 15.7^{ns}\ x_2 + 18.6^{*}\ x_3 + 19.8^{*}\ x_4 + 19.0^{*}\ x_5$	Multiple
	Lairage duration (hours)	$Y = 17.9^{*}\ b_1 + 17.9^{*}\ b_2 + 18.0^{*}\ b_3$	Multiple
Yellowness	Distance travelled (km)	$Y = 13.5^{ns}\ x_1 + 13.0^{ns}\ x_2 + 15.7^{ns}\ x_3 + 16.3^{ns}\ x_4 + 15.9^{ns}\ x_5$	ns
	Lairage duration (hours)	$Y = 14.9^{ns}\ b_1 + 14.7^{ns}\ b_2 + 15.0^{ns}\ b_3$	ns
Warner Bratzler Shear	Distance travelled (km)	$Y = 25.0^{**}\ x_1 + 45.3^{***}\ x_2 + 16.0^{*}\ x_3 + 19.4^{*}\ x_4 + 37.5^{***}\ x_5$	Multiple
Force	Lairage duration (hours)	$Y = 82^{***}\ b_1 + 61.5^{***}\ b_2 + 49.7^{***}\ b_3$	Multiple

Significance levels = *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns = not significant.
Distance travelled: x_1 - 366km, x_2 - 877km, x_3 - 922km, x_4 - 1012km, x_5 -1263km.
Lairage duration: b_1 - 12hours, b_2 - 14hours, b_3 - 22hours

There is a reduction of glycogen reserves at this point resulting in an increase in pH_u and tougher meat (Mohrhauser *et al.*, 2014). Muscle fatigue or activity reduces the energy stores (glycogen depletion) which results in high pH_u (> 5.8) (Allen *et al.*, 2008; Neath *et al.*, 2007). The results reported by Hofbauer *et al.* (2010) indicated that tenderness increases with decrease in pH_u. As the animals stood on the truck for more than 1263 km, the connective tissues would become tougher hence the meat became tough. In addition, during transportation, animals show a progressive decline in performance of muscles which can recover after a period of rest. At this stage, transportation becomes of importance because the management practices for livestock production are generally inherently stressful leading to reduced carcass weights, increased bruises and dark cutting beef (Werner *et al.*, 2013).

The amount of time given to animals on arrival at the abattoir until slaughter was 12, 14 and 22hrs (Lairage duration). Lairage duration had a significant ($P < 0.001$) effect on L* (34.3, 35.9 and 39.5), a* (17.9, 17.9 and 18) and WBSF (88, 61.5 and 49.7 N respectively) values. It was observed that DFD beef was reduced in animals that rested for 22 h as compared to others. Mounier *et al.* (2006) reported that longer rest periods reduces pH to normal values thereby reducing the incident of DFD`s. It is understood that DFD is a serious problem in beef production. Animals normally regain their energy reserves with appropriate lairage period without any feed supplementation but water supplied at *ad-libitum*. Contrasting results by Liotta *et al.* (2007) and Dokmanović *et al.* (2014) reported that animals rested over 16 hours and 28 hours may not recover from stress instead their pH_u levels will increase. Enough resting in animals is beneficial in the sense that their glycogen reserves are restored after being exhausted during transportation. The reduction during transport is aggravated by the release of catecholamines which also lead to lower *post-mortem* lactic acid production (Muchenje *et al.*, 2009).

Animals kept in lairages for 22 h had improved (88 down to 49.7 N) WBSF values (Table 4.1). Similar results (Liste *et al.*, 2009) were reported where longer hours of lairages had an improvement on the tenderness of rabbit meat. Contradicting results were reported by Lukić *et al.* (2014) where instrumental WBSF was higher in longer rested animals. The reason for high WBSF values in 12 and 14 h rested animals is that, during transportation, muscles become active, muscle temperature rise above 30 ^{o}C causing a reduction in the adenosine tryphosphate (ATP). Once ATP is hydrolysed, pH rises above 6.0 leading to the formation of cross-bridges between thin and thick filaments. Due to the actomyosin effect of myofibrillar proteins, background effect of connective tissue and the bulk density of fat, tenderness is still a complex multi-factorial trait that requires more attention (Joo *et al.*, 2013).

4.3.3 Creatine kinase, lactate dehydrogenase and physico-chemical attributes of beef

The PCA was done to show the relationship between CK, LDH and physico-chemical attributes of beef. The results of eigenvalues and cumulative percentages that were revealed by PCA are presented in Table 4.2. The eigenvalues are used to determine the linear combinations of the PCs that have a greater variance. The first PC had the highest eigenvalue of 2.3 while the 2nd (1.6), 3rd (1.2) 4th (1.0) and 5th (1.0) PCs were less than 2. As observed (Table 4.2), the PCs had decreasing variances to predict those with great significance in the total variance. If a graph were to be plotted, PC 1 would be rated as the important meat quality attribute which determines the rest of them followed by meat temperature. Literature indicates that pH_u is the key to quality beef and is the standard measurement that determiners colour and tenderness of beef (Werner *et al.*, 2013). According to Ferguson *et al*. (2001); Hoffman *et al.* (2003) and Muchenje *et al.* (2009), pH_u that is higher (>5.8) than the normal values leads to undesirable beef colour.

Table 4.2 Eigenvalues and cumulative percentages of the principal component analysis

Number	Parameters	Eigenvalues	Percentage	Cumulative Percentage
1	pH_u	2.3454	23.454	23.454
2	T_m	1.6570	16.570	40.024
3	L*	1.2856	12.856	52.879
4	a*	1.1060	11.060	63.939
5	b*	1.0423	10.423	74.362
6	CL%	0.8635	8.635	82.997
7	TL%	0.7576	7.576	90.573
8	WBSF	0.4755	4.755	95.328
9	CK	0.3368	3.368	98.696
10	LDH	0.1304	1.304	100.000

pH_u - Ultimate pH, T_m - Meat temperature, L* - Lightness, a* - Redness, b* - Yellowness, TL% - Thawing loss percentage, CL% - Cooking loss percentage, WBSF - Warner Bratzler Shear Force, CK - Creatine kinase and LDH - Lactate dehydrogenase

Consumers use colour and tenderness to judge the quality of beef (Peña *et al.*, 2009; Guzek *et al.*, 2013). Principal components 5, 6, 7, 8 and 9 were less than 1.0 and the observed differences could imply that these parameters were least important when it comes to understanding the combinations of beef quality. Principal component analysis is a method that is used to reduce the number of correlated variables to a fewer number of variables without loss of any significant information (Karlsson, 1992; Cañeque *et al.*, 2004). The first 5 PC`s contributed about 74 % (23.5, 16.6, 12.9, 11.1 and 10.4 % respectively) of the total variance which can be the critical meat quality attributes that can be used to assess the acceptance of beef. From the above mentioned eigenvalues, further PCA was computed where the first 3 PCs were condensed since they make 53% of the variance of the cumulative percentage. As highlighted in Figure 4.2, a two dimensional relationship of the loadings for PC 1, 2 and 3, contributed to 23.5, 16.6 and 12.9% which represent parameters that determine the eating quality of beef.

The results reported by Destefanis *et al.* (2000) in beef quality measurements that included chemical analysis of meat, about 62.5 % that was explained by the first 3 components. The other parameters such as TL %, CL % and WBSF are not the first parameters to be considered as far as meat quality is concerned. This is explained by studies by Troy and Kerry (2010), Girolami *et al.* (2013) and Guzek *et al.* (2013) who indicated that colour is the first meat quality attribute in meat acceptance by consumers. There is a strong relationship between pH_u, muscle temperature and colour because of the amount of glycogen levels present in muscle. Glycogen levels may vary widely without any effect on pH and meat colour, provided they are above a critical level that will produce a bright cherry meat (Gispert *et al.*, 2000).

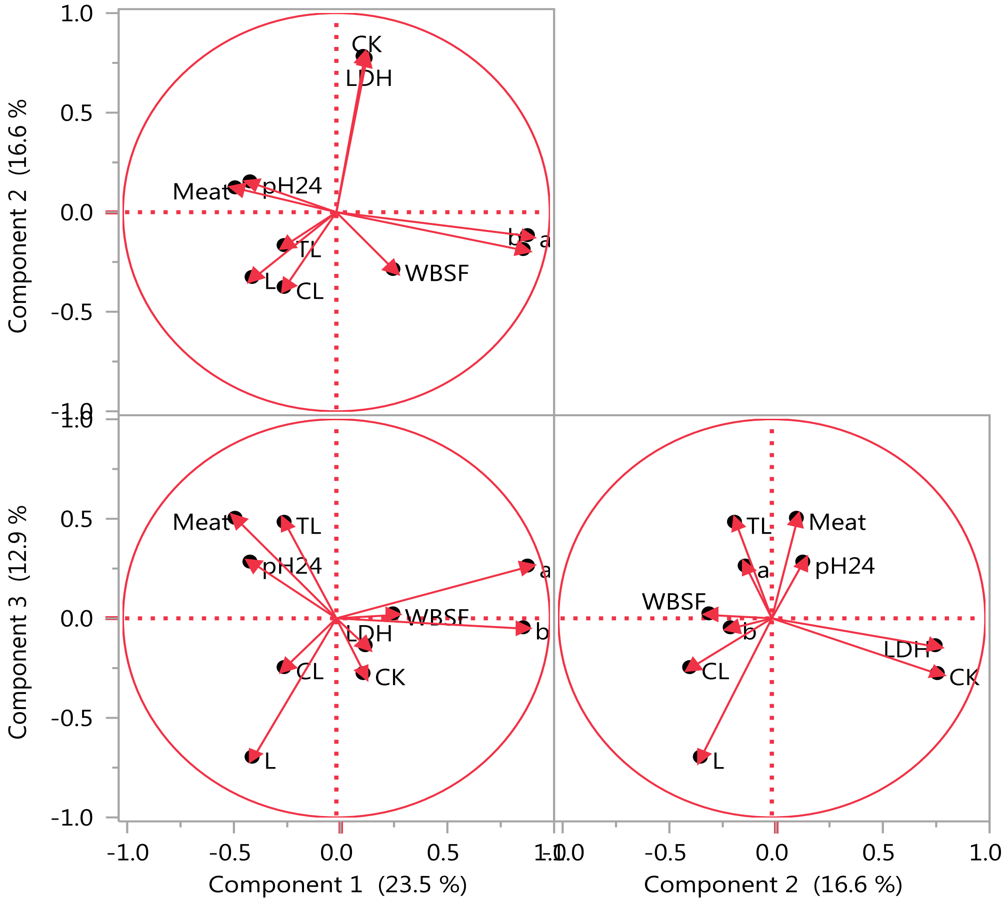

Figure 4.2 A two-dimensional relationship of the loadings for principal component 1, 2 and 3

pH_u - Ultimate pH, L* - Lightness, a* - Redness, b* - Yellowness, Meat - Meat temperature, TL - Thawing loss percentage, CL - Cooking loss percentage, WBSF - Warner Bratzler Shear Force, CK - Creatine kinase and LDH - Lactate dehydrogenase

Nonetheless, once the level is below the critical point, glycolysis speeds up causing an abrupt rise in pH and increased likelihood of dark meat colour (Geesink *et al.*, 2011). The relationship between PC 1 is that a*, b* and WBSF were positively related but were negatively related to CL, CK and LDH. The same relationship between meat quality attributes was observed in buffaloes by Kandeepan *et al.* (2013) explaining that tenderness is associated with the bright and attractive colour of meat. This is because of the low amount of collagen which is important in the muscle retaining reasonable amounts of water (Naveena *et al.*, 2011; Bhat and Pathak, 2012). In addition, nutrition, genetics, intramuscular fat, marbling score, myoglobin content and scattering proteins have a bigger role to play in improving the colour, tenderness and flavour of meat. Meat with the bright red colour indicates a long shelf-life and good eating quality (Kandeepan *et al.*, 2009).

4.4 Conclusions

It could be inferred from the current study that the activities of the CK were affected by distance travelled by cattle. Lactate dehydrogenase was not affected by distance travelled by the animals prior to slaughter. Distance travelled resulted in increased ultimate pH, darker and tougher beef. However, with long hours of lairage duration, there was an improvement in the quality of beef. The activities of CK and LDH were related since they are both released when an animal is experiencing stress in its environment. The first three PC`s explained about 53% of the total variability. The first PC was correlated with meat quality attributes where pH_u and T_m were positively correlated, but negatively related with a* and b* values. The activities of CK and LDH were related and useful to define the second PC, since CK and LDH are both released when an animal is experiencing stress in its environment. However, CK and LDH were not related to beef quality. Further research is needed to determne the relationship between carcass characteristics of cattle and their quality so as to identify beef breeds that can produce the required and acceptable beef by consumers.

