The influence of catecholamines on energy metabolism and selected meat quality attributes of three commercial beef breeds

By

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DECLARATION

I declare that this thesis for the PhD (Animal Science) degree at the University of Pretoria has not been submitted by me for a degree at another University

Signed_____

Hester Adriana O'Neill

ABSTRACT

Feed restriction and handling of animals destined to be slaughtered have are two factors that affect meat quality negatively. Where handling of slaughter animals is inevitable, fasting is applied to meat producing animals in the *ante mortem* period to minimise microbial contamination of carcasses or to lower the metabolic rate of certain cattle breeds as an adaptive mechanism to hot climates. From the present study, animals that are historically more docile had higher urinary catecholamine concentrations *per se*. By quantifying the relationship between catecholamines and animal temperament in cattle, selection of animals with a more favourable catecholamine profile, could enhance the ease of handling slaughter animals and reduce carcass bruising.The difference in urinary catecholamines of three commercial beef breeds types; Brahman, Nguni and Simmental; retained in lairages at an abattoir for either 24 or 3 hours *ante mortem* was measured. This was done in order to investigate whether longer feed withdrawal periods will lead to increased urinary catecholamine concentrations; a shift in *post mortem* energy metabolism and negative effects on meat quality.

Catecholamine turnover rate from dopamine to norepinephrine and epinephrine, differed between breed types and feed withdrawal periods. Results indicated a relationship between energy metabolism, feed withdrawal period and catecholamine turnover. Twenty four hours feed withdrawal increased the rate of catecholamine turnover for Brahman and Nguni. Lower early *post mortem* glycogen together with higher glucose-6-phosphate indicated that glycogenolysis increased for this Brahman and Nguni just before slaughter. Twenty four hours feed withdrawal triggered glycogenolysis via increased catecholamine turnover from dopamine to norepinephrine and epinephrine for Brahman and Nguni. In contrast, feed restriction had no effect on catecholamine turnover. Slightly higher early *post mortem* glycogen and lower glucose-6-phosphate indicated lower energy metabolism for the Simmental breed type just before slaughter. It seemed as if feed restriction had an energy sparing effect on Simmental type cattle with a consequent negative effect on meat quality.

Warner-Bratzler shear force results were the same among breed types when feed withdrawal was not longer than 3 hours and the values emphasise the importance of providing feed to animals kept in lairages or transported over long distances. When feed is available up until 3 hours before slaughter, irrespective of breed type, meat tenderness was not affected negatively.

When feed was restricted, additional sarcomere shortening and creatine phosphate depletion while the carcass was still warm, occurred to such a degree that the meat from Simmental was tougher. It is clear that feed withdrawal of 24 hours has a significantly negative impact on Warner-

Bratzler sheer force of Simmental. In this study with non-electrically stimulated carcasses it is clear that feed must be provided to Simmental up until 3 hours before slaughter to prevent excessive sarcomere shortening and meat toughness. This was explained by a probable lowered metabolic rate and changes in mitochondrial ATP turnover for the Simmental breed type. Cattle from differend breed types react differently to feed withdrawal periods and this knowledge should be applied in the meat industry to assure more consistent meat quality.

SUMMARY

The influence of catecholamines on energy metabolism and selected meat quality attributes of three commercial beef breeds

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1. My mommy and my sister, you were my inspiration and my wings. This is for you.

“Vir ons ma

Ons het saamgeloop
Op ‘n stofpad met klippe
Meestal hand aan hand

Ons het saamgeloop
In ‘n donker gang vol spoke
Meestal rug teen rug

Ons het saamgeloop
Deur die diep vallei van smart
Meestal op ons knieë

Maar ma ons het saamgeloop
Ons drie

Met jou ons middelpunt” ~ CM Laidlaw

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3. My supervisors, Prof EC Webb, Dr L Frylinck, Dr P Strydom for your patience and guidance.
4. The abattoir and laboratory staff at the Agricultural Research Council, Irene, Meat Industry centre.
5. The funding body for this research project, Red Meat Research and Development Trust

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
Br_{24}	Brahman breed type subjected to 24 hours feed withdrawal
Br_3	Brahman breed type subjected to 3 hours feed withdrawal
CIE	International Commission on Illumination
COMT	Catechol-O-methyltransferase
DHMA	3,4-dihydroxymandelic acid
DHPG	Dihydroxyphenylglycol
DOPAC	3,4-Dihydroxyphenylacetic acid
DOPAL	3,4-Dihydroxyphenylacetaldehyde
DOPEGAL	3,4-dihydroxyphenylglycolaldehyde
DOPET	3,4-dihydroxyphenylethanol
EDTA	Ethylenediaminetetraacetic acid
EPIDOP	Epinephrine to Dopamine ratio
FW_3	3 hours feed withdrawal
FW_{24}	24 hours feed withdrawal
G-6-P	Glucose-6-phosphate
GLM	General linear model
H^+	Hydrogen ion
Ha	Hue angle
L*	Lightness
MAO	Mono amine oxidase
MFL	Myofibrillar fragmentation length
$MgC\ell_2$,	Magnesium chloride
MHPG	3-Methoxy-4-hydroxyphenylglycol
MHPG-SO_4	3-Methoxy-4-hydroxyphenylglycol sulphate
mℓ	Millilitre
MN	Metanephrine
MN-SO_4	Metanephrine sulphate

Na^+ / H^+	Sodium to hydrogen ratio
Na^+	Sodium ion
K^+	Potassium ion
Ca^{2+}	Calcium ion
NAD^+	Nicotinamide dinucleotide (oxidised form)
NADH	Nicotinamide dinucleotide (reduced form)
$NaNO_3$	Sodium nitrate
ng / µmol creatinine	Nanogram per µmol creatinine
Ng_{24}	Nguni breed type subjected to 24 hours feed withdrawal
Ng_3	Nguni breed type subjected to 3 hours feed withdrawal
NMN	Normetanephrine
NMN-SO_4	Normetanephrine sulphate
NORDOP	Norepinephrine to dopamine ratio
pHu	Ultimate pH
Pi	Inorganic phosphate
pKa	Acid dissociation constant
PNMT	Phenyletanolamine-N-methyltransferase
RA	Rate of ATP hydrolysis
RC	Rate of creatine production
RG	Rate of ATP synthesis through glycolysis
Ri	Redness index
RM	Rate of myokinase ADP consumption
RO	Rate of aerobic ATP synthesis
Rpm	Revolutions per minute
SAS	Statistical analysis system
SEM	Standard error of mean
Si	Saturation index (Chroma)
Sm	Simmental breed type
Sm_{24}	Simmental breed type subjected to 24 hours feed withdrawal
Sm_3	Simmental breed type subjected to 3 hours feed withdrawal

SULT1A3	Sulfotransferase type 1A3
VMA	Vanillylmandelic acid
WBSF	Warner-Bratzler shear fore

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CHAPTER 1 INTRODUCTION

1.1 Project theme

To determine the difference in *post mortem* urinary catecholamines and its relationship to energy metabolism and meat quality between three commercial beef breed types used in the South African red meat industry subjected to two standard feed withdrawal periods

1.2 Project title

The influence of catecholamines on energy metabolism and selected meat quality attributes of three commercial beef breeds.

1.3 Hypothesis and justification

1.3.1 Economic importance of meat tenderness

For more than two decades the consumer's perception of red meat quality remained constant e.g. "Meat should be consistently tender" (Koohmaraie *et al*., 1995). According to Smith et al. (2003) the juiciness and flavour of beef have a significant effect on meat quality perception by the consumer, but there is twice as much variation in tenderness as in flavour or juiciness. Consumer preparation is another factor that determines juiciness and flavour more compared to tenderness. There is more opportunity to improve tenderness through genetic selection and management practices in the animal production industry.

1.3.2 Justification

Post mortem muscle is subjected to two processes namely the toughening phase and the tenderising phase as described by Koohmaraie (1996). The toughening phase is marked by a decrease in sarcomere lengths. Numerous practices to prevent and minimise toughness such as electrical stimulation (Bendall *et al*., 1979, Swatland, 1981; Strydom *et al*., 2005) and delayed chilling of carcasses (Savell *et al*., 2005) have been studied and reviewed.

The tenderising phase is marked by several factors that influence meat tenderness such as the Ca^{2+}-dependent proteolytic enzymes (Koohmaraie *et al*., 1987). The factors involved in *post mortem*

tenderising such as the genetic heritability of proteases (Frylinck *et al*., 2009) and programmed cell death through the caspases (Ouali *et al*., 2006) have been studied.

Although the relationship between stress responsiveness (Ferguson & Warner 2008; Muchenje *et al*., 2009), carbohydrate metabolism (Pösö & Puolanne, 2005) and tenderness have been investigated, the relationship between the catecholamine system, energy metabolism and meat tenderness still needs investigation.

1.3.3 History of carbohydrate metabolism, pH and meat tenderness

In the 1890's researchers have discovered an improvement in tenderness by submerging meat in a weak vinegar solution (Woods, 1896). Up until the late 1990's it was thought that factors that deplete glycogen content in muscle will result in lower lactate production during glycolysis with a subsequent high ultimate pH and should be considered as the culprit for tough meat (Tornberg, 1996).

Woods (1896) concluded that meat from poorly fed animals was tough to such an extent that prolonged boiling or roasting had little effect on improving tenderness. Unfortunately, feed withdrawal and the retainment of ruminants in lairages for up to 48 hours before slaughter is standard practice in the South African red meat industry (van Zyl, 1998). The rationale behind implementing fasting practices is to minimise microbial contamination of carcasses (Abouzeed *et al*., 2000) as well as to lower the metabolic rate of certain cattle breeds as an adaptive mechanism to hot climates (White *et al*., 2005).

1.3.4 Effects of feed withdrawal on metabolic rate

Feed withdrawal and fasting have different effects on the metabolic rate of animals from different species or even breeds within a species. As a general rule, feed withdrawal increases glycogenolysis in order to maintain glucose homeostasis (Voet & Voet, 1990). An animal's drive to maintain glucose homeostasis activates the hypothalamic-pituitary-adrenal axis which results in elevated circulating catecholamines. Stressors that activate the hypothalamic-pituitary-adrenal axis include mixing of animals from different groups, transport, and feed and water withdrawal. Activation of the hypothalamic-pituitary-adrenal axis evokes a number of biochemical changes such as energy depletion through increased glycogenolysis (Schaefer *et al*., 2001).