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Chapter 5 Carcass and meat quality characteristics of bovine species slaughtered at a high-throughput abattoir

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Abstract

The objective of the study was to compare carcass weights and meat quality of bovine cattle slaughtered at a high through-put abattoir. Slaughter weight (SWT), warm carcass weight (WCW) and cold carcass weights (CCW) were recorded. Dressing percentage (DP) was also calculated. At 48 hours after slaughter, the *muscularis longissimuss thoracis et lumborum* (LTL) samples of beef carcasses were removed from the loin region to measure ultimate pH (pH_u), T_m (Meat temperature), lightness (L*), redness (a*), yellowness (b*), and Warner Bratzler Shear force (WBSF) values. There were significant genotype effects on SWT, WCW, CCW and DP. The Charolaise (CH) cattle were the heaviest (289.5 ± 6.29) than Non-Descript (ND) and Brahman (BR) cattle. There were significant ($P < 0.001$) class effects on carcass weights with bulls having the heaviest SWT (721.6 ± 19.50), WCW (353.4 ± 10.92) and CCW (354.5 ± 10.17) while cows and heifers were the lightest. Significant genotype effects on T_m ($P < 0.001$), L* ($P < 0.001$), a* ($P < 0.001$), b* ($P < 0.001$) and WBSF ($P < 0.05$) were observed. Principal component analysis (PCA) showed positive relationships between carcass weights and beef quality characteristics for cattle where the cumulative percentage of the first six principal component`s (PC`s) (SWT, WCW, CCW, DP, pH_u and T_m contributed to 93.4 % of the total variance. Therefore, the correlations observed between carcass weights and beef quality characteristics can be used to improve the quality of beef by maintaining beef genotypes and animal classes that produce a higher dressing out percentages.

Key words: Animal class, genotypes, correlations, dressing percentage, principal component analysis, Warner Bratzler Shear Force.

5.1 Introduction

Bovine species are widely known for the provision of protein. However, domestic animals fail to meet the existing protein and meat demands of the growing human population. A large number of cattle genotypes are kept in South Africa (Nqeno *et al.,* 2010). About 5% of meat is imported from Namibia, Botswana, Swaziland, Australia and New Zealand and from the European Union. Most cattle genotypes that are used for beef production include indigenous breeds (Afrikaner, Sanga, Tuli, Nguni, Bonsmara and Drakensberg) and the European breeds such as Angus, Charolaise, Hereford, Limousine, Simmentaler, Sussex, Brahman and Santa Gertrudis (Strydom *et al.,* 2000; Vieira *et al.,* 2007; Eltahir *et al.,* 2008; Farmers Weekly, 2014; Chapter 3 and 4). Nowadays, even the dairy breeds (Jersey and Holstein-Friesian) are also included in the meat logistic chain (Jurie *et al.,* 2007; Węglarz, 2010). Beef genotypes contribute the major part in meat production due to their genetic diversity (Scholtz, 2005; Kamieniecki *et al.,* 2009; Scholtz and Theunissen, 2010).

Beef cattle are always characterised by higher slaughter value and meat quality than the cattle that are utilised for milk production. In the communal areas, farmers normally practise inbreeding which leads to a number of non-descript cattle which affect the yield, especially in areas where there is limited feeding (Tada *et al.,* 2013).The quality and quantity of domestic beef is poor and low due to limited and inadequate veld management practises, resulting in overgrazing, overstocking and loss of weight especially during the dry season (Muchenje *et al.,* 2008; Musemwa *et al.,* 2008; Mapiye *et al.,* 2009; Iwanowska and Pospiech, 2010). Moreover, the transportation of animals to the abattoir for slaughter is known for its adverse effects on the welfare of animals and meat quality (Chapter 3 and 4). The quality of meat begins at the farm, feed-lot, transportation, lairage period, slaughter process, sex, age and genotype. There is a link between sex, feeding and slaughter weight (Ferguson and Warner, 2008; Silva *et al.,* 2010; Węglarz, 2011; Magolski, 2012). Losses in the quantity of meat as a result of bruises which are not suitable for human consumption have been observed and recorded (Strappini *et al.,* 2009).The slaughter value of cattle considers the amount of muscles excluding trotters, intestines and the head. The carcass quality together with slaughter value is associated with carcass components with higher commercial value. Animal classes have different carcass characteristics such that steers are characterised by a slower growth rate, lower feed efficiency although they have a higher intramuscular fat content as compared to bulls. They are known for tender meat and higher water holding capacity (Nogalski *et al.,* 2014).

The demand for meat animals and meat products is increasing due to the ever increasing human population, urbanisation and economic development (UN, 2011). However, the required improvement to meet the population needs is causing a serious challenge to animal scientists, farmers, butchers, supermarkets and the industry as well. Węlgarz (2010) indicated that the existing problems during transportation of animals to the abattoir cause a reduction in carcass weights which negatively affects the dressing out percentage. The slaughter weight including warm and cold carcass weights and dressing percentage are collectively termed as carcass characteristics (Iwanowska and Pospiech, 2010). It is a measure of traits that are sought and valued by the consumer. According to Cañeque *et al.* (2004), carcass weight is one of the most important factors that affect carcass and meat quality. Collectively, they are used to determine the amount of saleable meat and retail price obtainable by the farmers as well as the abattoir. Dressing percentage is probably the most important attribute in beef marketing since it determines the amount of saleable meat harvested from slaughtered animals (van Marle-Köster *et al.,* 2013).

Research has been conducted to compare the meat quality and carcass characteristics of Nguni, Bonsmara and Angus steers raised on natural pasture (Muchenje *et al.,* 2008), optimum finishing weight of young crossbred steers and bulls, based on their slaughter value (Nogalski *et al.,* 2014), carcass and meat quality of light lambs (Cañeque *et al.,* 2004). Sañudo *et al.* (2004) also conducted a study to determine the effects of slaughter weight, genotype and ageing time on beef quality. It was observed that live weight, genotype and ageing had a significant effect on Warner-Bratzler shear force values. Longer ageing periods of meat reduced the effect of genotype on tenderness, however; heavier (live-weight) animals had tougher meat. Surprisingly, there is little information on the correlations of carcass weights and physico-chemical attributes of beef at the point of slaughter. Therefore, the objective of the current study was to compare carcass weights and meat quality characteristics of cattle slaughtered at a high through-put abattoir.

5.2 Materials and Methods

5.2.1. Study Site

The study site is described in Section 4.2.1.

5.2.2 Animal Management

One-hundred and forty-six (n = 146) cattle (35 Brahman, 52 Charolaise and 59 Non-descript) that were brought to the abattoir for slaughter were used in the study. The number of animal classes (Heifers, bulls and cows) per genotype is presented in Figure 5.1. Animals were allowed to rest at the lairages while having *ad-libitum* access to water for 14 to 16 hours (overnight).

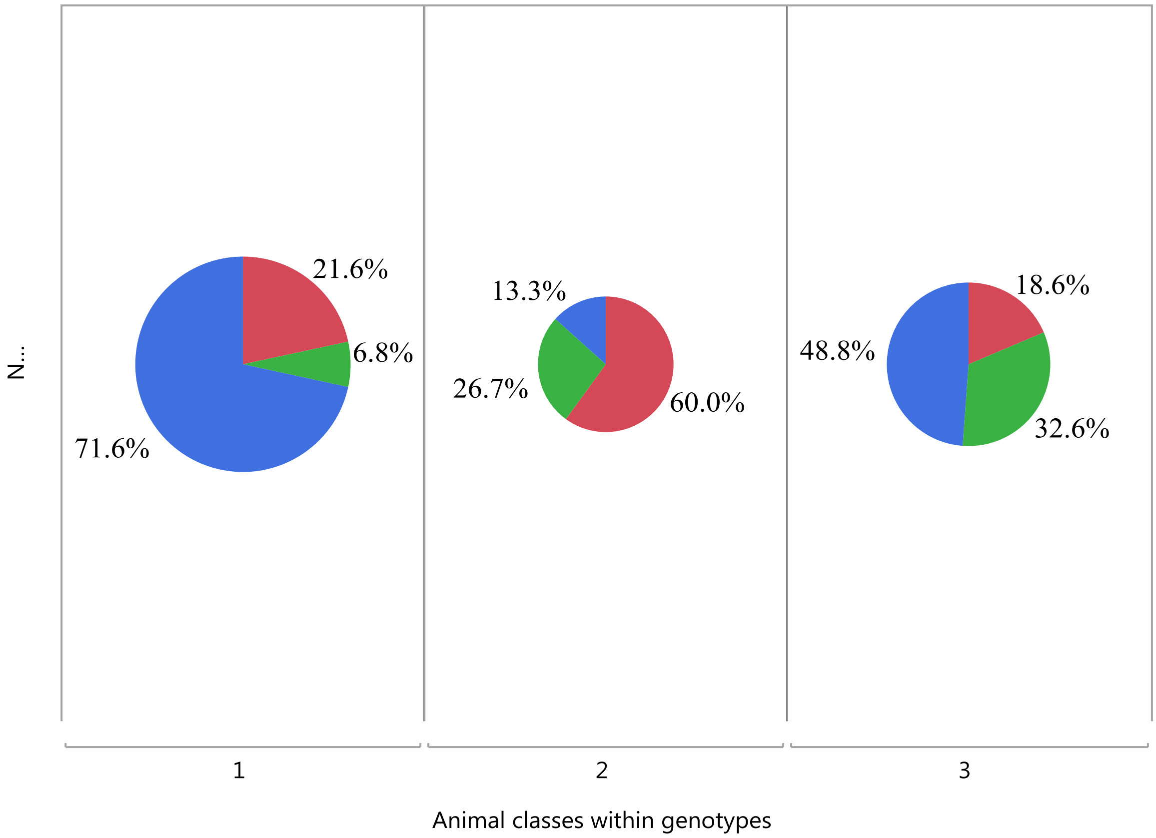

Figure 5.1 Percentage of animal classes within genotypes

Red – Heifers, Green – Bulls and Blue – Cows

N – Number

1 –Brahman, 2 – Charolaise and 3 – Non-descript cattle

5.2.3 Determination of carcass weights, conformation and fatness

On the day of slaughter, the animals were weighed to obtain the slaughter weight which was approximately 450 – 750 kg depending on breed and animal class (heifer, cow or bull). The captive bolt method of stunning with a voltage of 300 V and a current of 5A was used to stun the animals and then slaughtered. After evisceration, non-carcass components (head, skin, feet, lungs and trachea, liver, heart, spleen, gastro-intestinal tract and testicles) were removed. The carcasses were weighed individually to obtain warm carcass weight of each animal. The carcasses were chilled at 4 °C for 24 hours and the cold carcass weight was measured after chilling. Dressing percentage was calculated using slaughter weight and the cold carcass weight as follows:

$$\text{Dressing percentage} = \frac{\text{Cold carcass weight (kg)}}{\text{Slaughter weight (kg)}} \text{x } 100\,\%$$

Carcass grading classification of South Africa which considers age, conformation and fatness was determined for each animal. The mark used during meat classification is a quality assurance tool indicative of the differences in the monetary value between meat quality attributes (SAMIC, 2006). Age and class were considered as one character simply because most carcasses were ranging from AB, B and C. After sorting the data, it was observed that all the AB`s were heifers, B`s were bulls then C`s were dairy cows. Beef conformation (1 – very flat carcass, 2 – a flat carcass, 3 – medium carcass, 4 – a round carcass and 5 – a very round carcass) and fatness (0 – no visual fat, 2 – very lean, 2 – lean carcass, 3 – medium carcass, 4 – a fat carcass, 5 – over fat carcass then 6 represents an excessively over fat carcass) scales were used.

5.2.4 Beef quality measurements

Representative samples (100mm thick) of the *muscularis longissimuss thoracis et. lumborum* (LTL) were sampled from the 10^{th} rib to the third lumbar vertebra at 48 hours after slaughter (Section 4.2.4) to determine ultimate pH, meat temperature, colour (L*, a* and b*) and Warner Brazlter Shear Force (WBSF).

5.2.5 Statistical analysis

The data for the effects of animal class and genotype on carcass and beef quality characteristics (SWT, WCW, CCW, DP, conformation, fatness, pH_u, T_m, L*, a*, b* and WBSF) were analysed using PROC GLM. Significant means for animal class and genotype effect on carcass weights and beef quality were further compared by the PDIFF procedure of SAS (2003). Principal Component Analysis (PCA) was computed using JMP 9.0 (SAS, 2010) to determine the important carcass weights and beef quality characteristics and how they relate to each other (SWT, WCW, CCW, DP, pH_u, T_m, L*, a*, b* and WBSF).

5.3 Results and Discussion

5.3.1 Genotype effect on carcass weights

Table 5.1 show the effect of genotype and animal class on the carcass weights of beef (SWT, WCW, CCW and DP). There were significant genotype effect on SWT, WCW, CCW and DP. The CH cattle were bigger (289.5 ± 6.29) than ND and BR when considering their WCW although ND's SWT was the highest (554.9 ± 12.71). Considering the weight at 24hours after slaughter (CCW), ND had again the lowest weight (227.5 ± 4.33) as compared to CH and BR. The difference in slaughter weight is due to animal weight at maturity, genotype and how the animal has been fed. Vieira *et al.* (2007) and Muchenje *et al.* (2008) demonstrated that the live weight of cattle genotypes is largely due to their size at maturity, biological type and their growth rate.