On the other hand, feed restriction can be applied to lower the metabolic rate in some cattle breeds. South Africa often experiences long periods of extreme heat and drought. Brahman cattle (*Bos indicus*) and their crossbreds are widely used in the beef industry because of their heat tolerance and adaptability (White *et al*., 2005). In order to survive excessive heat, European cattle breeds - such as the Simmental (*Bos taurus*) – need to be able to regulate its body temperature. Heat production in some *Bos taurus* breeds can be lowered through restricted feed intake. A reduction in metabolisable energy intake lowers the metabolic rate in some breed types (Purwanto *et al*., 1990).

1.3.5 Involvement of catecholamines in meat tenderness

The purpose of this study was to investigate the difference in urinary catecholamines of three commercial beef breeds types, retained in lairages at an abattoir for either 24 or 3 hours *ante mortem*. It was hypothesised that longer feed withdrawal periods will lead to increased urinary catecholamine concentration; glycogen depletion and subsequent increased Warner-Bratzler shear force of muscle. It was speculated that muscle from smaller breeds with more oxidative fibers, subjected to longer feed withdrawal will become glycogen depleted more extensively. This will lead to significant sarcomere shortening (cold shortening) and extremely tough meat.

Except for glycogen depletion, stress experienced by an animal as acute stress during transport and handling was investigated. Although the relationship between stress responsiveness and hormones have been quantified in pigs (Foury *et al*., 2005), most reports on stress responsiveness and meat quality (Mota-Rojas *et al*., 2006; O'Neill *et al*., 2006; van Schalkwyk *et al*., 2000) are largely speculative and do not quantify the magnitude of the relationship between stress hormone levels and meat quality.

1.3.6 Involvement of catecholamines in animal welfare issues

Animal welfare issues became an important determinant of meat quality as perceived by the consumer (Ferguson & Warner, 2008), although stress experienced by livestock is an inevitable result of transport, handling and mixing of animals from unfamiliar groups (Warriss, 1990; Apple *et al*., 2005). Some breed types are more temperamental than others (Ferguson & Warner, 2008). Selection for improved temperament or less fearful cattle will facilitate both the human handler and animal (Ferguson & Warner, 2008). Catecholamines have been correlated with animal temperament and the development of fearful behaviour. Cannon introduced the term *fight-or-flight* to the catecholamine system (Cannon, 1929; From: Kvetnansky *et al*., 2009) and therefore this group of

neurotransmitters is thought to have mainly negative effects on stress, fear and aggression. One purpose of this study was to determine whether urinary catecholamine concentrations differ between breeds.

CHAPTER 2 LITERATURE REVIEW

2.1 Biochemistry and physiology of catecholamines

2.1.1 Historical discovery of catecholamines

The historical "discovery" of catecholamines was described in the review by Kvetnansky *et al*. (2009). Catecholamines, the neurotransmitters and hormones of the adreno-medullary, sympatho-neuronal, and brain catecholaminergic systems, were discovered at the beginning of the 20th century (Kvetnansky *et al*., 2009). The discovery was based on experiments by Walter Cannon and the importance of adreno-medullary secretions of "adrenaline" during various types of stimulation in animals and humans. Cannon introduced the terms *homeostasis* and *fight-or-flight* to the scientific literature. These terms remain in use today (Cannon, 1929; From: Kvetnansky *et al*., 2009). The perception of a unitary sympatho-adrenal system is incorrect as separate pathways and different changes in sympathetic nervous and adreno-medullary activities with various stressors have been described (Paćak *et al*., 1998; Paćak & Palkovitz, 2001; Goldstein & Kopin, 2007).

2.1.2 Physiological aspects of catecholamines

To date there are more than 30 neurotransmitters classified and each of these neurotransmitters is used in discrete and often highly localized regions of the nervous system according to Voet & Voet (1990). Many of them have distinctive physiological roles. For example γ-amino butyric acid and glycine are inhibitory rather than excitatory neurotransmitters. The difference between the ligand-gated channels for the inhibitory or excitatory receptors is that they are either selectively permeable to anions (inhibitory) or cations (excitatory). The actual nature of a neuron's response to a neurotransmitter depends more on the characteristic of the receptor than on the neurotransmitter's identity (Voet & Voet, 1990).

The biological effects of catecholamines are mediated by two classes of plasma trans-membrane receptors, α- and β-adrenoreceptors (also known as adrenergic receptors). An agonist is a substance that binds to a hormone receptor to trigger a hormonal response. An antagonist is a substance that binds to a hormone receptor, but fails to elicit a hormonal response and blocks any agonist action (Voet & Voet, 1990). α- and β-adrenoreceptors, which occur on separate tissues in mammals, generally respond differently and often oppositely to catecholamines (Voet & Voet, 1990).

Stimulation of α-adrenoreceptors by α-agonists causes intracellular effects that are mediated by the inhibition of adenylate cyclase (α2 receptors) or via the phosphoinositide cascade (α1 receptors). This leads to the stimulation of smooth muscle contraction in blood vessels supplying peripheral organs such as skin and kidney, the smooth muscle relaxation in the gastrointestinal tract and blood platelet aggregation.

Stimulation of β–adrenoreceptors by β-agonists, activates adenylate cyclase, stimulates glycogenolysis and gluconeogenesis in liver and skeletal muscle, lipolysis in adipose tissue, smooth muscle relaxation in the bronchi and blood vessels supplying the skeletal muscles and increased heart rate. Most effects are directed towards a common end: the mobilization of energy resources and their shunting to where they are most needed to prepare the body for sudden action (Voet & Voet, 1990).

2.1.3 Release of catecholamines from neurons

With the arrival of an action potential, voltage gated Ca^{2+} channels open. The elevation of cytoplasmic Ca^{2+} concentration is the essential trigger for the exocytotic release of transmitter substances from neurons (Boehm & Huck, 1995). Intravesicular catecholamines (concentration ~0.5M) are rapidly released into the synaptic cleft via exocytosis as soon as an action potential occurs (Ressler & Nemeroff, 1999).

2.1.4 Catecholaminergic systems

All living beings preserve their internal environment in the face of a life threatening experience. One way in which mammals preserve their internal environment is by anticipatory and compensatory recruitment of the sympatho-adreno-medullary system (Kagedal & Goldstein, 1988).

Catecholamines can be divided into:

- the sympathetic neurotransmitter norepinephrine,
- the adreno-medullary hormone epinephrine,
- the central nerve transmitter, dopamine
- and catecholamine precursor, dihydroxyphenylalanine

Catecholamines are found in body fluids such as blood, urine and cerebrospinal fluid (Kagedal & Goldstein, 1988). These neurotransmitters are hormones which occupy key positions in the regulation of physiological processes as well as the development of neurological, psychiatric, endocrine and cardiovascular responses and diseases (Eisenhofer, 2001).

The systems that employ norepinephrine and epinephrine as chemical signals consist of networks within the central nervous system and peripheral catecholaminergic system.

Brain catecholaminergic systems are divided into noradrenergic system, adrenergic system, dopaminergic system and L-dihydroxyphenylalanine neurons. The noradrenergic neurons (as termed by Kvetnansky *et al*., 2009) in the central nervous system are involved in information processing, as well as in the control of endocrine responses (Kvetnansky *et al*., 2009).

There are three distinct peripheral catecholaminergic systems (Kvetnansky *et al*., 2009):

- Sympatho-adreno-medullary system – a remote unit operating as an endocrine organ (the adrenal medulla). The adrenal medulla of the systems controls metabolic processes (Haller *et al*., 1998).
- Sympatho-neuronal system (Haller *et al*., 1998) or somatic neural network (the sympathetic nervous system). The somatic division controls effector systems, e.g. muscle (effectors for behaviour), several internal organs (such as liver or brown adipose tissue) and endocrine glands by means of local adrenergic terminals.
- Dihydroxyphenylalanine-dopamine autocrine / paracrine system (Kvetnansky *et al*., 2009).

The abovementioned systems react very rapidly to environmental changes (catecholamines are amongst the fastest to react). The receptors which recognize chemical signals are distributed widely in the periphery and the central nervous system, so that the entire organism can be mobilized to respond to changes (Haller *et al.,* 1998).

2.1.5 Biosynthesis of catecholamines in different regions

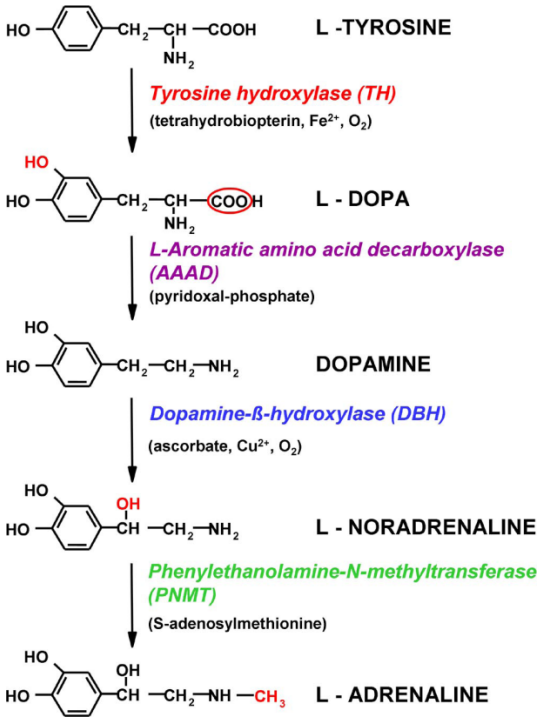

Figure 2.1 Pathway for catecholamine biosynthesis in sympathetic and some brain neurons and its enzymatic steps

(From: Kvetnansky *et al*., 2009)

Catecholamines are synthesized from the essential amino acid, L-tyrosine. The steps for the conversion of L-tyrosine to L-noradrenaline are typical for sympathetic and some brain neurons and the conversion of L-noradrenaline to L-adrenaline is typical for the adreno-medullary cells and some peripheral and brain neurons (Kvetnansky *et al*., 2009). Noradrenaline and adrenaline are terms used by Kvetnansky *et al*. (2009) and will further be referred to as norepinephrine and epinephrine respectively.

Catecholamine biosynthesis starts with the uptake of tyrosine into the neuronal cytoplasm and conversion to dihydroxyphenylalanine by tyrosine hydroxylase. Tyrosine hydroxylase is highly localized to catecholamine-synthesizing cells such as the adrenal medulla, sympathetic nerves and particular nuclei in the brain. Tyrosine hydroxylase activity is subject to feedback inhibition by its products - dihydroxyphenylalanine, norepinephrine and dopamine (Kagedal & Goldstein, 1988).