The differences were also due to the fact that some of the animals were pregnant, and others had their carcasses trimmed-off during inspection because of bruises incurred during transportation resulting in reduced dressing percentage (Chandra and Das, 2001; Miranda-de la Lama, 2013). According to Riehn *et al.* (2010), there is an increasing incidence of slaughtering pregnant animals that are destined for meat production. Fayemi and Muchenje (2013) further stipulated that slaughtering of pregnant animals challenges the ethical evidence of such animals especially in areas where there is no law which encourages their slaughter. Besides that, slaughter of pregnant animals reduces the amount of saleable meat produced. McKiernan *et al.* (2007) also indicated that pregnancy, bruises and genotype are part of the causes of reduced dressing percentage in slaughter animals. According to Wheeler *et al.* (1996), Non-descript cattle tend to have fluctuating growth and carcass weights which negatively affect the dressing percentage.

Table 5. 1 Least square means and standard errors of carcass weights and beef quality from Brahman, Charolaise and Non-descript cattle

	Genotype			
Variables	**Brahman** (n = 35)	**Charolaise** (n = 52)	**Non-descript** (n = 59)	**P-values**
Slaughter weight	471.7^{a}±16.51	512.7^{b}±12.91	554.9^{b}±12.71	0.000
Warm carcass weight	255.3^{a}±11.4	289.2^{c}±6.90	262.1^{b}±4.41	0.0718
Cold carcass weight	237.1^{a}±6.44	258.5^{b}±11.75	227.5^{c}±4.33	0.0335
Dressing percentage	54.2^{b} ±0.60	55.4^{b}±0.50	50.3^{a}±0.47	<0.0001
Conformation	3.2±0.03	3.0±0.03	2.93±0.03	0.0843
Fatness	2.57±0.15	2.3±0.12	2.5±0.11	0.0465
Ultimate pH	5.7±0.04	5.7±0.03	5.8±0.03	0.0452
T_m	14.5^{a} ±0.22	16.5^{c}±0.18	15.5^{b}±0.17	0.0001
Lightness	32.1^{a}±1.40	38.6^{b}±0.76	33.5^{a}±0.54	<0.0001
Redness	18.8^{b}±0.52	16.1^{a}±0.42	18.4^{b}±0.48	<0.0001
Yellowness	15.7^{b}±1.46	13.3^{a}±0.37	18.4^{c}±0.39	0.0002
WBSF	51.5^{b}±2.20	48.6^{a}±1.80	54.4^{c}±1.69	0.043

^{abc}Means with different superscript within a row are significantly different.
P-values less than 0.05 are significantly different from each other
T_m – Meat temperature
WBSF – Warner Bratzler Shear Force

The dressing percentage for BR and CH was significantly higher (54.2 ± 0.60 and 55.4 ± 0.50) as compared to the ND cattle (50.3 ± 0.47). The poor performance of Non-descripts or local genotypes in terms of dressing percentage was reported by Li (2009). The reason for this difference in DP is much higher because head and trotters, the skin, udder, testicles, offals such as intestines, fat, lungs and liver are not included in the carcass. Dressing percentage is a factor that is used to calculate the carcass weight from a known parameter or estimate live-weight (McKiernan *et al.,* 2007; Khalafalla *et al.*, 2011). In abattoirs, slaughter weight of cattle is normally calculated as an average group weight of animals. This value is used to compare the live-weight price which allows a producer to estimate the carcass weight of a live animal (van Marle-Köster *et al.,* 2013).

Animals with more bruises normally, have reduced carcass weights. Bruised carcasses tend to have darker meat which is not ideal beef for the meat industry sector as well as consumers (Romero *et al.*, 2011). It was also highlighted that areas in the carcass that are dark in colour are an indication of injuries that occurred within 24 hours (Werner *et al.,* 2013). Strappini *et al.* (2009) stated that bruises do not affect the quality of meat but the quantity since the bruised areas are trimmed off. This has economic implications on the quantity of meat because of the increasing production demand by humans (Webb, 2013).

Significant (P < 0.001) effects of animals class on carcass weights were observed where bulls were the heaviest SWT (721.6 ± 19.50), WCW (353.4 ± 10.92) and CCW (354.5 ± 10.17). Heifers were bigger (267.0 and 256.7 kg) than cows when considering the WCW and CCW respectively. In the current study, heifers had highest DP (55.2 %) than cows (52.4 %) and bulls

(49.0 %). The lower DP in bulls could be attributed to the bruise occurrence that led to trimming of the carcass during meat inspection. Beef genotypes are characterised by higher DP as compared to dairy breeds (Kluyts *et al.,* 2007; van Marle-Köster *et al.,* 2013; Nogalski *et al.*, 2014). Bulls are also known for a broader, bigger body and tough muscles that affect the DP and their quality of meat. As for heifers, they can be fattened to heavy live weight without the risk of reducing carcass quality due to excessive fatness, while achieving very good dressing percentage and favourable tissue composition of the carcass (Mach *et al.,* 2008).

5.3.2 The effect of animal class on beef quality characteristics

The effects of animal class on beef quality characteristics of cattle are presented in Table 5.2. Significant genotype effects on T_m ($P < 0.001$), L* ($P < 0.001$), a* ($P < 0.001$), b* ($P < 0.001$) and WBSF ($P < 0.05$) were observed. Meat temperature (16.5 ± 0.18) and Lightness (38.6 ± 0.76) values for CH were higher as compared to ND and BR cattle. The difference in the L* values was due to the amount of myoglobin pigment in the muscle. The L* values determine the visual appearance of meat which also influences the purchasing decisions of consumers as reported by Brewer *et al.* (2004).

The Non-descript cattle had the highest values for yellowness (18.4 ± 0.39) and WBSF (54.4 ± 1.69) values as compared to BR (15.7 ± 1.46 and 51.5 ± 2.20) and CH (13.3 ± 0.37 and 48.6 ± 1.80) respectively. The reason for higher values of WBSF in the ND cattle was that, genotype is one of the factors that determines the quality of meat especially tenderness which is one of the most important cause of consumer satisfaction (Casas *et al.,* 2006; Silva *et al.,* 2010).

Monsón *et al.* (2004) reported that double-muscled animals had improved tenderness as compared to others. In this case, Charolaise had more tender meat than Brahman and Non-descript cattle. This is because of the lower collagen content and less stable cross-links which cannot be reduced. Sañudo *et al.* (2004) also indicated that the high WBSF values from heavy animals leading to tougher beef as a result of higher intake of grass all year round (Silva *et al.*, 2010). The amount of collagen in the muscle may be reduced and transformed into soluble collagen during cooking (Wyrwisz *et al.*, 2012). Therefore, an improved value of tenderness will improve the value of the final product.

There were no animal class effects (P > 0.05) on fatness, conformation, pH_u, L*, a* and b* values. Meat temperature and WBSF values for bulls were significantly higher (16.0 ± 0.36 and 57.7 ± 3.30 respectively) than for heifers (16.2 ± 0.26 and 44.2±1.80, respectively) and cows (115.2 ± 0.16 and 48.8 ± 1.42 respectively). This is because, bulls are characterised by high lean meat content, lower fat and higher content of bones as compared to heifers and cows resulting in lower DP. Bull meat has undesirable traits such as high pH_u, dark colour and tougher meat hence it becomes less desirable for direct market sales (Holló *et al.*, 2007; Węglarz, 2010; Schiavon *et al.*, 2011). Bulls that are raised with other cows and heifers tend to have a greater advantage of leaner meat grades as a result of less fat gained during feeding. The metabolism of both fat and protein in dairy animals is poor due to the production of nutrients for lactation (Hamada *et al.*, 2012).

Table 5.2 Least square means and standard errors of combined carcass weights and beef quality from Brahman, Charolaise and Non-descript cattle

	Animal class			
Variables	**Heifers** (n = 62)	**Bulls** (n = 29)	**Cows** (n = 55)	**P-values**
Slaughter weight	$484.5^{a}\pm10.68$	$721.6^{b}\pm19.50$	$497.8^{a}\pm8.39$	<0.0001
Warm carcass weight	$267.0^{a}\pm6.00$	$353.4^{b}\pm10.92$	$260.6^{a}\pm4.70$	<0.0001
Cold carcass weight	$256.7^{a}\pm5.57$	$354.5^{b}\pm10.17$	$254.5^{a}\pm4.38$	<0.0001
Dressing percentage	$55.2^{c}\pm0.50$	$49.0^{a}\pm0.99$	$52.4^{b}\pm0.43$	<0.0001
Conformation	3.0 ±0.03	3.0±0.05	2.9±0.02	0.3579
Fatness	$2.3^{a}\pm0.12$	$2.9^{b}\pm0.22$	$2.5^{a}\pm0.10$	0.0443
Ultimate pH	5.7 ±0.04	5.7±0.07	5.7±0.03	0.0689
T_m	$16.2^{b}\pm0.20$	$16.0^{b}\pm0.36$	$15.2^{a}\pm0.16$	0.0007
Lightness	32.3±0.56	31.7±0.03	31.7±0.44	0.6457
Redness	17.0 ±0.45	18.1±0.82	18.1±0.36	0.0629
Yellowness	13.9±0.40	15.5±0.73	14.8±0.37	0.0712
WBSF	$44.2^{a}\pm1.80$	$57.7^{c}\pm3.30$	$48.8^{b}\pm1.42$	0.0089

[abc]Means with different superscript within a row are significantly different.
P-values less than 0.05 are significantly different from each other
T_m – Meat temperature
WBSF – Warner Bratzler Shear Force

Dairy animals have poor carcass weights, meat quality and muscle traits. Heifers and steers are recorded to have better meat quality in terms of tenderness and average pH_u. Higher intramuscular content in muscles and smaller diameter of muscle fibre influences the tenderness of beef resulting in improved palatability due to juiciness and more flavour in meat (Li *et al.*, 2006; Weglarz, 2010).

5.3.3 Principal component analysis between carcass weights and beef quality characteristics

Table 5.3 indicate the correlations between carcass weights and beef quality characteristics for bovine cattle. PC 2 (WCW) contributed 23.2 % while PC 3 (CCW) explained about 13.1 % of the total variance. Eigenvalues greater than 5 % (0.5) indicate important parameters to be considered for improving the quality of products (Pinto *et al.*, 2006; Kamieniecki *et al.*, 2009). In this case, the first six principal components (PC's) were greater than 0.5 which is in agreement with the rule for principal component analysis (PCA) (Kopuzlu *et al.*, 2011). They contributed about 93.4 % of the total variance both carcass weights (SWT, WCW, CCW and DP) and beef quality (pH_u and T_m). The results indicate that carcass weights and ultimate pH can be used in the beef breeding programs due to the high consumer demand for quality grade, leaner and tender beef (Miar *et al.*, 2014). This can be achieved by estimating the animal genetic and phenotypic parameters. In this case, carcass weights determine the amount of fat and meat yield one would get which is correlated to ultimate pH (Utrera and Van Vleck, 2004). Ultimate pH is known by the role it plays in the conversion of muscle to meat, the improvement of colour and tenderness of beef (Hoffman *et al.*, 1990; 2007; Muchenje *et al.*, 2008).

Table 5.3 Eigenvalues and cumulative percentage for carcass weights and beef quality characteristics

Number	Eigenvalue	Percent	Cumulative Percent
1	3.0	29.4	29.4
2	2.3	23.2	52.6
3	1.3	13.1	65.7
4	1.1	10.7	76.4
5	1.0	10.2	86.6
6	0.7	6.8	93.4
7	0.4	4.1	97.5
8	0.2	1.4	98.9
9	0.1	0.9	99.8
10	0.0	0.2	100.0

A combined cumulative percentage of the first 3 PC`s (SWT, WCW and CCW, respectively) contributed to 65.7 % of the total variance which indicates the importance of carcass weights. This is in agreement with the report by Kluyts *et al.* (2007) and Burrow (2014) who highlighted that the value of the carcass is a function of the carcass quality. In cattle, Liu *et al.* (2013) and Miar *et al.* (2014) made use of an ultrasonic machine to detect carcass fat which is a good measure of animal cutability and the amount of intramuscular fat for improved beef quality. In addition, Vote *et al.* (2009) made use of Video Image Analysis (VIA) on the *lumborum* region to determine backfat thickness, intramuscular fat and meat yield since they determine the value of the saleable carcass per animal. Heifer and mature cow weights and dressing percentage are one of the variables with a high heritability value (h^2 = 0.32, 0.43 and 0.39 respectively) followed by carcass fatness in millimetres (h^2 = 0.44) as indicated by Kluyts *et al.* (2007).

Figure 5.2 and 5.3 are showing the bi-plot and screeplot of bovine carcass weights and beef quality characteristics. The biplot displays that these measurements are placed far from the beginning of the 1st PC and those that are close to each other indicate a high correlation. This means that carcass weights are determinants of meat quality even though ultimate pH might not be the major contributor to the normal values of colour and WBSF. In the current study, high positive correlations were observed between carcass weights; T_m, L*, DP and pH_u; between a* and b while there were negative correlations between carcass weights and a*, b* and WBSF. Those below the origin of the PC`s are negatively correlated. The observed relationship showed the efficacy of carcass weights for the improvement of meat quality and quantity. As observed in the previous study (Chapter 4), medium framed carcasses produced a tender and acceptable beef to consumers. This will supply tangible information to the genetic improvement programs.