By the action of an aromatic amino acid decarboxylase, dopamine is synthesized from dihydroxyphenylalanine. Norepinephrine differs from dopamine in having a β-hydroxy group which

is introduced by the enzyme - dopamine-β-hydroxylase. Phenyletanolamine-N-methyltransferase (PNMT) introduces a methyl group onto the amino group to give epinephrine (Kvetnansky *et al*., 2009).

Dihydroxyphenylalanine can be seen as the catecholic amino acid; dopamine and norepinephrine as the primary amines and epinephrine the secondary amine. Epinephrine in plasma and urine is derived from adreno-medullary secretion. The cytoplasm of adreno-medullary cells contains PNMT, which catalyses the conversion of norepinephrine to epinephrine (Kvetnansky *et al*., 2009).

Exposure to stress increases the utilization and synthesis of dopamine, norepinephinre, and epinephrine and the availability of tyrosine for catecholamine synthesis can become rate limiting under these conditions (Schaefer *et al*., 2001).

When catecholamine synthesis is compromised, animals become less resistant to stress and develop an array of counterproductive behavioural (learned helplessness) and physiological changes. These animals are unable to respond appropriately to stimuli and are unable to function normally, to the extent that even eating and sleeping are disturbed (Anisman & Zacharko, 1986; Lieberman, 1994; From: Schaefer *et al*., 2001)

2.1.6 Release of catecholamines in fluids

Catecholamines are continually in complex dynamics. Multiple processes are involved in the synthesis, storage, release, inactivation and metabolism of catecholamines. These processes differ among tissue and cell types before and after release into extracellular fluid and general circulation (Eisenhofer, 2001). In order to understand the presence of catecholamines in body fluids, some metabolic pathways will be discussed.

2.1.6.1 Intraneuronal metabolism of catecholamines

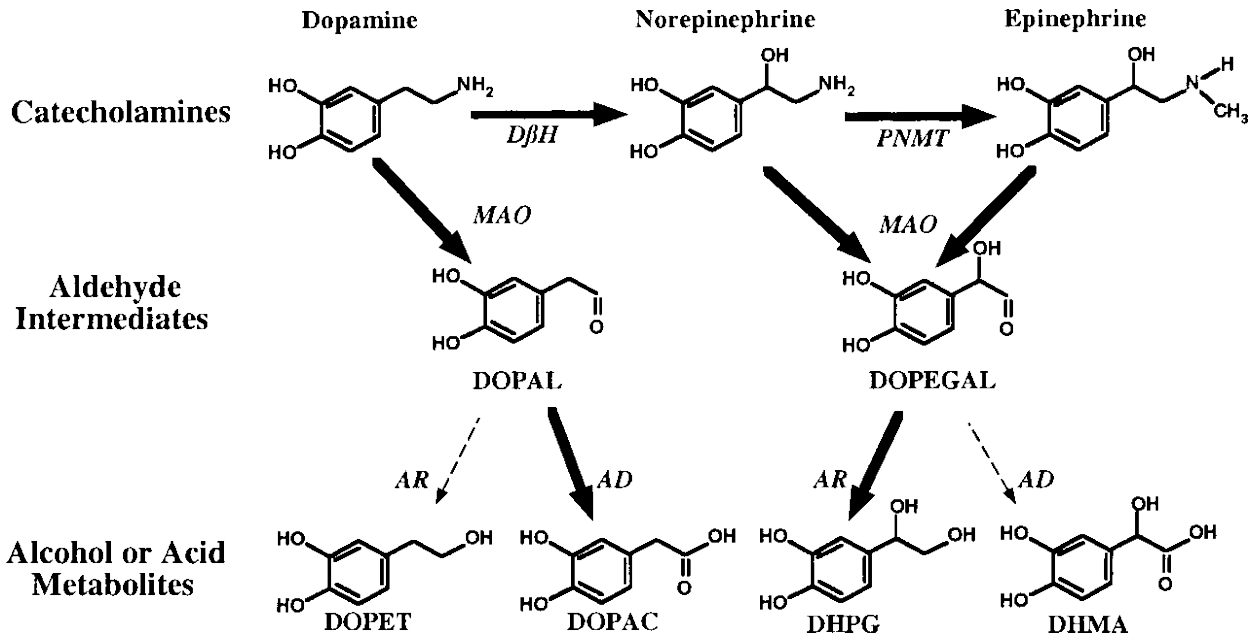

Figure 2.2 Pathways for the oxidative deamination of catecholamines to their corresponding biogenic intermediates and subsequent alcohol metabolites

DHMA ~ 3,4-Dihydroxymandelic acid; DOPAL ~ 3,4-Dihydroxyphenylacetaldehyde; DHPG ~ 3,4-Dihydroxyphenylglycol; dopamine ~ Dopamine; DOPA ~ Dihydroxyphenylalanine; DOPAC ~ 3,4-Dihydroxyphenylacetic acid; DOPEGAL ~ 3,4-dihydroxyphenylglycolaldehyde; DOPET ~ 3,4-Dihydroxyphenylethanol

(From: Eisenhofer *et al*., 2004).

The large contribution of intraneuronal deamination to catecholamine turnover and the dependence of this on the vesicular-axoplasmic monoamine exchange process helps explain how synthesis, release, metabolism, turnover and stores of catecholamines are regulated in a coordinated way during stress (Eisenhofer, 2001)

Eisenhofer *et al*. (2004) explained that the metabolism of catecholamines occurs within the same cells they are synthesized. Before metabolism commences, catecholamines leak from the vesicular stores into the cytoplasm from the different regions they were synthesized. The vesicular stores exist in a highly dynamic equilibrium, with passive outward leakage counterbalanced by inward active transport controlled by vesicular monoamine transporters (Eisenhofer *et al*., 2004).

In catecholaminergic neurons, the presence of monoamine oxidase (MAO), norepinephrine and epinephrine are preferentially metabolized to alcohol metabolites and dopamine to acid

metabolites (Eisenhofer *et al*., 2004). Production of these toxic and insTable aldehydes depends on the dynamics of vesicular-axoplasmic monoamine exchange and enzyme-catalysed conversion to non-toxic acids or alcohols. In sympathetic nerves, the aldehyde produced from norepinephrine is converted to 3,4-dihydroxyphenylglycol (DHPG). Deaminated aldehyde metabolites are short-lived intermediates and undergo further metabolism in a second step to produce alcohol or acid metabolites (Eisenhofer *et al*., 2004).

2.1.6.2 Extraneuronal metabolism of catecholamines

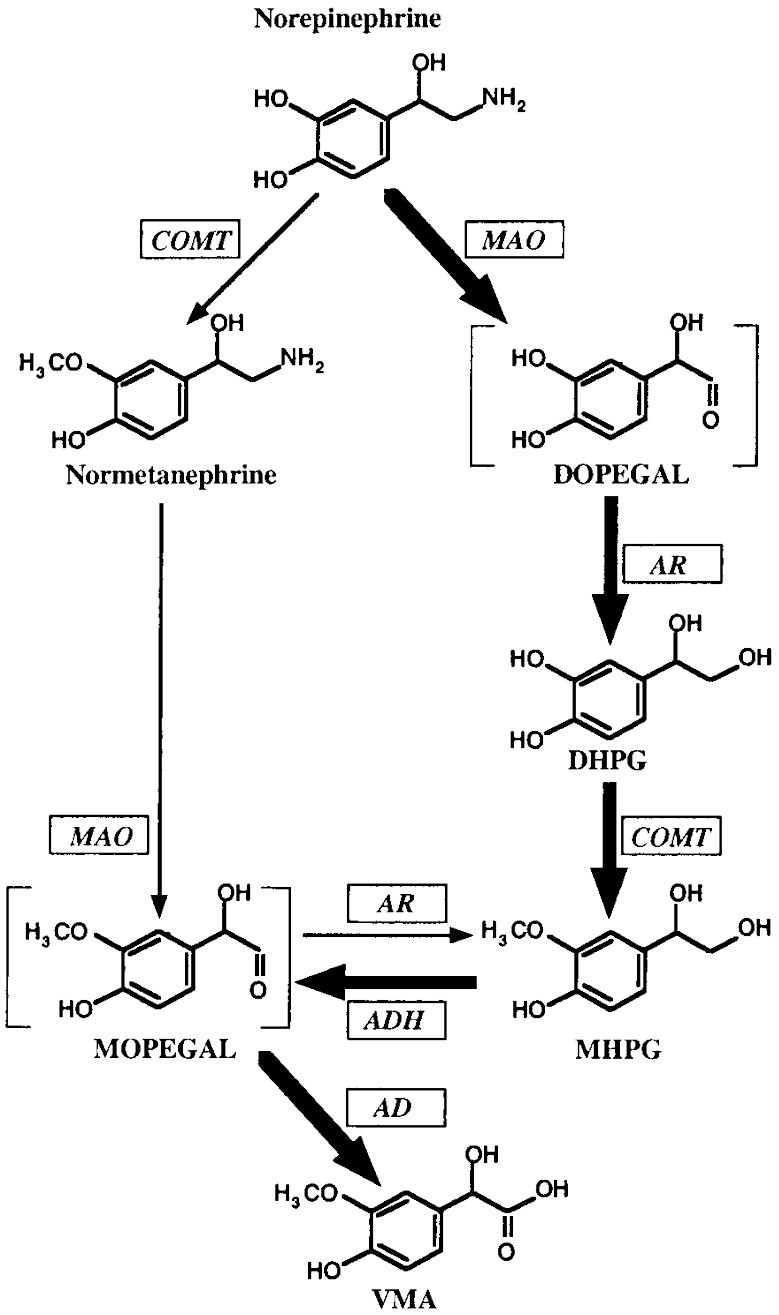

Figure 2.3 Deamination of norepinephrine to 3,4-dihydroxyphenylglycol aldehyde (DOPEGAL) by monoamine oxidase (MAO) and subsequent reduction to 3,4-dihydroxyphenylglycol (DHPG) by aldose or aldehyde reductase (AR)

(From: Eisenhofer *et al*. 2004)

Extraneuronal O-methylation of DHPG leads to the production of 3-methoxy-4-hydroxyphenylglycol (MHPG). Vanillylmandelic acid (VMA) is formed in the liver by oxidation of 3-methoxy-4-hydroxyphenylglycol (MHPG) and is catalysed by alcohol and aldehyde dehydrogenase (AD). Compared to intraneuronal deamination, extraneuronal O-methylation of norepinephrine and epinephrine to metanephrines represent minor pathways of metabolism. The single largest source of metanephrines is the adrenal medulla (Eisenhofer *et al*., 2004). Catechol-O-methyltransferase (COMT), catalyses DHPG to MHPG and also norepinephrine to normetanephrine in extraneuronal tissue. Alcohol dehydrogenase (ADH) converts MHPG to 3-methoxy-4-hydroxyphenylglycolaldehyde (MOPEGAL) and AD converts MOPEGAL to VMA in the liver (Eisenhofer *et al*., 2004).

2.1.6.3 Contribution of vesicular leakage to catecholamine metabolism

As mentioned previously, catecholamine metabolism occurs mainly in the cytoplasm of the same cells they were synthesized as a result of passive leakage into the cytoplasm. Monoamine oxidase sequesters 90% of the leaked catecholamines back into the vesicles and only 10% are metabolized further. Furthermore, only 30% of norepinephrine that escapes the sympathetic nerves is metabolized further. The rate of recapture and leakage of norepinephrine exceeds those of baseline exocytotic release and reuptake. Most of the norepinephrine released by sympathetic nerves is recaptured and recycled into vesicles, so that only about 30% is lost to metabolism (Eisenhofer, 2001). Although only a small proportion of norepinephrine that leaks from vesicles is metabolized the leakage rate considerably exceeds those of baseline exocytotic release and reuptake. Thus, under resting conditions much more catecholamines are metabolized secondary to leakage from vesicles than are metabolized after exocytotic release (Eisenhofer, 2001).

Therefore, catecholamine turnover, reflecting the continuous loss by metabolism and replenishment by synthesis, is driven primarily by leakage of catecholamines from vesicular stores (Eisenhofer 2001).

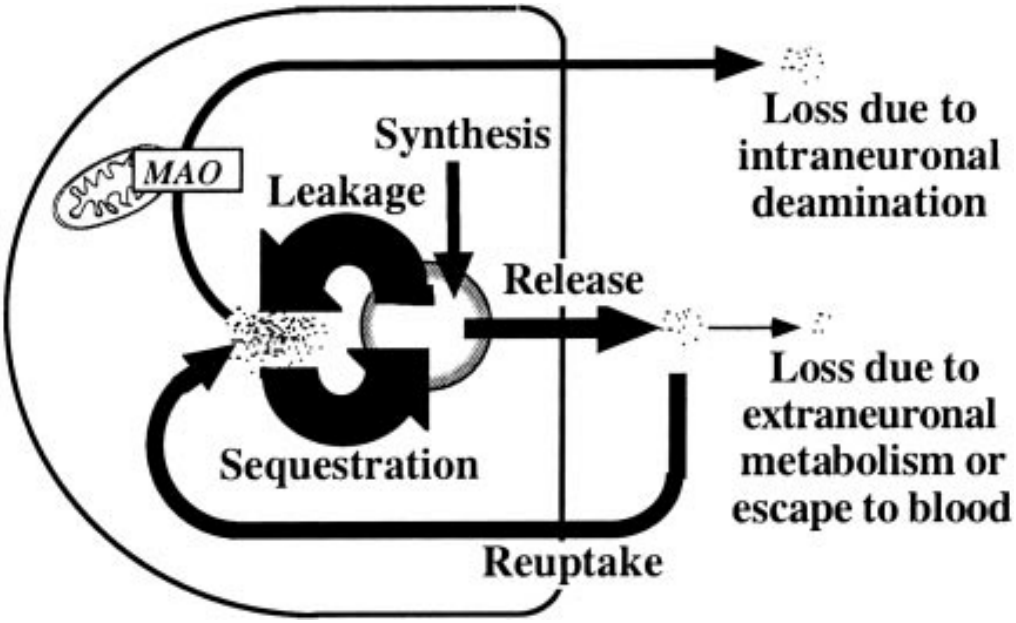

Figure 2.4 Contribution of vesicular-axoplasmic monoamine exchange process (leakage counter-balanced by sequestration) to catecholamine metabolism and turnover

MAO ~ mono amine oxidase

(From: Eisenhofer *et al*., 2004)

To maintain vesicular stores of catecholamines constant the rate of catecholamine turnover (i.e. loss of catecholamine due to metabolism or escape to the bloodstream) must be balanced by an equal rate of synthesis. The contribution of exocytotic release to catecholamine turnover is minimized by highly efficient neuronal reuptake and vesicular sequestration. This efficient sequestration also minimizes the contribution of vesicular leakage to turnover. Because rates of leakage are larger than baseline rates of release, the contribution of leakage to catecholamine metabolism and turnover exceeds that of release (Eisenhofer *et al*., 2004).

2.1.6.4 Metabolic pathways contributing to urinary catecholamine concentration

Eisenhofer (2001) explained how the sympatho-neuronal pathway is seen as the major pathway of catecholamine metabolism. The sympatho-neuronal pathway involves intraneuronal deamination of norepinephrine leaking from storage granules or of norepinephrine recaptured after release by sympathetic nerves.

The extraneuronal pathway is a relatively minor pathway of metabolism of catecholamines released from sympathetic nerves or the adrenal medulla, but is important for further processing of metabolites produced by neuronal and adreno-medullary pathways (Eisenhofer, 2001).

The adreno-medullary pathway involves O-methylation of catecholamines leaking from storage granules into the cytoplasm of adreno-medullary cells (Eisenhofer, 2001).

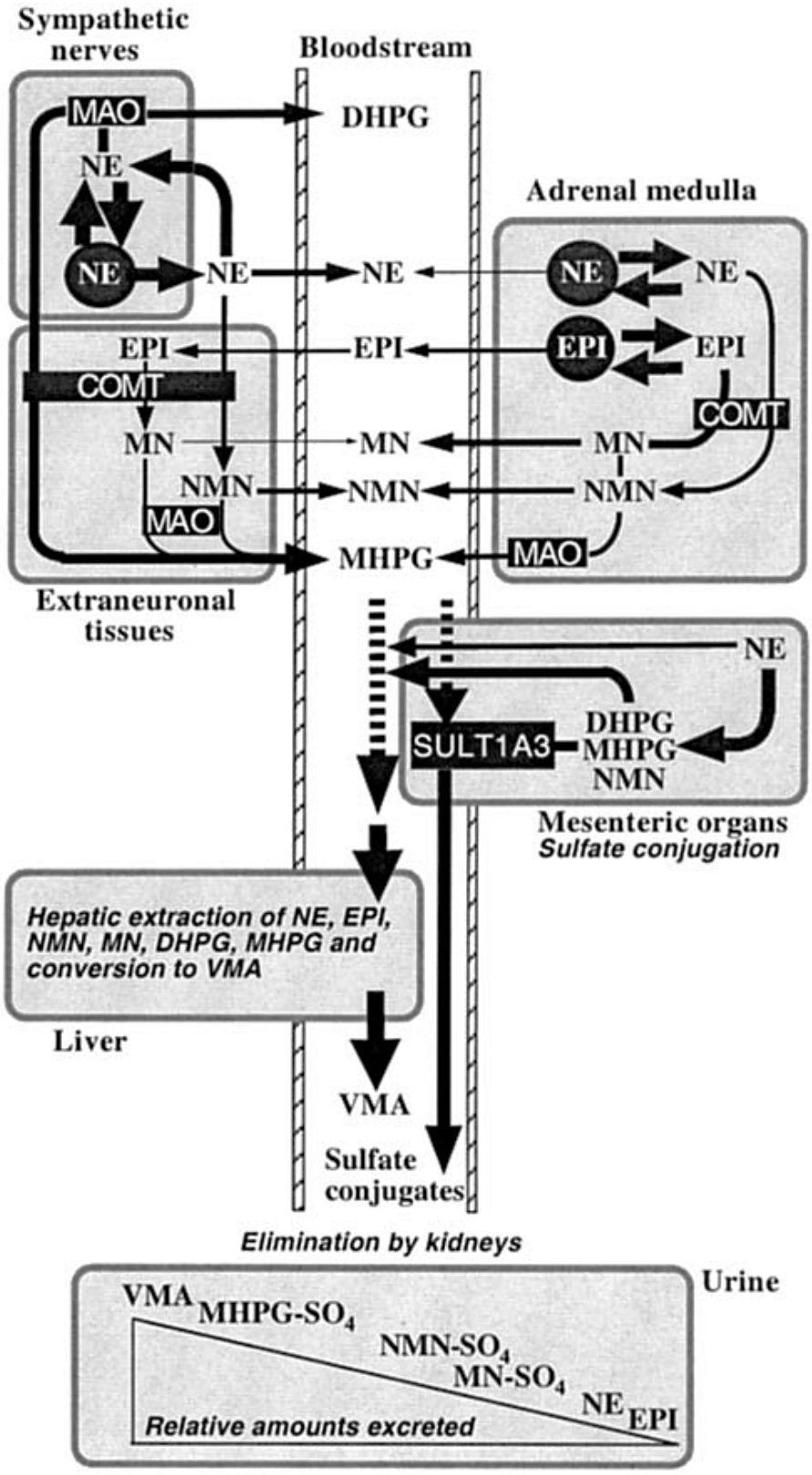

Figure 2.5 Regional catecholamine metabolism that explains the relative amounts excreted in urine by the kidney

MAO ~ Mono amine oxidase; MHPG ~ 3-Methoxy-4-hydroxyphenylglycol; VMA ~ Vanillylmandelic acid; SULT1A3 ~ Sulfotransferase type 1A3; NE ~ Norepinephrine; epinephrine ~ Epinephrine; NMN ~ Normetanephrine; MN ~ Metanephrine; NMN-SO4 ~ Normetanephrinesulphate; MN-SO_4 ~ Metanephrinesulphate; MHPG-SO_4 ~ 3-Methoxy-4-hydroxyphenylglycol sulphate

(From: Eisenhofer *et al*., 2004)

Most norepinephrine is released and metabolized within sympathetic nerves, including up to a half produced in sympathetic nerves of mesenteric organs. Sulphate conjugation of catecholamines and catecholamine metabolites, particularly MHPG, occurs mainly in mesenteric organs, whereas production of VMA occurs mainly in the liver. These represent the main metabolites excreted in urine (Eisenhofer *et al*., 2004).