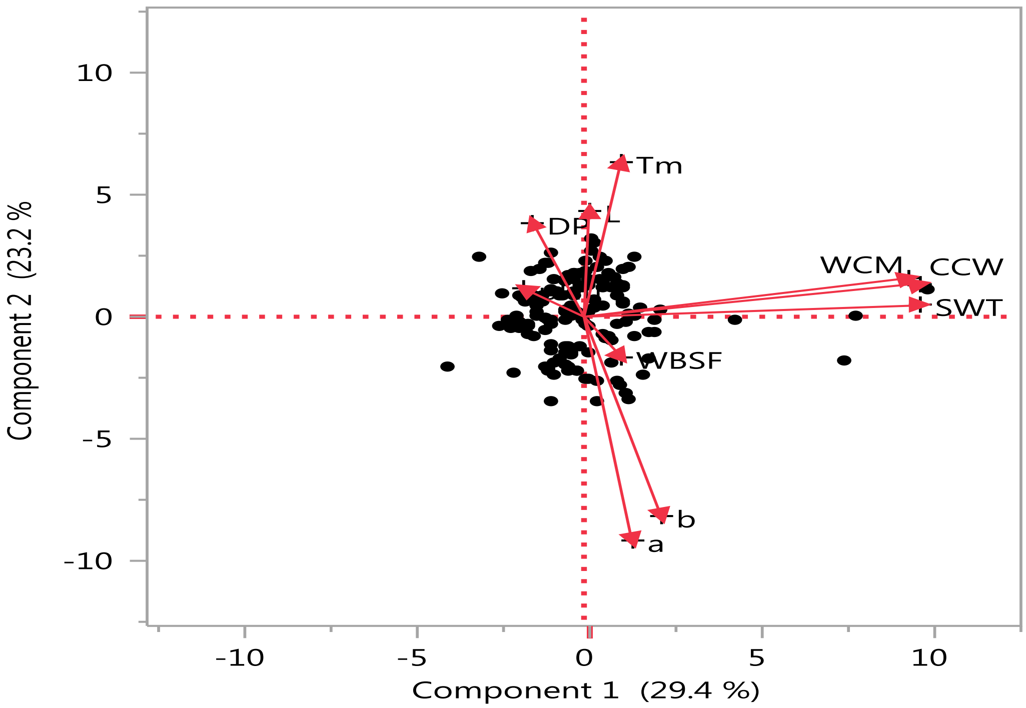

Figure 5.2 The scores and biplot of beef carcass characteristics showing the variance explained by component 1 and 2

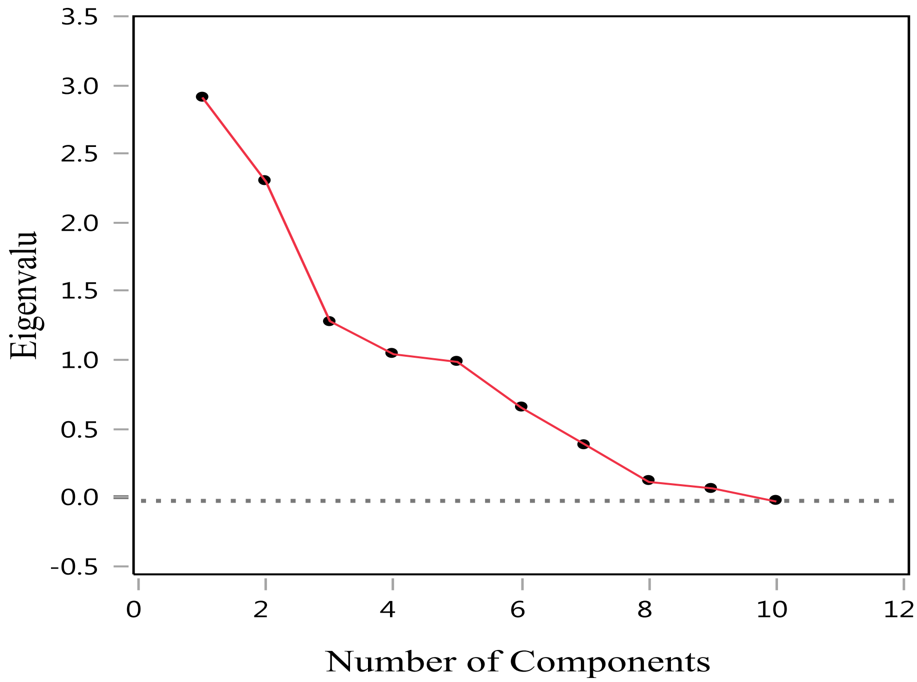

Figure 5.3 The Scree plot of the eigenvalues for beef carcass characteristics

5.4 Conclusions

In general, carcass weights were positively correlated to each other of which the relationship indicated the importance of early recording of weights as a basis for selection of animals. Therefore, the correlations observed from PCA between carcass quality traits can be used to improve the quantity and quality of beef by maintaining beef genotypes and animal classes that produce high dressing out percentages to boost the meat industry sector. Although the method does not give an analytical output to estimate the effects and differences between genotypes and classes, a better understanding of the underlying relationships were achieved. Further research is needed to understand the differences between the skeletal muscles of animals from different genotypes.

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Chapter 6 The effect of animal class, genotype and sex on the nanostructure of beef and meat quality

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Abstract

The objective of the study was to determine the effects of animal class, genotype and sex on the nanostructure of *muscularis longissimuss thoracis et. lumborum* (LTL) and beef quality. One-hundred and seventy (n = 170) cattle composed of 56 Bonsmara, 65 Non-descript and 49 Nguni were slaughtered at an A-throughput commercial abattoir in the Eastern Cape Province of South Africa. The animals were identified at the lairages and classified into genotype, class (heifers, bulls and cows) and sex (male and female). At slaughter, the captive bolt method was used to stun the cattle and the LTL muscle was removed after 24 hours *post-mortem*. Physico-chemical attributes of beef; ultimate pH (pH_u), lightness (L*), redness (a*) and yellowness (b*), Warner Bratzler Shear Force (WBSF) and histology of tissue structure as affected by animal-related and pre-slaughter stress were determined. The results showed significant correlations between distance travelled (DT) (r = 0.15), lairage duration in hours (LD_{hr}) (r = 0.29), ambient temperature (T_a) (r = -0.52), meat temperature (T_m) (r = 0.26), L* (r = 0.33), a* (r = 0.32) and WBSF (r = -0.49). The Brahman (BR), Non-descript (ND) and Nguni (NG) cows and heifers had visible skeletal fibres which were thin and long indicating improved tenderness of beef. The first important principal component's (PC's) as they appear from the analysis were pH_u, T_m, L*, a*, b* and WBSF. Therefore, animals classes; cows and heifers did not affect the nanostructures of beef, instead, the meat was tender due to longer and visible muscle fibers.

Keywords: Animal class, beef quality, genotype, muscle fiber, Scanning Electron Microscope, Warner Bratzler Shear Force.

6.1 Introduction

From farm to fork, different factors such as sex, age, nutrition, rearing conditions, and weight at slaughter, genetic and environmental condition usually interplay to determine the quality of meat including the amount of external and intramuscular fat, appearance and sensory properties (Iwanowska and Pospiech, 2010; Chapter 3, 4 and 5). Humans appreciate meat with a bright red colour, medium fat which improves flavour, juicier and tender meat. This is because meat is an important source of nutrients and most people consume it to also enjoy flavour, aroma and the tenderness it provides (Maltin *et al.*, 1998; Vimiso *et al.*, 2012; Muchakilla *et al.* 2014). Meat is composed of about 300 distinct muscles of the carcass including the fat deposited in the muscles and connective tissues (Sisson and Grossman, 1953). Muscles are differentiated into smooth, cardiac and skeletal depending on their structure, contractile properties and control. They are responsible for support and movement of the animal skeleton which is initiated voluntarily by some impulses of the neurons in nervous system (Loeb and Ghez, 2000; Fayemi, 2013).

The chemical composition and other biological properties of these muscles are significant for a better understanding of the major causes of variation in meat quality, particularly colour, intramuscular fat and tenderness. Meat tenderness is influenced by the amount of myofibrillar and connective tissue of the muscle tissue (Xia *et al.*, 2007). Mapiye *et al.* (2011) highlighted that, the appropriate utilisation of cattle genotypes with the necessary dietary regimes could be the way forward in order to meet the quality that is required by consumers. According to Strydom *et al.* (2008) and Muchenje *et al*. (2008), among the local genotypes, Nguni cattle has favourable genes that contribute to better performance of this genotype in terms of meat quality. However, besides genotypic characteristics, there are some factors such as pre-slaughter conditions, stunning, animal class, age and nutritional factors which distort the acceptability of beef and pork by consumers (Terlouw, 2005; Terlouw and Rybarczyk, 2008; Robinson *et al.*, 2001; Chapter 3; 4 and 5). In addition, Swatland (2003) highlighted that it becomes a challenge to improve tenderness through genetics if the nanostructural componets such as sarcomere length, muscle fibre orientation and fibre texture are not known. This is because the meat becomes darker when it is affected in the pre-slaughter environment leading to dark firm dry meat (DFD).

Some beef muscles have received less attention due to reduced tenderness and discolouration defects whereas the meat industry is looking for reliable meat quality information throughout the production process that will guarantee high-quality meat products for the consumers (Damez and Clerjon, 2008). This is because; the variability in the meat quality prevents the industry from marketing its produce according to its quality (tenderness, colour, water-holding capacity and juiciness). Studies have been conducted on the improvement for meat using genetic variation (Robinson *et al.,* 2001; Greeff *et al.,* 2008), optical scattering and absorption coefficients of beef (Xia *et al.*, 2007), sensorial consumer evaluation (White *et al.,* 2006; Muchenje *et al.,* 2008), use of a microscope attached to the video image analysis (VIA) (Muchenje *et al.,* 2008; Mapiye *et al.,* 2011) to measure sarcomere length where a longer sarcomere resulted in more tender beef as a good measure of tenderness. However, there is little information regarding the use of the scanning electron microscopy to evaluate the tenderness of beef according to genotype and distance travelled by animals prior to slaughter. Therefore, the main objective of this study was to determine the quality of beef as affected by genotype, animals and distance travelled using the scanning electron and an Instron machine.

6.2 Materials and Methods

6.2.1 Study site

The study was conducted in Buffalo City Municipality at a commercial East London abattoir found in Eastern Cape Province of South Africa. The abattoir is governed by the Meat Safety Act of 2002 and SAMIC (2006). The abattoir is located at 120 km South East of Alice (Nkonkobe Municipality).

6.2.2 Animal Management/ Experimental design

One-hundred and seventy (n = 170) cattle composed of 56 Bonsmara, 65 Non-descript and 49 Nguni cattle that were brought to the abattoir from different environments were used in the study. Upon arrival at the abattoir, distance travelled by the animals was obtained from the drivers. The minimum and maximum distance travelled (DT) and lairage duration (LD_{hr}) recorded ranged between 183 – 300 km and 12 – 16 hours. The low and high ambient temperatures ranged from 14.5 – 19.5 ^{o}C between May and July 2014 (Autumn-winter season). Animals were allowed to rest at the lairages while having *ad-libitum* access to water overnight. The captive bolt method of stunning was used to stun the animals before they were slaughtered following slaughter procedures at the abattoir.

6.2.3 Meat quality parameters

At 24 hours after slaughter, the split carcasses were used to cut representative samples (100 mm thick) of the *muscularis longissimuss thoracis et. lumborum* (LTL) between the 10th rib and the third lumbar vertebra at 48 hours after slaughter. The measurements of pH_u and colour coordinates (L*, a* and b*) were carried out at 48 hours after slaughter using the same sample.

6.2.4 Determination of ultimate pH, colour and Warner Bratzler Shear Force

As described in Chapter 4.2.4.

6.2.5 Scanning Electron Microscopy Analysis

During the period of dehydration, each sample was kept in ethanol for 20 minutes in an ascending order of 10% up to 100% respectively. In order to improve the electrical conductivity of the sample surface in the SEM, a thin film of gold palladium was used for sputter coating to enhance the analysis. Critical Point Drying (CPD) was performed using the Hitachi critical point dryer HCP-2 (Hitachi Koki Co Ltd, Tokyo Japan) to prevent the samples from alteration and to boost good structural preservation. This was done by mounting the samples on aluminium stubs with double-sided carbon tape then the sputter coating with gold-palladium (Au-Pb) using the Eiko IB.3 Ion Coater (EIKO Engineering Co TD, Japan). The samples were then observed under the JEOL JSM-6390LV scanning electron microscope (SEM) for the determination of the skeletal surface area of beef muscles.