Conversion of dopamine to norepinephrine by dopamine-β-hydroxylase in sympathetic nerves is 90% efficient (Eisenhofer *et al*., 1996). A substantial amount of dopamine produced in the body is produced in mesenteric organs. Dietary constituents have repeatedly been shown to influence plasma and urinary levels of dopamine metabolites (Hoeldtke *et al*., 1974; Eldrup *et al*., 1997; Goldstein *et al*., 1999).

2.2 Catecholamines and animal temperament

2.2.1 Importance of animal temperament in the meat industry

According to Price (1999) tameness towards humans and associated ease of handling are important aspects of the domestic phenotype. In the livestock industry, temperament is defined as the fear response of cattle towards human handling (Fordyce *et al*., 1985; Petherick *et al*., 2003). Researchers have developed methods to evaluate animal temperament in order to measure stress response to handling. Temperament scores were calculated as the animal's flight speed or exit velocity (Boles *et al*., 2015). Flight speed was measured as the amount of time it took for an animal to cover 1.7m after leaving a crush. Animals with a slow flight speed were classified to be tamer (with a better temperament) and scored lower temperament scores (Fordyce *et al*., 1988). According to Curley *et al*. (2006) blood cortisol levels were related to how fast an animal left the handling

chute. This suggested that the use of exit velocity was a viable tool to evaluate stress during livestock handling.

Temperament affects the economic return to a producer as cattle breeds with aggressive temperaments tend to have lower average daily gains in feedlots compared to docile breeds (Hammond *et al.,* 1998). Fordyce *et al*. (1988) reported that better temperament scores of animals in feedlots correlated with higher growth rates compared to animals with poor temperaments. These observations were made regardless whether favourable scores resulted from intensive, long-term handling or animals that are naturally docile. Burrow & Dillon (1997) indicated a relationship between growth rate and temperament in *Bos taurus* steers and heifers grazed on pasture. Decreased growth rates were also reported in animals with more excitable temperaments by Behrends *et al*. (2009) and Voisinet *et al*. (1997). Cafe *et al*. (2011) found that cattle with more excitable temperaments (as measured by exit velocities), had consistently lower feed intake and slower growth rates, resulting in smaller carcasses.

In addition to improved growth rate, animal temperament also influences the dressing percentage of meat producing animals. *Bos indicus* type cattle are perceived as less docile than *Bos taurus* types and according to Burrow & Dillon (1997), bruise trim per carcass increases by about 0.3 kg per unit increase in temperament score *(P<0.05).* Fordyce *et al.* (1988) reported that after cattle were transported, aggressive or temperamental cattle displayed more bruises along the back and around the *tuber coxae* and *tuber ischii* areas.

Along with decreased growth rates, researchers reported that meat from animals possessing a more excitable temperament had higher shear force values (Cafe *et al*., 2011 and Voisinet *et al*., 1997). The relationship between excitable temperaments and higher shear force values were seen to be stronger in *Bos indicus* than *Bos taurus b*reeds (Cafe *et al*., 2011 and Voisinet *et al*., 1997). Fordyce *et al*. (1988) found that animals with poor temperament scores had tougher meat *(P<0.05)* where animals with better temperament scores were not only easier to handle but obtained better meat tenderness values..

2.2.2 Catecholamines and domestication

It is clear that animal tameness and docility affects growth performance, ease of handling, bruise trim per carcass as well as meat quality attributes and the catecholamine system plays a crucial role in an animal's ability to cope with its environment. From the results of domestication studies it was found that selection for tameness in foxes and rats influenced the catecholamine system of the

brain (Cuomo-benzo *et al*., 1977; Nikulina, 1990; From: Price, 1999). Similarly, domestication of Sprague Dawley and Long Evans rats resulted in significant increments in norepinephrine and epinephrine content in whole brain (Cuomo-benzo *et al*., 1977). In rats and foxes, selected, tame animals had higher levels of norepinephrine in the hypothalamus than unselected non-tame control lines. Lower levels of norepinephrine were found in wild rats compared to domesticated rats. Researchers concluded that selection pressure favoured high norepinephrine content in the domesticated rat and silver fox. This enabled the domesticated rat to perform effectively in laboratory test situations (Cuomo-benzo *et al*., 1977). To conclude, catecholamine's played a crucial role in an animal's ability to cope with stressful situations as selection for tameness resulted in increased catecholamine synthesis which is linked to more tame animals.

2.2.3 Catecholamines and hyperactivity in humans

Studies on temperament and its correlation with catecholamine's of beef cattle have not been investigated to date. In humans dysfunctional catecholaminergic neurotransmitter systems contributed to the symptoms of attention deficit hyperactivity disorder (Sontag *et al*., 2010). The validity of animal models for attention deficit hyperactivity disorder was evaluated and validated by Mefford & Potter (1989), Pliszka *et al*. (1996) and Arnsten *et al*. (1996) emphasized the role of norepinephrine in focusing on relevant stimuli or tasks. In a study by (Sirvio *et al*., 1993; From: Haller *et al*., 1998) agents that increased brain norepinephrine improved attention in rats.

Increased blood flow to the brain as a result of the activation of the catecholamine system makes an animal more able to cope with stressful situations Sontag *et al*. (2010). The mechanisms that involve norepinephrine and corticotrophin releasing factor act in tandem in such a way that norepinephrine systems regulate the release of brain corticotrophin releasing factor via a α1-adrenoreceptor (Clark *et al*. 1988).

2.2.4 Catecholamine heritability

Dopamine-β-hydroxylase is the enzyme responsible for the conversion of dopamine to norepinephrine and is released as a response to exposure to stressful situations. Dopamine-β-hydroxylase activity is derived largely from sympathetic nerves and is measured in human plasma. Galvin *et al*. (1995, 1997) measured decreased activities of dopamine-β-hydroxylase in serum and

urine in patients with attention deficit hyperactivity disorder and concluded that low dopamine-β-hydroxylase levels were indirectly correlated with the seriousness of the hyperkinetic syndrome in children (Galvin *et al*., 1995, 1997). According to Kopeckova *et al*. (2006) polymorphisms occur frequently in the dopamine-β-hydroxylase gene, the G444A, G910T, C1603T, C1912T, C-1021T, 5'-ins/del and TaqI. These polymorphisms affect the function of gene products or modify gene expression and thus influence the progression of attention deficit hyperactivity disorder (Kopeckova *et al*., 2006), and possibly animal temperament.

2.3 The role of catecholamines in energy metabolism of living animals

2.3.1 Factors that activate catecholaminergic systems

In some scientific communities, catecholamines are referred to as coping hormones. Catecholamines provide energy to the brain and a deficiency may lead to "energy deficiency syndromes" in the brain (Todd & Botteron, 2001).

2.3.1.1 Acute stress

Lacourt & Tarrant (1985) explained that if an animal is stressed through transport and handling, there is a quick release of catecholamines which leads to changes in carbohydrate metabolism in order to provide glucose for important functions. The two central integrated processes namely the autonomic nervous system and hypothalamic-pituitary-adrenal axis are activated as neuroendocrinal responses to fear-eliciting stimuli. Acute stressors, such as human contact, activate an autonomic response in order to initiate reaction that requires a rapid response. By activating the autonomic nervous system, tachycardia, increased respiration rate, elevated body temperature and redistribution of visceral blood volume towards skeletal muscle and the brain are some of the physiological changes that incur. The sympatho-adrenal component of the autonomic response is mediated by catecholamines - epinephrine and norepinephrine. Activation of the hypothalamic-pituitary-adrenal axis is manifested by the release of glucocorticoids e.g. cortisol, from the adrenal cortex and operates independently of stressful situations (Ferguson & Warner, 2008).

According to Parkinson *et al*. (2002), dopamine is involved in an animal's attraction to a stimulus that predicts reward and dopamine has been shown to be involved in gluconeogenesis (Matsumura *et al*., 1980). It was also shown that intermittent tail-shock stress in mice increased

extracellular dopamine relative to the baseline, and researchers concluded that dopamine concentration increases during aversive conditions (Abercrombie *et al*., 2006).

2.3.1.2 Hunger

Ferguson & Warner (2008) stated that the need for energy is a basic need and animals that were transported may experience hunger. The inability of an animal to resolve this state may invoke further psychological distress (Ferguson & Warner, 2008). Catecholamine secretion results in significant changes in energy metabolism including lipolysis, glycogenolysis in muscle and gluconeogenesis. Epinephrine is an effective initiator of glycogenolysis. It mediates glycolysis through a series of biochemical changes that amplify the cyclic adenosine monophosphate-mediated pathway. In this way, epinephrine activates vast amounts of phosphorylase rapidly (Ylä-Ajos, 2006). The primary source of energy to the brain is blood-borne glucose (Todd & Botteron, 2001) and therefore catecholamines are crucial in providing the brain with "energy". Exposure to mixing, transport, and feed and water withdrawal increases the utilization and synthesis of dopamine, norepinephrine, and epinephrine implying that with feed restriction there may be a shortage of catecholamines (Schaefer *et al*., 2001).

2.3.1.3 Transport and increased activity

Animals that are transported may experience increased activity Ferguson & Warner (2008). Contracting muscle needs glucose and adenosine trisphosphate (ATP) as energy source. The rate of glycogenolysis in resting living mammalian muscle is low. In contracting muscle, glycogenolysis is triggered by a cascade of activating glycogenolytic enzymes. This is initiated by, amongst other, increased catecholamine release, a subsequent increased Ca^{2+} content in the cytosol or by a combination of both processes (Newsholme & Leech, 1983; Tarrant, 1989; Spriet *et al*., 1990). At an early stage of exercise, muscle contraction stimulates glycogenolysis (Richter *et al*., 1981; Richter *et al*., 1982). Muscle contraction induces the conversion of inactive phosphorylase to the active form through an allosteric activation caused by Ca^{2+} release from the sarcoplasmic reticulum (Roach, 2002; Nelson & Cox, 2005). This triggers immediate glycogen breakdown. Continued glycogenolysis needs the direct effect of epinephrine on muscle (Richter *et al*., 1982); or phosphorylase will revert back to resting levels (Chasiotis *et al*., 1982; Richter *et al*., 1982; Ren *et al*., 1990; Hespel & Richter, 1992; Parolin *et al*., 1999).

2.3.1.4 Pain

Norepinephrine is involved in the perception of pain by an animal. Pain sensitivity (beyond opioidergic control) decreases through descending noradrenergic pathways according to Devor *et al*., (1984); From: Haller *et al*. (1998). The main role of norepinephrine release and the onset of a socially relevant stimulus is the establishment of both somatic and neural conditions that ensures maximal efficiency in coping.

2.4 Glucose homeostasis in a living animal and its effect on meat quality

2.4.1 Catecholamine induced glucose homeostasis in a living animal

Catecholamine infusion under physiological conditions was associated with enhanced rates of aerobic glycolysis and ATP production in one study. Glucose was released from glycogenolysis and gluconeogenesis and these effects were termed: catecholamine-induced effects on glucose homeostasis by Barth *et al.* (2007).

Maddison *et al.* (1968) stated that increased plasma free fatty acid concentration increases during feed withdrawal as a result of fat reserves being mobilized to supply energy requirements. According to Shaw & Tume (1992), increased plasma free fatty acid concentration may also occur in response to catecholamine release following acute stress.

2.4.2 Energy restriction and glycogen depletion in muscle of living animals

Numerous studies have investigated pH changes and other biochemical parameters in *post mortem* mammalian muscle (Bate-Smith & Bendall, 1949; Lawrie, 1953; Howard & Lawrie, 1956; Tarrant *et al*., 1972; Hamm 1974). Although glucose homeostasis is essential in order for a live animal to cope with its environment, researchers concluded that *ante mortem* glycogen depletion in muscle resulted in meat with higher pHu values which is detrimental for the conversion of muscle to good quality meat (Purchas *et al*., 1999). Beef with pHu values higher than 6.0 was undesirable

because of its dark colour (Bartoš *et al*., 1993; Mounier *et al*., 2006), high variation in tenderness (Silva *et al*., 1999) and increased water binding capacity (Apple *et al*., 2005). *Ante mortem* stressors and factors (i.e. breed type and feed withdrawal) as well as the control and standardization of the *post mortem* environment (e.g. *rigor* temperature) that influence key traits like tenderness have also been extensively researched (Bendall, 1973; Bendall, 1979). Immonen & Puolanne (2000) stated that the determination of factors that result in a low pHu and to establishing the limits for residual glycogen levels obtained under various conditions are central to stress and meat quality research.

Stressors encountered by livestock cause catecholamine release in the immediate pre-slaughter period (Lacourt & Tarrant, 1985). Epinephrine activates muscle adenylate cyclase which stimulates glycogen breakdown (Voet & Voet, 1990). Lowered glycogen prevents an accepTable decrease in pH and attainment of pHu for optimal conversion of muscle to meat (Purchas *et al*., 1999; Warriss, 1990). This statement should rather be viewed within breed types as metabolic rate (Frisch & Vercoe, 1977) and stress responsiveness (Muchenje *et al*., 2009) vary between breeds.

2.4.3 Energy restriction in live animals and hypometabolism

Contrary to the previous paragraph, Bronnikov *et al*. (1990) explained that some triggers (i.e. cold or hunger) in some mammalian species lower its metabolic rate in order to preserve energy. When there is a change in metabolic fuelling, there may be a deactivation of mitochondrial functions. This may lead to a reduction in ATP production counterbalanced by decreased ATP consumption (Malan *et al*., 1988). Thus, in some animals, decreased food intake or increased energy expenditure may result in greater hunger, but subsequent energy conservation (Stephens & Caro, 1988).

2.5 *Post mortem* carbohydrate metabolism in the conversion of muscle to meat

2.5.1 Glycogen and muscle fiber types

The concentration, activity and ratio of different enzymes used for contraction and energy metabolism differ in various types of muscle. Fiber diameter varies from 10 to 100mm. Thinner fibers (Type I) are adapted for sustained activity that require sub-maximal tension generation. Type

IIA fibers have an intermediate diameter and the thickest fibers (Type IIb) are adapted for short bursts of near-maximal activity (Klont *et al*., 1998).

The function of muscle depends on the oxygen supply and its potential to remove waste products such as L-lactate (Saltin & Gollnick, 1985). There is an inverse relationship between fiber diameter and the oxidative capacity of fibers. Myoglobin is the molecule that provides a storage capacity for oxygen within muscle cells (Hopkins, 2006). Myoglobin is pigmented because of haem that is responsible for its oxygen-binding capability. Muscle with predominantly Type I fibers contain more myoglobin and therefore appear deep red in colour compared to muscle with few Type I fibers (Klont *et al*., 1998). A combination of fiber type and size is important with regards to *ante mortem* muscle metabolism and its relationship with meat quality (Henckel *et al*., 1997; From: Klont *et al*., 1998).

Immonen & Puolanne (2000) described the concentration of glycogen in muscle at the time of slaughter as essential because of its key role in preventing a beef quality defect known as dark-cutting. Mammalian muscle glycogen has been studied intensively because of its role in *in vivo* stress, *ante mortem* glycogen depletion, exercise-related energy metabolism as well as *post mortem* anaerobic glycolysis in muscle. It should be kept in mind that *post mortem* energy metabolism in different species, breeds and muscle types may differ as determined by the metabolic fiber type i.e. oxidative or glycolytic fiber types. The metabolic pathway of fibers affects its glycogen concentration so that slow twitch/red fibers have low and fast twitch/red and fast twitch/white fibers have high glycogen concentrations (Judge *et al*., 1989; From: Immonen & Puolanne, 2000).

2.5.2 Phases of *post mortem* glycogen metabolism

In *post mortem* muscle, glycogen is converted to L-lactate through two phases namely glycogenolysis and glycolysis (Campbell, 1995; Ylä-Ajos, 2006). These two phases will be discussed in order to understand the allosteric control of glycogen breakdown, the enzymes involved and possible breed differences.

2.5.2.1 Glycogenolysis

The differences in glycogenolytic enzymes (glycogen debranching enzyme and phosphorylase) between different muscles and fibers, affect the rate of glycogenolysis. This will depend on the relative ratio of these enzymes within a fiber (Ylä-Ajos, 2006).

Phosphorylase and glycogen debranching enzyme

The reason for discussing the enzymes involved in this phase of glycogenolysis is because concentrations are fiber type specific as well as pH and temperature sensitive.

The first step of glycogen degradation involves the enzyme glycogen phosphorylase which rapidly releases glucose as glucose-1-phosphate (G-1-P) (Newsholme & Start, 1979). As described by Walker & Whelan (1960) and Meléndez-Hevia et al. (1993) the action of glycogen phosphorylase stops when it reaches the fourth glucose unit from the branching point of a glycogen chain. In other words, glycogen phosphorylase, cleaves α (1→4) linkages in glycogen. No ATP is hydrolysed in this step and because ATP hydrolysis is proposed to be the major source of proton (H^+) load during anaerobic metabolism (Gevers, 1977; From: Scheffler et al., 2011) pH is not affected in this phase.

Glycogen debranching enzyme degrades α (1→6) linkages for complete glycogen breakdown (Campbell, 1995) and glycogen become susceptible to the action of glycogen phosphorylase again (Brown & Illingworth-Brown, 1966; Hers et al., 1967; Taylor & Whelan, 1968; Nelson et al., 1969). Thus, glycogen degradation is accomplished by the combined action of phosphorylase and glycogen debranching enzyme acting in concert.

Both glycogen debranching enzyme and phosphorylase are extremely pH and temperature sensitive. Temperatures at 39 ˚C with pH values at around seven are optimal for the action of phosphorylase and its activity sharply reduces at lower or higher temperatures (Cori et al., 1943; Schwägele et al., 1996). Glycogen debranching enzyme is active at a pH range of 5.0 to 7.4 in m. masseter and *m. longissimus*, peaks slightly at around pH 6.5 for m. longissimus, and increases slowly as pH decreases. At temperatures below 15 °C in m. longissimus, glycogen debranching enzyme activity may drop at 25 °C and even reach zero activity (Ylä-Ajos, 2006).

Allosteric control of glycogenolysis

The rate of glycogenolysis is regulated allosterically. Glycogen phosphorylase is a dimer that exists in two forms - the inactive T-form and the active R-form. In the T-form glycogen

phosphorylase can be modified by the phosphorylation of a specific serine residue. Esterification of the serines to phosphoric acid is catalysed by phosphorylase kinase; the dephosphorylated form is catalysed by phosphoprotein phosphatase. The phosphorylated form of glycogen phosphorylase is termed phosphorylase a; the dephosphorylated form is termed phosphorylase b (Campbell, 1995).

Phosphorylase a does not respond to the allosteric effectors that control behaviour of phosphorylase b and vice versa. Glucose is an allosteric inhibitor of phosphorylase a; ATP and glucose-6-phosphate are allosteric inhibitors of phosphorylase b. Adenosine monophosphate (AMP) is an activator of phosphorylase b. These differences ensure that glycogen will be degraded when there is a need for energy and ATP i.e. low ATP and low glucose-6-phosphate (Campbell, 1995; Ylä-Ajos, 2006).

Activation of glycogenolytic enzymes

Activation of glycogenolytic enzymes can be triggered by either increased catecholamines or muscle contraction or by both acting in concert (Tarrant, 1989). A number of factors influence the amount of glycogen debranching enzyme in muscle. Glycogen debranching enzyme activity differs between animal species and between different fiber types of an animal and possibly between breeds of the same species. Higher glycogen debranching enzyme and phosphorylase activity (in fibers of the same animal) exist in muscle with predominantly fast twitch glycolytic fibers. The high activity of these enzymes enables a faster rate of glycogenolysis in glycolytic- compared to oxidative fiber types (Ylä-Ajos, 2006).

The relative ratio between phosphorylase and glycogen debranching enzyme activity plays a role in the rate of glycogenolysis (Kivikari, 1996; From: Ylä-Ajos, 2006). The ratio between phosphorylase and glycogen debranching enzyme is higher in fast twitch glycolytic than in slow twitch oxidative fibers. Relatively lower glycogen debranching enzyme activity compared to phosphorylase activity in fast twitch glycolytic fibers may be a protection mechanism in living muscle against a fast decrease in pH (Kivikari, 1996; From: Ylä-Ajos, 2006).

2.5.2.2 Glycolysis

Compared to glycogenolysis, which is the breakdown of glycogen to glucose-1-phosphate and glucose, glycolysis is the stepwise degradation of glucose-1-phosphate and glucose to produce

pyruvate. Glucose-1-phosphate is converted to glucose-6-phospate by the enzyme phosphoglucomutase (Campbell, 1995).