6.2.6 Statistical Analysis

PROC CORR (SAS, 2003) was performed to measure correlations between pre-slaughter conditions (distance travelled, lairage duration and ambient temperature) and meat quality characteristics (fatness, conformation, pH_u, Tm, L*, a*, b* and WBSF). The nonastructures of the skeletal surface area for beef samples (Nguni, Brahman and Non-descript) were sputtered with gold for visibility of the image using JEOL JM-5600 scanning electron microscope (SEM) at x 5,000 magnification. The relationship between pH_u, L*, a*, b* and WBSF among animals classes where genotype was used as a random variable, were determined using the Principal Component Analysis (PCA) of JMP 9.0 (SAS, 2010).

6.3 Results and Discussion

6.3.1 Animal-related factors, pre-slaughter conditions and beef quality

Table 6.1 shows the correlations between pre-slaughter conditions (Distance travelled, lairage duration and ambient temperature) and meat quality characteristics (pH_u, T_m, L*, a*, b* and WBSF). Positive correlations were observed between DT (r = 0.15), LD_{hr} (r = 0.29), T_m (r = 0.26), L* (r = 0.33), a* (r = 0.32) while DT was negatively correlated with T_a (r = -0.52) and WBSF (r = -0.49). Animal classes of different genotypes of cattle do not exhibit the same body weight, conformation and fatness. Genotype, age and weight of the animal at slaughter determine the type of meat grade that will be produced (Hulof and Ouhayoun, 1999; Sañudo *et al.*, 2004; Hanzelková *et al*., 2011). The Nguni cattle has been reported to produce leaner meat grades as compared to Bonsmara and Angus genotypes due to variations in intramuscular fat, fibre types and moisture content which is appreciated by most consumers (Muchenje *et al.,* 2008). However, due to different fibre types that skeletal muscles possess, this could be the cause of variation between these 3 genotypes. A similar report by Ryu *et al.* (2008) who indicated that the type of breed has an influence on fibre composition, proteins availability, molecular, and contractile texture of the muscle.

It has also been reported that as the animal ages, the meat becomes reddish, tougher and darker due to the amount of collagen, haem pigment and myoglobin content in the muscles (Hulof and Ouhayoun, 1999; Martínez-Cerezo *et al.*, 2005; Sañudo *et al.*, 2014).

Table 6.1 Pearson correlations between pre-slaughter conditions (distance travelled and lairage duration) and meat quality characteristics

	DT	LD_{hr}	pH_u	T_m	L*	a*	b*	WBSF
DT	1							
LD_{hr}	0.83***	1						
T_a	-0.45***	-0.54***						
pH_u	Ns	0.17*	1					
T_m	0.52***	0.39***	-0.21**	1				
L*	0.25**	0.32***	-0.19*	0.32***	1			
a*	-0.23***	-0.31***	ns	-0.39***	-0.38***	1		
b*	ns	0.18**	ns	-0.21**	ns	0.70***	1	
WBSF	ns	-0.22**	ns	-0.22**	-0.23**	0.30***	0.26***	1

Significance level: *P < 0.05, **P < 0.01, ***P < 0.001 and ns – not significant (P > 0.05)

DT - Distance travelled, LD_{hr} - lairage duration, pH_u - Ultimate pH, T_m - Meat temperature, L* - Lightness, a* - Redness, b* - Yellowness and WBSF - Warner Bratzler Shear Force

The relationship observed between genotype, DT, LD_{hr} and T_a is linked to the response of cattle to different environmental conditions prior to slaughter. There is a decline in muscle performance during long hours of transportation. This is associated with muscle activity which can be recovered after enough hours of rest at the lairages (Allen *et al.,* 2008). However, if care is not taken, some animals arrive dead at the abattoir which is a loss to the industry as well (dalla Costa *et al.,* 2007; Hoffman and Lühl, 2012). Stressed animals have darker meat, tougher and reduced water holding capacity (Van der Wal *et al.*, 1999). There were no significant correlations between pH_u and other meat quality parameters except for T_m (r = -0.21) and L* (r = -0.19) while T_m was significantly correlated with L* (r = 0.32), a* (r = -0.39), b* (r = -0.21) and WBSF (r = -0.22). Similar results were reported by Kušec *et al.* (2003) in pigs where there was no correlation between pH_u and other meat quality parameters. The observed relationship between pH_u and L* was also reported by Li *et al.* (2013) and Vimiso and Muchenje (2013) in beef. This implies that the amount of myoglobin content is largely affected by pH decline in the muscles after slaughter (Byrne *et al.,* 2000). Muscle pH is lowered during *post-mortem* storage of meat as a result of accumulation of lactic acid during glycolysis. However, contrasting reports by Purchas (1990) indicated that ultimate pH and colour co-ordinates were not related.

6.3.2 Nanostructure of beef muscles

Figure 6.1 and 6.2 shows the nanostructure of LTL muscle from the Non-descript, Bonsmara and Nguni cattle of different classes (bull, cow and heifer). The Non-descript, Bonsmara and Nguni bull revealed less visible, visible and thick, and visible and thin fibres with intercalated discs, respectively.

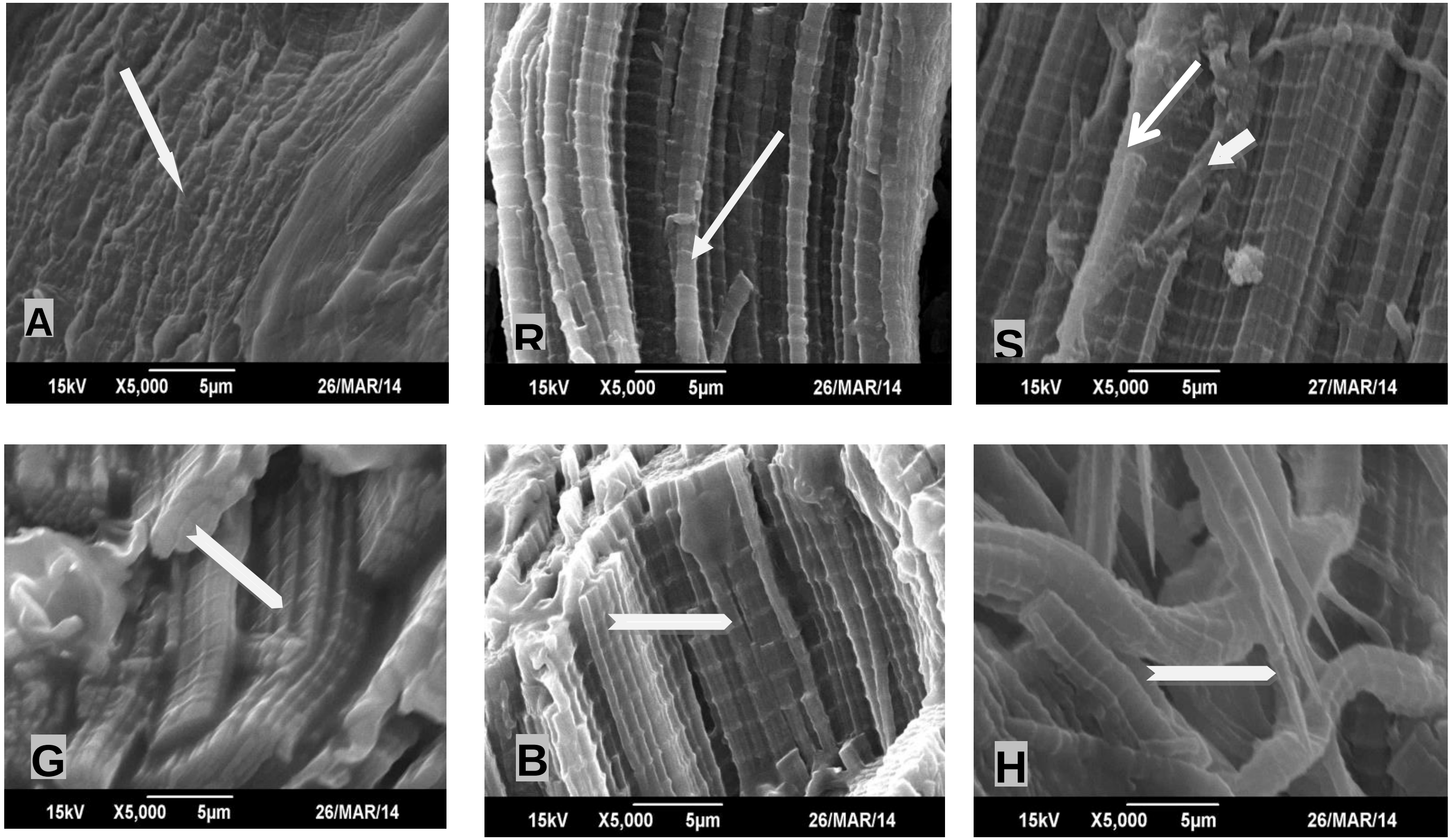

Figure 6.1 The micrograms of different cattle genotypes
A (Bull), R (Cow) and S (Heifer) – Non descript cattle; G (Bull), B (Heifer) and H (Cow) – Bonsmara cattle

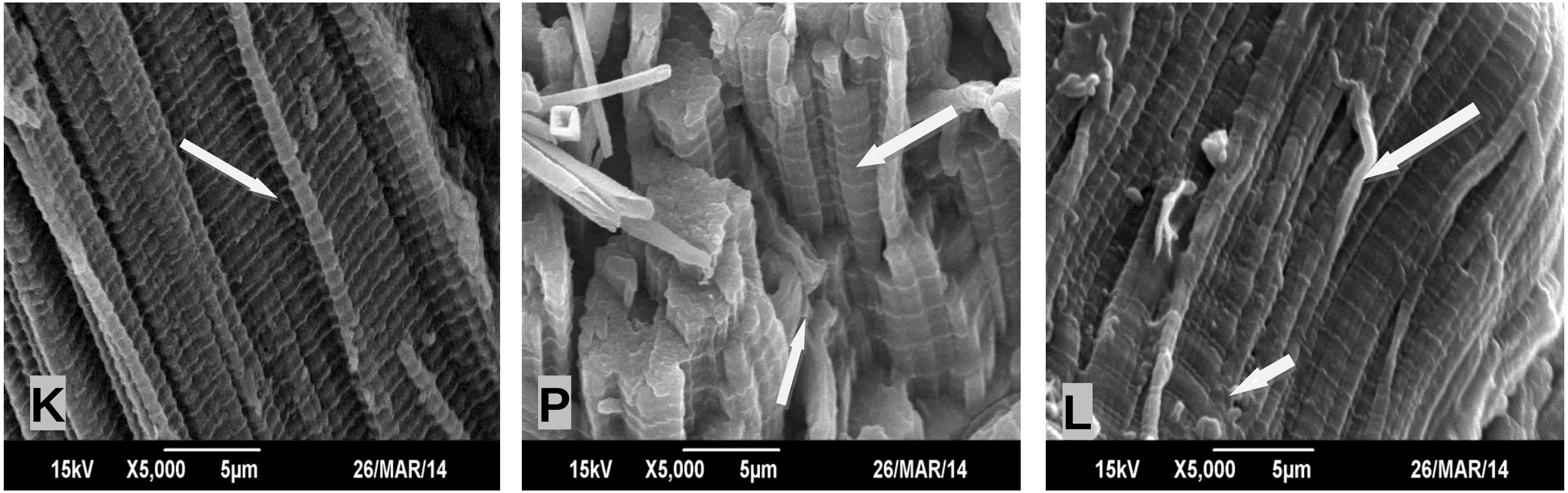

Figure 6.2 The micrograms of different animal classes of Nguni cattle genotype

K (Bull), P (Cow) and L (Heifer)

It was observed that BR, ND and NG cows had visible fibres which were thin and long for improved tenderness. The observed differences in the muscle fibres was anecdotally been reported to be due to finer grain some genotypes possess, making the muscle fibres to be visible at the microscopic level (Damez and Clerjon, 2008; Christensen *et al.*, 2011). This visibility is much more appreciated by consumers because it favours tenderness. As for heifers, it was only the NG and ND cattle with thin and visible intercalated discs, with few traces of intramuscular fat that enhances flavour and tenderness. This is because intramuscular fat consist of fat cells which are situated in the perimysium and the endomysium that sorrounds myofibrils and muscle fibre bundles. Intramuscular fat increases with an increase in animal age because it develops late in the maturation of cattle (Koohmaraie and Geesink, 2006; Ahnström, 2008; Webb and O'Neill, 2008). However, the BR heifers had visible thick muscle fibre which symbolizes tougher meat. This is linked to variations in glycolytic rate of fibre types between animal classes of different genotypes, changes in growth, carcass and meat quality traits. As the animal advances in age, the amount of collagen content and the stability of cross-bridges increase causing reduced tenderness (Damez and Clerjon, 2008; Petracci and Cavani, 2012)

Figure 6.3 indicates the length between intercalated discs of beef muscles. The Non-descript cow length between the z-line was ranging between 967.47 nm to 1.33µm whereas the width was between 441.81 and 684.69 nm. This is an indication of a significant difference between fibre orientations of cattle genotypes. Fibre orientation is linked to the tenderness of meat as it is greatly influenced by muscle structure of different genotypes and age groups (Muchenje *et al*., 2009). This was indicated by Koohmaraie and Geesink (2006) where it was observed that proteins such as nebulin and desmin also play a part in the *post-mortem* tenderisation of meat since they lie next to the z-line.

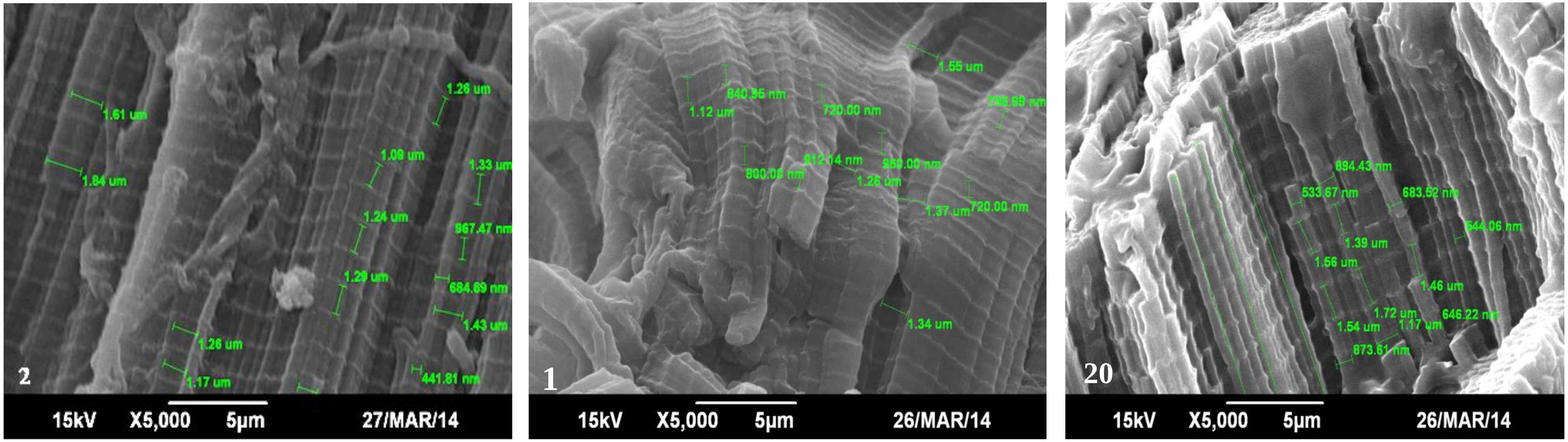

Figure 6.3 The length and width between the intercalated discs of the Non-descript cow, Nguni and Brahman cow, respectively.

The sliding motion of many cross-bridges forces the thin filaments (actin) towards the centre of a sarcomere, making the short fibres to affect the sarcomere length hence meat becomes tough (Loeb and Ghez, 2000). A study that was conducted in chicken by Łukasiewicz *et al.* (2014) indicated that genotype has an influence on the histological structure of muscles such that tenderness, moisture content and muscle pH becomes affected. Tougher meat is associated with lean and older animals especially those that have been stressed in the pre-slaughter environment (Purchas *et al.*, 2001).

The length between the z-line of the Nguni male was between 720 nm and 1.12 µm whereas the Brahman cow had a length of 1.39-1.72 µm. The shorter distance between the z-lines for the Nguni males indicate tougher meat because the length falls below the normal values of 1.5-3.5 µm (Loeb and Ghez, 2000). The results reported by White *et al.* (2006) in hot-boned and non-electrically stimulated beef muscles had shorter sarcomeres resulting in higher values for Warner Brazler Shear Force (WBSF). In addition, it should be noted that, males usually have tougher meat than females due to differences in the level of carcass fatness between sexes (Lind *et al.,* 2011; Guerrero *et al.,* 2013). Swatland (2003) indicated that it is important to understand the function of sarcomere length which forms fibres in order to be able to improve the tenderness of meat. Studies indicate that the microstructure alterations in beef intramuscular connective tissue were caused by highdrodynamic pressure processing (HDP) (Zuckerman *et al.*, 2013). Xia *et al.* (2007) characterized cooked beef muscles using an optical scattering and absorption coefficients to measure tenderness.

6.3.3 Beef quality parameters

Table 6.2 represents the eigenvalues of beef quality attributes according to the percentage they contribute to the total variance. The first important principal component's (PC's) as they appear from the principal component analysis (PCA) were pH_u, T_m, L*, a*, b* and WBSF with eigenvalues greater than 0.5. Literature indicates that PC's with the eigenvalue greater than 0.5 are considered to be important to contribute to the quality of beef (Kopuzlu *et al.,* 2011; Chapter 5). In agreement with the PCA rule, the cumulative percentage of the first 6 PC's contributed about 95 % of the total variance. The technological or physico-chemical properties of meat include meat pH, water holding capacity, colour, tenderness, thawing and cooking loss, evaporation loss and water distribution (Muchenje *et al.,* 2008; Gajana *et al.,* 2013; Li *et al.,* 2013). According to Hoffman *et al*. (2007), meat quality is the sum of all quality factors of meat in terms of the sensory, nutritive, hygienic and toxicological and technological properties. Muchenje *et al.* (2008) indicated that pH_u is the first meat quality attribute that affect colour and tenderness of beef. As observed in this study, pH_u contributed about 32 % of the total variance. However, it was reported that pH_u is the weak predictor of the final pork quality because it was not related to either of the meat quality traits (Kušec *et al.,* 2003).

Table 6.2 The overall eigenvalues of beef as shown by principal component analysis from cows, heifers and bulls

Principal component`s	Eigenvalue	Percentage	Cumulative Percentage
Ultimate Ph	2.6	32.0	32.0
Meat temperature	1.4	17.0	49.0
Lightness	1.2	14.0	63.0
Redness	1.1	12.8	75.8
Yellowness	1.0	12.0	87.7
WBSF	0.6	7.2	95.1
Fatness	0.3	4.1	99.2
Conformation	0.1	0.8	100.0

WBSF – Warner Bratzler Shear Force

In Table 6.3 - 6.5, shows the eigenvalues of PCA results from heifers, bulls and cows, respectively. The first 2 PC's (pH_u and T_m) of beef from heifers contributed about 95 % of the total variance while bulls and cows contributed about 66 % and 62 %, respectively. This indicates that the quality of beef from heifers is determined by only 2 meat quality parameters which are pH_u and T_m while meat from cows can be determined by 4 parameters (pH_u, T_m, L* and a*). Ultimate pH from heifers, bulls and cows had the highest percentage (56, 40 and 41 %, respectively) in comparison to the other meat quality parameters. There is little or no research conducted around this area. However, based on the observations, ultimate pH which made the highest contribution in all animal classes (heifers, bulls and cows) is the major determinant of beef quality (Muchenje *et al*., 2008; Chapter 3 and 4). This agrees with the reports by Andrés-Bello *et al*. (2013) who indicated that pH is the major determinant of functional foods because of the influence it has in other meat quality characteristics.

The concentration of hydrogen ions in meat is the determinant of the proceeding chemical reactions that will occur especially when the animal is in a stressful environment which apparently reduces the quality of meat (Dewi *et al*., 2011; Egan *et al*., 2015). Ultimate pH has an effect on the content of myoglobin which is responsible for the bright colour of meat. In addition, it is responsible for the improvement of growth and mortality of mircoorganisms and enzymatic activities which determine the shelf life of meat. Meat temperature is involved because at high temperatures, the conditions favours the growth of microorganisms which eventuallty deterioriates the quality and shelf-life of meat. Moreover, increased meat temperature also increases the mobility of hydrogen ions as a result of the dissociation that occurs in the molecules leading to a change in pH (Gould, 2000; Hambrecht *et al*., 2004; Lin *et al*., 2011; Egan *et al*., 2015; Singh *et al*., 2015).

Table 6.3 The eigenvalues of beef from heifers

Principal component`s	Eigenvalues	Percentage (%)	Cumulative Percentage
Ultimate pH	3.353	55.894	55.894
Meat temperature	2.332	38.871	94.765
Lightness	0.314	5.235	100.000

Table 6.4 The eigenvalues of beef from bulls

Principal component`s	Eigenvalue	Percentage (%)	Cumulative Percentage
Ultimate pH	2.410	40.166	40.166
Meat temperature	1.546	25.768	65.934
Lightness	1.477	24.620	90.554
Redness	0.395	6.595	97.149
Yellowness	0.171	2.851	100.000

Table 6.5 The eigenvalues of beef from cows

Principal component`s	Eigenvalue	Percentage (%)	Cumulative Percentage
Ultimate pH	2.487	41.460	41.460
Meat temperature	1.261	21.024	62.485
Lightness	0.942	15.704	78.189
Redness	0.714	11.914	90.103
Yellowness	0.547	9.124	99.226
WBSF	0.046	0.774	100.000

Lightness of beef form heifers contributed about 5 % although in bulls and cows it contributed about 24 and 15 % of the total variance, respectively. This simple means that even though L* is less important in the improvement of meat for heifers, for bulls and cows, it is an important parameter which needs to be considered for beef improvement. The inclusion of L* is determined by the age of the animal which is also determined by the accumulation of fat, amount of myoglobin content and the type of feed consumed. Myoglobin is the principal protein (water soluble) found in muscles which is responsible for the bright colour of meat (Mancini and Hunt, 2005; Heinz and Hautzinger, 2007). Once the colour of meat is reduced, the acceptability of meat is aslo affected. According to Fernandez *et al*. (1997), Dewi *et al.* (2011) and Andrés-Bello *et al*. (2013); colour is one of the important meat quality characteristics which may affect even the sensory perception and consumer acceptance of meat. Furthermore, consumers associate discolouration of meat as an indicator of its freshness and wholesomeness. Mancini and Hunt (2005) further indicated that the formation of metmyoglobin depends on temperature and pH hence it was observed that meat lightness was the 3^{rd} PC`s after pH_u and T_m. however, further research is needed to determine the amount of myoglobin that is required to give a bright and acceptable beef from different genotypes of cattle and animal classes.

Figure 6.4 is a 3-D scatter plot of the first 3 eigenvalues of beef by genotype. The three genotypes (Bonsmara, Non-descript and Nguni) of cattle had almost similar PC’s as observed in the scatter plot. The colour of beef from BR was less than the normal values (< 34 %) while the colour of ND and NG was within the expected values.

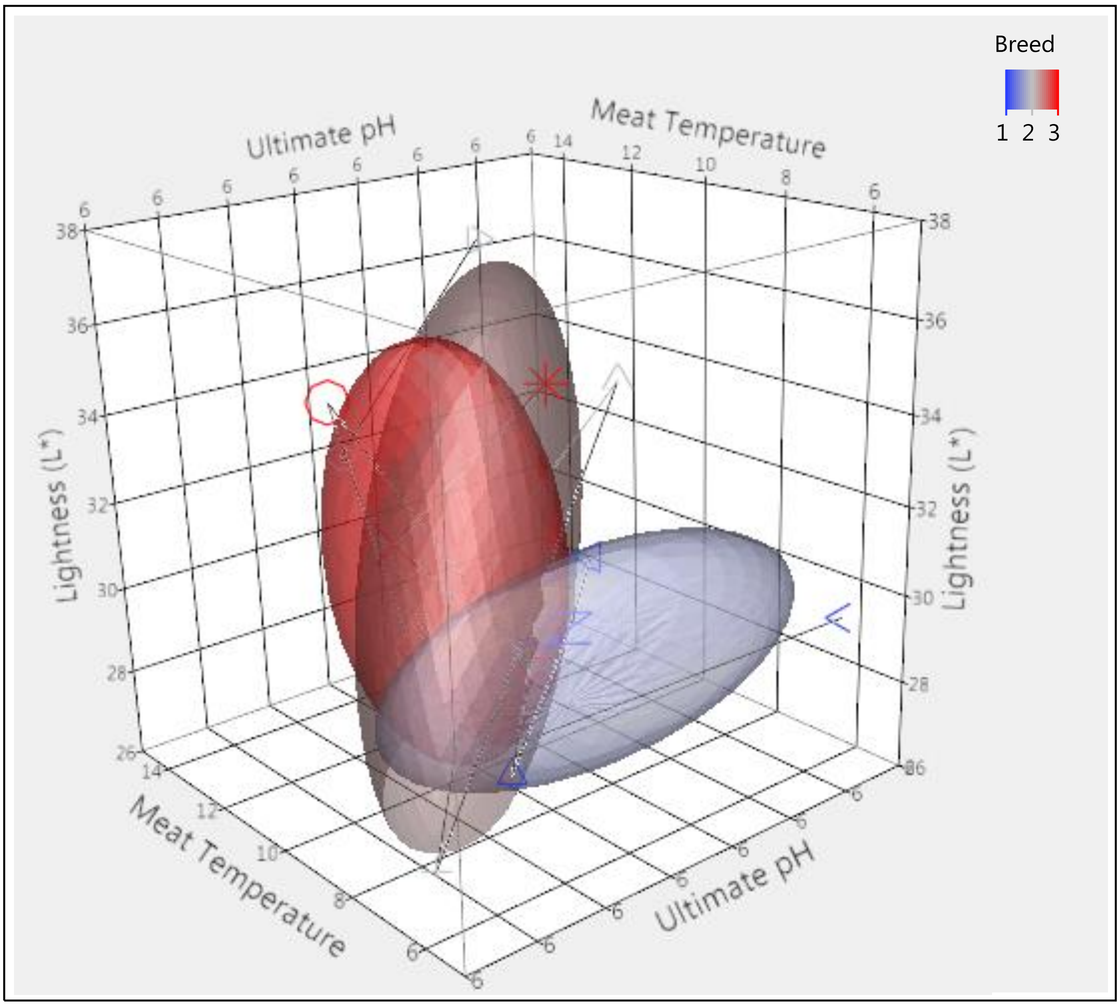

Figure 6.4 The overall 3-D scatter plot of the first three principal components of beef from all genotypes of cattle

1 – Bonsmara, 2 – Non-Descript, 3 – Nguni

In Figure 6.5 – 6.7, the first 3 eigenvalues of beef from heifers, bulls and cows, respectively are presented. Heifers, bulls and cows of the Nguni cattle had normal pH_u, T_m and L*. Sañudo *et al.* (2014) reported that, meat becomes darker owing to higher consumption of the mitochondria resulting in poor colour stability. The results are also as a result of age differences between these genotypes as it was observed that older animals tend to have a higher myoglobin content which lowers the L* value resulting in darker meat (Mancini and Hunt, 2005). The observed darker meat in BR cattle was linked to reduced intramuscular fat as it also plays a role in the brightness of meat (Mapiye *et al.,* 2011). In addition, darker meat is caused by an increase in ultimate pH as a result of glycogen levels and reduced production of lactic acid. Li *et al.* (2013) highlighted that pH_u influences the extent of protein denaturation, colour and water holding capacity of fresh meat. In addition, the pH variation is due to the mitochondria consumption (Hudson, 2012), size, thickness and the location of the muscle.