Pyruvate as the junction point in glycolysis

Newsholme & Leech (1983) explained the synthesis of lactate as follows: when oxygen is available and the density of mitochondria is not a limiting factor, pyruvate enters the mitochondrion and is converted to Acetyl-coenzyme-A. Acetyl-coenzyme-A is further oxidized in the Krebs cycle. Under anaerobic conditions the Krebs cycle is halted and lactate dehydrogenase catalyses the reduction of pyruvate to lactate in the fiber cytosol (Newsholme & Leech, 1983). Some glycogen always remains unconverted in muscle after the *post mortem* reaction sequence has ended (Lawrie, 1955; Lawrie et al., 1959; van Laack & Kauffman, 1999; Immonen & Puolanne, 2000).

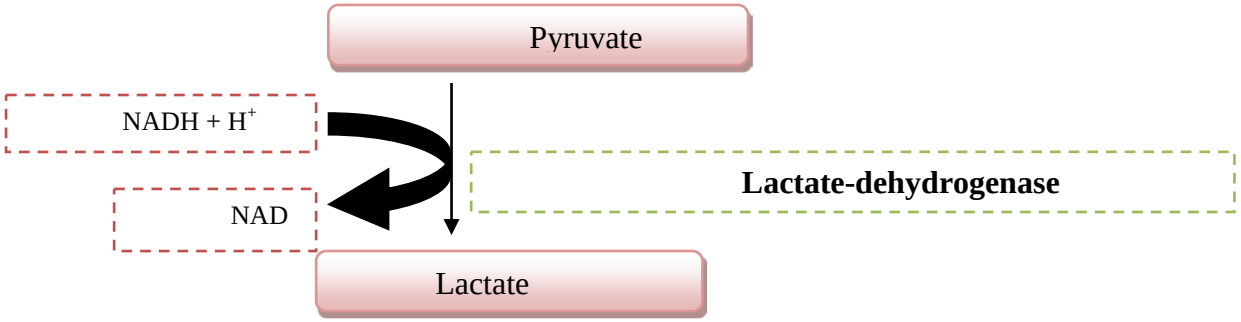

Figure 2.6 Conversion of pyruvate to lactate through the action of the enzyme lactate dehydrogenase

NAD^+~ Nicotinamide dinucleotide (oxidized form); NADH ~ Nicotinamide dinucleotide (Reduced form); H^+~ Proton

(Adapted From: Scheffler *et al*., 2011)

The rate of *post mortem* glycolysis is slowed down by several factors which include AMP deficiency; shortage of adenosine diphosphate (ADP) or glucose and inhibition of glycolytic enzymes by a low pH (Kastenschmidt *et al*., 1968; Scopes, 1971; Lundberg *et al*., 1987; Pearson & Young, 1989a; van Laack *et al*., 2001; Rhoades *et al*., 2005). The inhibition of glycolytic enzymes, and specifically glycogen debranching enzyme, will lead to insufficient glycogen debranching enzyme action resulting in a decrease of glycosyl units that are needed by phosphorylase to complete glycogenolysis (Ylä-Ajos, 2006).

2.5.3 Metabolic acidification

In the living cell, ATP is maintained at a constant level. Anaerobic degradation of glycogen to lactate is a rapid metabolic source of ATP, as ATP is synthesized via the glycolytic pathway (Scheffler *et al*., 2011). Anaerobic ATP synthesis makes swift movements possible in living muscle. Thus, anaerobic energy production by glycolysis allows for fast adjustment to increased energy demand, as it can be accelerated faster than ATP production from oxidative phosphorylation (Juel, 1997). Although ATP production is faster during anaerobic metabolism it is an inefficient way to produce ATP. Complete aerobic oxidation of glucose-6-phosphate yields 37 ATP molecules whereas only 3 ATP molecules are regenerated anaerobically (Ylä-Ajos, 2006).

Small amounts of lactate are produced almost constantly in the muscle of living resting animals and resting man (Bendall, 1973; Richter *et al*., 1982; Henckel, 2002). In living muscle, lactate is used for energy production and should not be regarded as a waste product (Scheffler *et al*., 2011). When oxygen is available, lactate is oxidized for energy in the heart, brain, liver, dark (oxidative) muscle, and to a lesser extent in light (glycolytic) muscle (Juel, 1997). The conversion of lactate to pyruvate and vice versa is catalysed by the enzyme, lactate dehydrogenase (Scheffler *et al*., 2011).

The relationship between acidification and meat quality characteristics have been covered in detail by several researchers (Briskey, 1964; Bendall, 1973; Hamm, 1974; Asghar & Pearson, 1980; Seideman *et al*., 1984; Offer & Knight, 1988; Pearson & Young, 1989; Warner *et al*., 2001). Generally, both the rate and extent of *post mortem* pH decrease determine the palatability of meat by affecting meat quality properties such as drip loss, colour development, shelf life, water binding capacity, texture and tenderness.

Living mammalian muscle pH at rest is between 7.0 and 7.3 (Tarrant *et al*., 1972; Bendall, 1973; Bendall, 1975; Renou *et al*., 1986; Kivikari, 1996 From: Ylä-Ajos, 2006). *Ante mortem* stress reduces muscle glycogen (Tarrant *et al*., 1972; Henckel *et al*., 2000). Fatigued muscle pH may fall to between 6.5 and 6.3 (Lovell *et al*., 1987; Juel, 1996) and can be lower at the time of slaughter compared to muscle at rest.

In living muscle, homeostasis and pH are maintained by strong metabolic and structural buffering mechanisms (Bate-Smith, 1938; Davey, 1960; Bendall, 1973; Rao & Gault, 1989; Kivikari, 1996 From: Ylä-Ajos, 2006). pH is maintained by H^+ and lactate transport out of the fiber mainly

with the help of monocarboxylate transporters and Na^+ / H^+ exchange (Juel, 1996; Juel, 1997; Halestrap & Price, 1999; Sepponen *et al*., 2003).

Anaerobic energy production leads to lactate and H^+ accumulation, which in turn leads to decreased pH values. Metabolic acidosis in living muscle occurs when ATP demand (i.e. the rate of ATP hydrolysis) exceeds the rate of ATP production in the mitochondria (Bendall, 1973; Honikel & Hamm, 1974; Hamm, 1977; Robergs *et al*., 2004).

According to Bendall (1973) the tendency of fibers to maintain ATP at constant levels even after death leads to the process whereby muscle is converted to meat. In the anoxic cell, ATP is replenished by the breakdown of creatine phosphate and degradation of carbohydrates via anaerobic pathways (Bendall, 1973).The initial splitting of ATP to ADP plus inorganic phosphate (Pi) and H^+ (during the first biochemical steps of glycolysis), determines the rate and magnitude of carbohydrate catabolism. Without this reaction glycolysis and acidification can come to a halt very rapidly (Bendall, 1973). Reactions for the resynthesis of ATP and ATP hydrolysis inside a cell include the creatine kinase pathway, glycolytic, myokinase reaction and the aerobic synthesis of ATP (Vetharaniam *et al*., 2010).

There are several ATP hydrolases (ATPases) present in skeletal muscle:

1. myosin ATPase
2. sarcoplasmic reticulum Ca^{2+}-ATPase
3. mitochondrial ATPases
4. ATPases of the sarcoplasmic reticulum which are activated by Na^+, K^+ or Ca^{2+} ions (Bendall, 1973; Pearson & Young, 1989; Nelson & Cox, 2005).

In living muscle, the energy released by action of these enzymes is mainly used for contraction, to keep the cytoplasmic Ca^{2+} content low and to maintain a balance between Na^+ and K^+ (Savell *et al*., 2005).

ATP that is synthesized and hydrolysed in order to provide chemical energy for contraction is as follows:

1. ATP hydrolysis: (RA) rate of ATP hydrolysis
$ATP^{n-1} + H2O \rightarrow ADP^{(n-1)-} + \gamma Pi^{2-} + (1-\gamma)Pi^{1-} + \gamma H^+$
2. Creatine kinase pathway: (RC) rate of creatine production
$CP^{2-} + ADP^{(n-1)-} + H^+ \leftrightarrow ATP^{n-} + Cr$
3. Glycolysis: (RG) rate of ATP synthesis through glycolysis
$ADP^{(n-1)-} + 1/3\ glycogen + \gamma Pi^{2-} + (1-\gamma)Pi^{-1} + \gamma H^+ \rightarrow ATP^{n-} + H2O + 2/3La^- + 2/3H^+$

4. Myokinase: (RM) rate of myokinase ADP consumption

$ADP^{n-} \leftrightarrow \frac{1}{2} ATP^{(n+1)-} + \frac{1}{2} AMP^{(n-1)-}$

5. Aerobic ATP synthesis: (RO) rate of aerobic ATP synthesis,

$ADP^{(n-1)-} + \gamma Pi^{2-} + (1-\gamma)Pi^{1-} + \gamma H^{+} \rightarrow ATP^{n-} + H2O$

(where γ represents the fraction of free phosphate existing as PO^{2-}_4 in equilibrium with PO^{1-}_4, with pKa = 6.70 (Kushmerick, 1997; From: Vetharaniam *et al*., 2010).

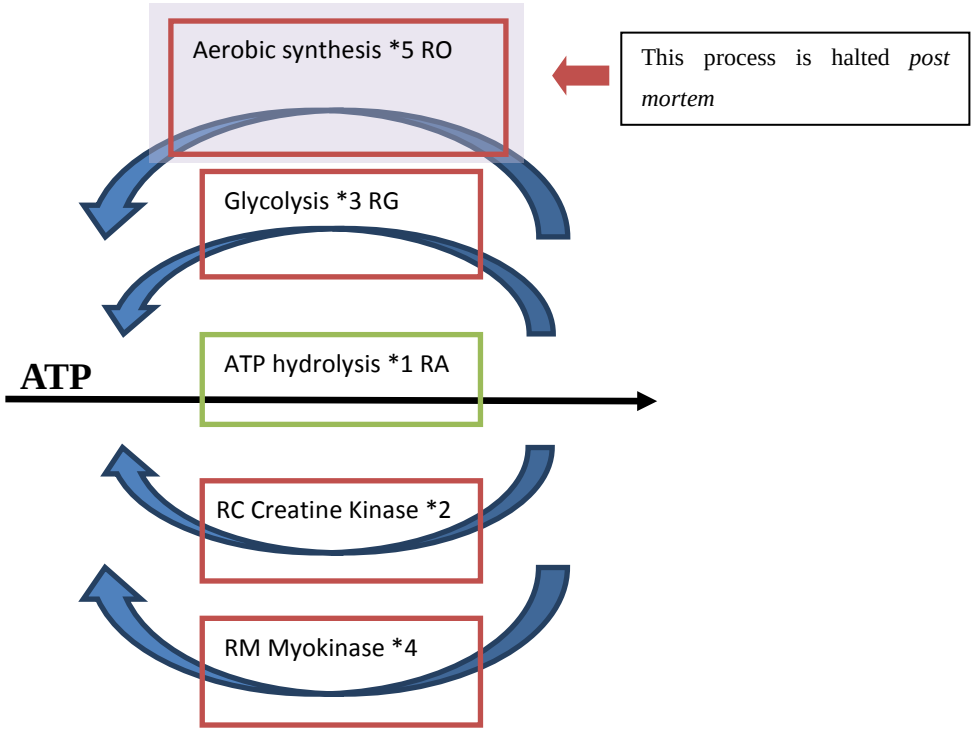

Figure 2.7 Synthesis of ATP through ATP hydrolysis, creatine kinase, glycolysis, myokinase and aerobic synthesis of ATP

RA ~ rate of ATP hydrolysis; RC ~ rate of creatine production; RG ~ rate of ATP synthesis through glycolysis; RM ~ rate of myokinase ADP consumption; RO ~ rate of aerobic ATP synthesis

(Adapted From: Vetharaniam *et al*., 2010).