As observed in Table 6.2, pH_u is the primary attribute that may control the outcome of the other meat quality attributes. This implies that when considering the improvement of beef, one has to make sure that the factors that influence pH are minimised as much as possible. In agreement with this study, reports by Scholtz (2007); Li *et al.* (2013) and Vimiso and Muchenje (2013) indicated that pH_u is the major influence of meat quality and the extent of protein denaturation. In addition, the amount of protein in the muscles is negatively affected with reduced metmoyoglobin which affects the amount of water holding capacity (WHC) and colour stability of the muscle (Kim *et al.,* 2010; Andrés-Bello *et al*., 2013). This was in agreement with reports by) where the variation of the pigment and oxidation state led to differences in the L* and a* values.

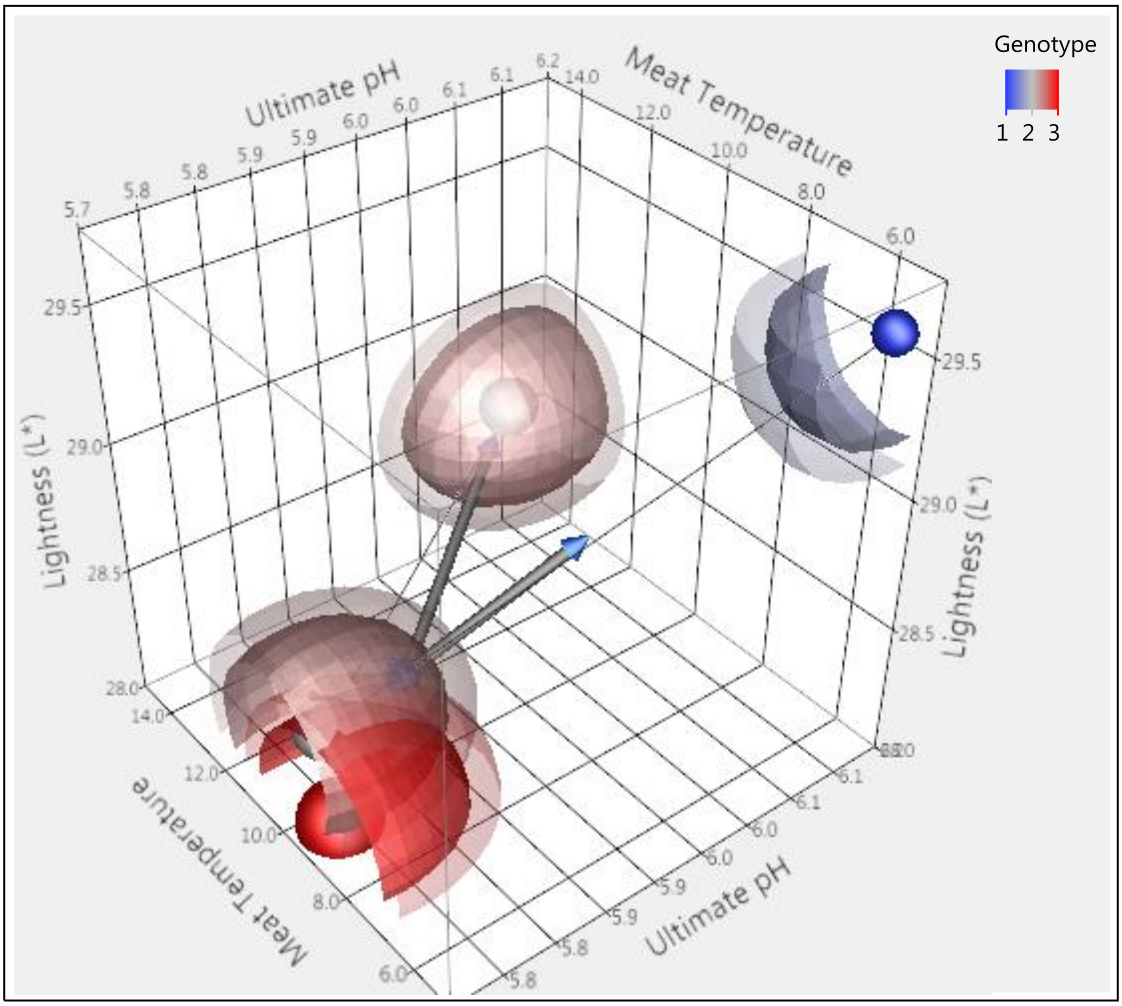

Figure 6.5 The 3-D scatter plot of the first three principal components from heifers

1 – Bonsmara, 2 – Non-Descript, 3 – Nguni

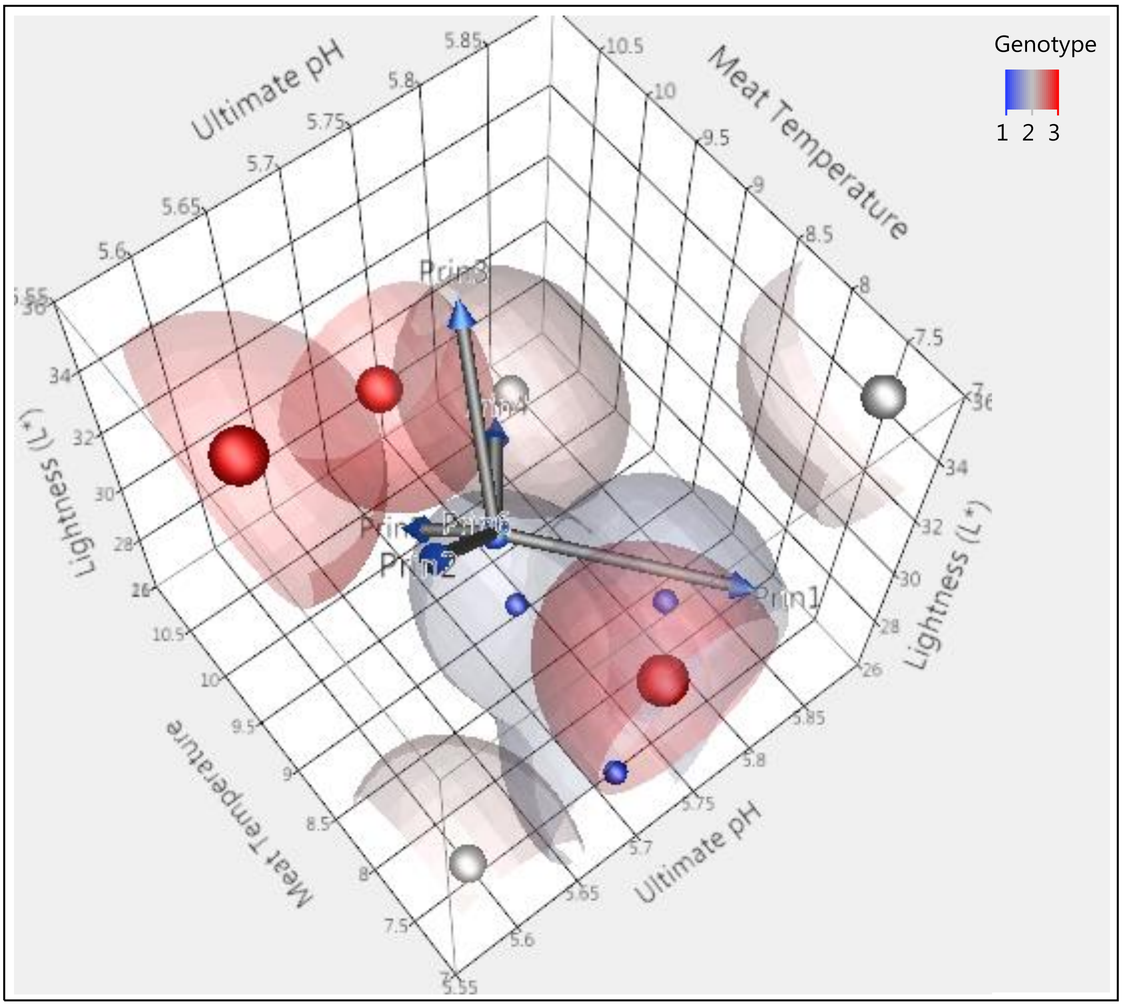

Figure 6.6 The 3-D scatter plot of the first three principal components from bulls

1 – Bonsmara, 2 – Non-Descript, 3 – Nguni

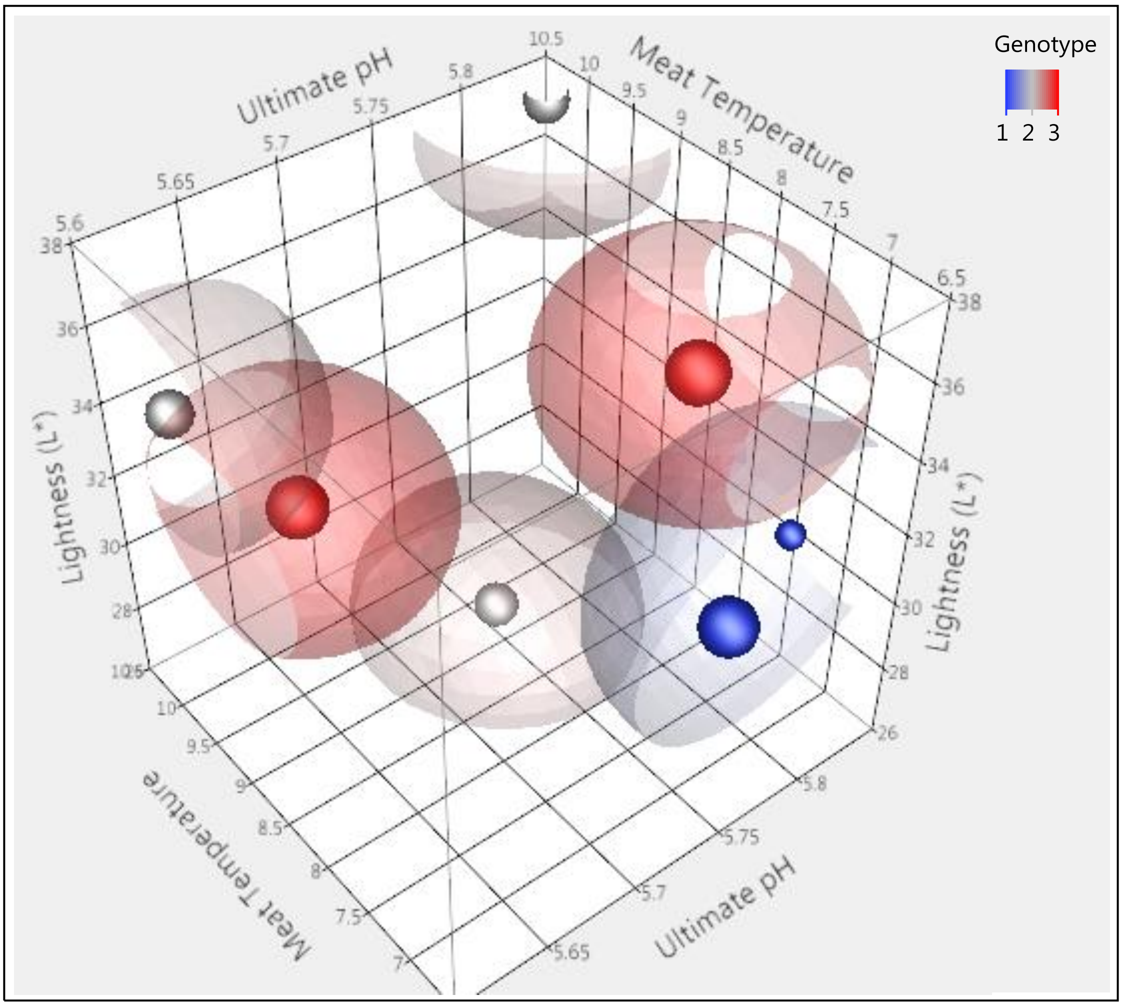

Figure 6.7 The 3-D scatter plot of the first three principal components from cows

1 – Bonsmara, 2 – Non-Descript, 3 – Nguni

6.4 Conclusions

It could be inferred from the study that, animal class, genotype and sex affected the nanostructures of beef. Principal component analysis showed positive relationships between distances travelled and beef quality. Among the BR, ND and NG cattle, heifers had better meat quality than bulls and cows. The first two PC`s of beef from heifers had the highest contribution of the total variance followed by bulls and cows. Therefore, the nanostructures of beef were not affected by animal's class with heifers having the best meat than bulls and cows. Considering the most important beef quality triats, heifers had better meat with pH_u, T_m and L* contributing the highest percentages in the total variance.

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Chapter 7 General Discussion, Conclusions and Recommendations

7.1 General Discussion

The objective of the study was to determine the effects of pre-slaughter stress on the biochemical conditions, carcass characteristics, technological properties and nanostructure of beef. Demand of animal proteins has been mainly influenced by emerging markets worldwide especially in Brazil, Russia, China and South Africa (Mapholi *et al.*, 2014). This demand has led to transportation of animals for slaughter and marketing of food products worldwide. The necessity for increased cattle production globally is perceived as another contributing factor to the poor handling of animals prior to slaughter. The period of transportation has been reported to affect livestock production due to high ambient temperatures, truck movements and change in acceleration leading to stress.

The response of animals to the stressor is a pattern of endocrine, neural, immune, hematologic, and metabolic changes for restoration of homeostasis (Muir, 2004; Węglarz, 2011; Nakyinsige *et al.*, 2013). The imbalance of homeostasis especially in animals with heavy muscular work has a negative impact on the glycogen content, lactic acid accumulation, and colour of meat, water holding capacity, flavour, juiciness and tenderness (Terlouw *et al.*, 2008). Stress also increases body temperature which negatively weakens meat quality leading to higher drip loss and a lighter meat. Meat tenderness is a well-known problem that is sought to be resolved and improved by the scientific community for the consumers and the industry as well (Ferguson *et al.*, 2000; Bourguet *et al.*, 2011; Li *et al.*, 2014).

In Chapter 3, the expression of heat shock proteins, glucose and cortisol in slaughtered bovine species and their correlations with beef quality were determined. Heat shock proteins are important during heat stress because they protect other proteins from being aggregated and also prevent irreversible accumulation of large aggregates from forming within the cell. The results obtained revealed the relationship between, HSPA1A, GLU, CORT and the quality of meat of bovine species (Schrönder *et al.,* 1993). The relationship was due to the binding effect caused by HSPA1A with ATP when animals are stressed. Once animals are stressed, there is a rapid release of cortisol through the adrenal cortex which is stimulated by the HPA axis resulting in reduced muscle glycogen. This in turn affects the targeted ultimate pH, the key to good quality meat (Muchenje *et al.*, 2008). Increased pH_u results in tougher and darker meat, meaning that WBSF and the colour of beef are affected negatively of which this is not ideal for human consumption (Dubost *et al.* 2013). The production of CORT, GLU and HSPA1A as affected by animal class (heifers, bulls and cows) was also observed with animals that travelled long distances. During transportation, animals loose the energy levels due to exhaustion, starvation, and heat stress and truck movements. Animal respond accordingly to various stress stimuli leading to a set of heat shock genes which synthesise Hsp (Christian *et al.* 2003; Pawar *et al.*, 2014). The expression of Hsp in bulls indicates that they were stressed during transportation and at the lairages. However, with maximum amount of time at the lairages, energy reserved can be restored for the improvement of beef quality. This will reduce the occurrence of DFD beef since lactic acid production will also increase for normal ultimate pH in the muscles.

The objective of Chapter 4 was to determine the activities of creatine kinase (CK) and lactate dehydrogenase (LDH) as welfare indicators and how they relate to beef quality. The results reported increased activities of creatine kinase in different genotypes with Non-descript genotype having the highest activity. This is related to acquaintance to handling during pre-slaughter. It is said that CK is a physical stress related enzyme whereas LDH is an environmental stressor (Werner *et al*., 2013). The observed production of enzymes (CK and LDH) was also linked with increased DT. However, it was noted that CK and LD were not related with beef quality. This was not clear because these enzymes are located in the muscles. The release of these muscles is due to muscle damage, such as bruises and muscle fatigue. The occurrence of bruises in animals indicates how stressful the DT was to the animal (von Holleben, 2009; Miranda-de la Lama et al., 2012; Strappini et al., 2012) which has a negative impact on the quality of beef.

According to Muchenje *et al.* (2009), meat with higher pH affects other meat quality traits such as colour, juiciness, flavour and tenderness of meat. Long hours of transportation resulted in the pH_u values that were higher than normal (> 5.9). Meat quality attributes such as the L*, a* and the b* values were affected negatively leading to darker meat. Incidents of darker meat are also increased by high stocking density and reduced lairage period especially with animals that are fasted for more than 22 hours. This results in meat deterioration due to muscle membrane damage (Yu *et al.*, 2009). In this scenario, the marbling percentage of meat is also reduced as reported by Muchenje *et al.* (2009) leading to tougher meat and reduced flavour. Troy and Terry (2010) further stipulated that undesirable meat by consumers has negative implications on the meat industry sector because it reduces profitability. Similar studies in cattle (Vimiso and Muchenje, 2013), pigs (Gajana *et al.*, 2013) and sheep (Ekiz *et al.*, 2012) indicated that long hours of transportation affect both animal welfare and meat production.

In Chapter 5, it was observed that genotype and animal class had an effect on carcass weights and some beef quality characteristics. Among the genotype effects, Non-descript cattle were heavier although their DP was low as compared to Brahman and Charolaise cattle. Research indicates that most animals are brought to the abattoir with foetuses which negatively affect DP. Dressing percentage is what abattoir managers use to calculate the farmer's returns. In addition, the amount of saleable meat is also determined by DP. Therefore, slaughtering of animals that will reduce production is not a way to go in approaching the increasing demand of beef production for 2050 (UN, 2011; Meissner *et al.*, 2013; Schönfeldt *et al.*, 2013).

Transportation issues that are related to livestock production of beef industry are critical due to the fact that management practise for productions are novel and stressful to the animal. Pre-slaughter stress begins at the farm, transportation and at the abattoir (Grandin, 1997; Mota-Rojas *et al.*, 2011). The methods and equipment used to handle animals and what they feed on has an ample effect on the welfare and beef quality. Therefore, reducing stress during handling of animal's prior to slaughter has a positive improvement in the health of the animals and prevents physiological changes that may reduce productivity. Tenderness, juiciness, and flavour are important factors that are used by consumers to judge and evaluate beef quality (Serra *et al.*, 2008). These parameters influence consumers buying power relative to making a repeated purchase (Xia *et al.*, 2007). In addition, Vimiso *et al.* (2012) indicated that meat colour and fat marbling are the quality cues used by consumers to determine the visual quality of a product while juiciness, tenderness, freshness and leanness are the expected quality indicators of meat.

Animals may not produce the same amount of fat, colour and tenderness due to different ages, sexes, genotypes and type of production systems, pre-slaughter conditions and the processing that is done *post-mortem* (Li *et al.*, 2014). The amount of collagen, connective tissue and muscle fibre length determines the tenderness of beef. Therefore, in Chapter 6, it was hypothesised that, animal class, genotype and sex do not affect the nanostructure of *muscularis longissimuss thoracis et. lumborum* (LTL) and beef quality There were significant correlations that were observed between, DT, LD_{hr}, T_a, T_m, L* a* and WBSF. The correlations imply that pre-slaughter stress has an ample effect on the quality of beef (Grandin, 1997; Honkavaara *et al.*, 2003; Schneider *et al.*, 2012). This is because as pre-slaughter conditions become stressful to the animal such that they are unable to cope, their energy levels are reduced leading to an increase in pH_u and consequently result in darker meat (Kemp *et al.*, 2010; Pethick *et al.*, 2004). The first important principal component's (PC's) as they appear from the analysis were pH_u, T_m, L*, a*, b* and WBSF. The Brahman (BR), Non-descript (ND) and Nguni (NG) cows had visible skeletal fibres which were thin and long indicating improved tenderness of beef. The observed nanostructure of Nguni cows and heifers was better than those from Bonsmara and Non-Descript cattle. The Nguni had longer fibres which favour tenderness and acceptance of beef by consumers (Heinz and Hautzinger, 2007; Marino *et al.*, 2014).

7.2 Conclusions

It was concluded that pre-slaughter stress and animal related factors affected the production of enzymes, glucose, cortisol, heat shock proteins, carcass characteristics and meat quality. Distance travelled increased the presence of heat shock proteins, cortisol, creatine kinase and lactate dehydrogenase leading to increased pH_u, darker and tougher beef. An adequate lairage period improved the quality of beef because the energy reserves were restored, giving room for lactic acid accumulation. On the other hand, carcass weights were positively correlated with beef quantity and quality. Principal component analysis showed the most important beef quality characteristics that can be used to improve the quality of beef when the pre-slaughter environment is improved. The nanostructure of beef was affected by animal class, genotype and sex of the animal. However, the eigenvalues observed indicated a similar trend of the first 2 PC`s that can be used to improve beef quality. Ultimate pH and meat temperature as well as lightness were among the PC`s that can be considered.

7.3 Recommendations

The study has produced the following recommendations:

1. The diets given to animals at birth, growth and development and a week before slaughter must be monitored and evaluated since it has been suggested that feed can alter the quality and amount of myoglobin content related to colour values of meat.
2. There is a need to modify and improve the transport logistic chain of meat animals to reduce the negative effects it has on the expression of heat shock proteins as they bind with ATP which is responsible for the production of lactic acid for improved ultimate pH, lightness and tenderness of beef. Therefore, research should focus on quantifying the amount of ATP that binds with Hsp during pre-slaughter stress.

3. In addition, research should also focus on detecting the gene that is involved during transcription. This will assist even during selection of animals to know which genes of genotypes are less susceptible to stress.
4. Due to the effect of genotype on some of the beef quality traits, ageing of meat and how it can reduce the tightness of connective and collagen tissues, it is therefore imperative to identify the activities that are involved in the transformation of muscles to meat such as mitochondria treason.
5. Among animal classes, heifers responded positively when they were exposed to pre-slaughter stress unlike bulls. This means that in the process of improving meat quantity and quality, genetic selection of heifers with higher dressing percentage should be targeted hence research in this area is needed.
6. Principal component analysis suggested the most important carcass weights and beef quality traits that can be considered when attempting to improve the quantity and quality of beef. In addition, animal welfare and the improvement of beef require proper selection of genotypes that will produce meat with longer sarcomere length for tender beef.
7. Furthermore, understanding the function of sarcomere length, muscle fibres and type of muscle for tender meat is important since different fibre type`s produces varying degree of tenderness.

7.4 References

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Appendix 1 The ethical clearance certificate

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ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number:	MUC012 1SCHU01
Project title:	**The effect of avoidance behavior of cattle during pre-slaughter and slaughter stress on bruising, catecholamines, plasma enzyme activity in relation to oxidative stress and beef quality**
Nature of Project:	PhD
Principal Researcher:	Amanda Yucca Chulayo
Supervisor:	Prof V Muchenje
Co-supervisor:	Prof A Hugo

On behalf of the University of Fort Hare's Research Ethics Committee (UREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the UREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document

- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research

The Principal Research must report to the UREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance.

The UREC retains the right to

- Withdraw or amend this Ethical Clearance Certificate if
  - Any unethical principal or practices are revealed or suspected
  - Relevant information has been withheld or misrepresented
  - Regulatory changes of whatsoever nature so require
  - The conditions contained in the Certificate have not been adhered to

- Request access to any information or data at any time during the course or after completion of the project.

The Ethics Committee wished you well in your research.

Yours sincerely

Professor Gideon de Wet
Dean of Research

27 November 2012

Appendix 2 The transport recording sheet

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TRANSPORT RECORDING SHEET

Breed	Sex	Class	Weight	Delivery date	Slaughter date	Arrival time	Distance from the farm to the abattoir (km)	Transport Time	Lairage hrs	Ambient Temperature ^{0}C

Appendix 3 Recording sheet for beef quality

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BEEF QUALITY																				
Date	Tag no	Breed	Sex	Age	SLW	WCW	CCW	Ph48	°C	L	a	b	Date Cooked	Meat Age	Frozen kg1	Melted kg2	TL %	After cooking kg3	CL %	WBSF