The rate of pH decrease reflects the intensity of *post mortem* metabolism. The intensity of metabolism is species, breed and muscle specific. Different species, breeds and muscle within the same species or breed have unique pH curves. *Post mortem* energy production results in acidification to pH values between 5.4 and 6.2 (Bate-Smith, 1938; Hamm, 1977; Monin, 2004; Robergs *et al*., 2004). In aerobic muscle, resynthesis of ATP, consequent to its enzymatic hydrolysis to liberate chemical energy, is dominated by the activity of the tricarboxylic acid cycle using carbohydrate, fatty acids or amino acids as substrates (Vetharaniam *et al*., 2010).

ATP hydrolysis with consequent metabolic acidification occurs in two phases.

Phase 1

ATP decline and eventual "disappearance" of ATP early *post mortem* is associated with an accumulation of H^+ and Pi and followed by pH decline (*1) (Figure 2.7 Vetharaniam *et al*., 2010). In anaerobic muscle, energy metabolism is maintained through glycolytic pathway activity (with glycogen as the initial substrate) and the creatine kinase reaction (Lohmann reaction; Lohmann, 1934). Maintenance of cellular ATP will continue as long as creatine phosphate (*1) and glycogen are available (*3).

Phase 2

Sustained anoxia (or later stages of *rigor*) results in depletion of ATP, leading to muscle stiffness as cross-bridges form between actin and myosin during the development of *rigor mortis* (Jeacocke, 1984). As soon as muscle temperature is reduced to 0 – 15 °C, the sarcoplasmic reticulum cannot function properly and is unable to bind Ca^{2+}, which leaves an abundance of Ca^{2+} in the sarcoplasm. If there is ATP left in the muscle, it contracts at a maximum force, causing the filaments to slide over one another basically eliminating the I-band of the sarcomere (Aberle *et al*., 2001 From: Savell *et al*., 2005). Myosin ATPase is Ca^{2+} dependent. Available Ca^{2+} in the sarcoplasm, activates myosin ATPase, which results in myosin moving from a high energy to low energy configuration (contraction) (Huxley, 2000). At internal temperatures of 1 – 2 °C, the sarcoplasmic reticulum is least functional (Aberle *et al*., 2001 From: Savell *et al*., 2005).

Researchers have found that enzymes controlling ATP metabolism and glycolysis in living muscle are still active *post mortem*, but can only maintain *ante mortem* levels of ATP for as long as creatine phosphate is available (Bendall, 1951; Lawrie, 1953; Tarrant *et al*., 1972; Bendall, 1973; Bertram *et al*., 2002). Within a few hours *post mortem* ATP levels decrease to one third of the resting value (Bendall, 1951; Bendall, 1973) and onset of *rigor mortis* ensues (Bendall, 1951; Lawrie, 1953; Bendall, 1973).

During glycolysis the production of nicotinamide dinucleotide (oxidized form) NAD^+ (a biochemical step that uses H^+) ensures that glycolysis and ATP production continues (Scheffler *et al*., 2011). Lactate synthesis is therefore a prerequisite for continuing anaerobic energy production. Lactate maintains the rate of anaerobic glycolysis by maintaining the redox equilibrium between NAD^+ and nicotinamide dinucleotide (reduced form) NADH (Nelson & Cox, 2005).

The biochemical process of lactate synthesis retards acidification. During the last few biochemical steps of glycolysis, (including the conversion of pyruvate to lactate), H^+ is consumed and pH declines at a slower rate. But, although lactate synthesis retards acidification, it is still a good indirect marker for fiber metabolic conditions that induce increased H^+ release (Scheffler *et al*., 2011).

2.5.4 Influence of carbohydrate metabolism on meat colour

Post mortem glycolysis decreases pH, making the muscle appear brighter and superficially more wet. If the ultimate meat pH is high, the physical state of proteins will be above their iso-electric point. Proteins will associate with more water in muscle and therefore fibers will be tightly packed. This meat is dark because its surface does not scatter light to the same extent as the more open surface of meat with a lower ultimate pH (Seideman *et al*., 1984). Low temperatures delay metmyoglobin formation both directly and indirectly by suppressing the residual activity of oxygen utilizing enzymes (Lawrie, 1998).

Prolongation of high muscle temperature at lower pH causes proteins to denature and the precipitation of sarcoplasmic proteins is associated with a decrease in myofibrillar solubility, water is liberated and superficially wet meat reflects more light. The characteristic colour of meat is a function of its pigment content and light scattering properties. On exposure of meat surface to oxygen, the purple ferrous haem pigment, myoglobin, forms a bright red covalent complex, oxymyoglobin. Formation of desirable oxymyoglobin is enhanced by conditions that increase oxygen solubility such as low temperature, low pH (glycolytic potential), high oxygen tension and low enzyme activity (MacDougall, 1982). Meat pH has a great influence on meat colour development (Abril *et al*., 2001) through its effects on the physical state of proteins. At a high pHu (>6.0) myofibers hold a lot of water (Offer & Trinick, 1983). At a higher myofibrillar volume, light is able to penetrate to a deeper depth and be absorbed by myoglobin before it is scattered back to the eye (MacDougall, 1982). Meat appears translucent and dark. At normal pHu values (5.5) myofibers hold less water and meat appears brighter and glossier (Ledward, 1992).

2.6 Toughening and tenderising phases in the conversion of muscle to meat

Meat tenderness can be described in two phases that occur *post mortem*. The first phase is the toughening phase which is evident in sarcomere shortening and the second phase is the tenderising phase which involves proteolytic enzymes (Koohmaraie, 1996).

2.6.1 Toughening phase in the conversion of muscle to meat

2.6.1.1 Muscle contraction

In living, resting muscle, myosin is bound to ATP. ATP serves as energy source for the movement of cross-bridges which result in shortening of a sarcomere (contraction). In a resting phase, ATP is associated with the myosin head. Bonding of ATP moves the myosin head (cross-bridge) to a high energy (relaxed) configuration. Transfer of energy, when pivoting movement of the myosin head (cross-bridge) occurs, results in a low energy configuration. Thus, myosin in a high energy conformation is in a relaxed state. Chemical energy that is produced during ATP hydrolysis to form ADP, results in contraction. The enzyme responsible for conversion of myosin ATP to ADP is myosin ATPase (Gordon *et al*., 2000). If Pi becomes depleted and ADP cannot be converted to ATP anymore, the muscle stays in its contracted configuration which is known as *rigor* (Savell *et al*., 2005).

2.6.1.2 Different conditions for sarcomere shortening

Different conditions for additional sarcomere shortening have been summarised by Savell *et al* (2005). They are cold shortening, thaw and heat *rigor*.

Thaw *rigor* develops when muscle that was frozen before the onset of *rigor* is thawed. On thawing, contraction is produced by the sudden release of Ca^{2+} into the sarcoplasm resulting in 60–80% shortening of original muscle length (Aberle *et al*., 2001).

Heat *rigor* develops when muscle is maintained at elevated temperatures (up to 50 ˚C). This results in rapid ATP depletion which creates severe shortening and early onset of *rigor* (Aberle *et al*., 2001).

2.6.1.3 *Post mortem* sarcomere shortening and the sarcoplasmic reticulum

Chilling processes of livestock carcasses are primarily employed to ensure food safety (Savell *et al*., 2005). However, chilling of carcasses affects eating quality of beef negatively (Lawrie, 1998). Cold shortening has been studied since the 1960s (Locker, 1985). Locker & Hagyard (1963) defined cold shortening as a rapid decline in muscle temperature to less than 14–19 ˚C before onset of *rigor mortis*. When muscle temperature is reduced to 0–15 °C before the onset phase of *rigor*, the sarcoplasmic reticulum becomes unable to bind Ca^{2+}. With abundant Ca^{2+} in sarcoplasm together with ATP at lower temperatures, contraction occurs at a maximum level which causes filaments to slide over one another. This eliminates the I-band of a sarcomere (Aberle *et al*., 2001).

Muscle types vary in their potential to undergo cold shortening, with red being more susceptible than white fibers (Bendall, 1973). Because white fibers tend to have higher glycogen concentrations not all muscles are affected by cold shortening to the same degree (Hannula & Puolanne, 2004).

2.6.1.4 *Post mortem* sarcomere shortening and the mitochondria

In a study by Buege & Marsh (1975) researchers concluded that mitochondria in muscle fibers are also involved in sarcomere shortening. Ca^{2+} released under *post mortem* anaerobic conditions by mitochondria, cannot be removed rapidly enough by the sarcoplasmic reticulum at low temperatures which leads to further sarcomere shortening.

2.6.1.5 Debate around meat toughness and sarcomere shortening

Meat toughening and its causes have been debated and some researchers (Culler *et al*. (1978) have shown that sarcomere length does not affect tenderness (Culler *et al*., 1978; Seideman & Koohmaraie, 1987 and Shackelford *et al*., 1994). On the other hand, majority of research suggests that sarcomere shortening is the causative factor for a decrease in tenderness of muscle from the time of slaughter to 24 hours *post mortem* (Bouton *et al*., 1973). Koohmaraie (1996) showed that as sarcomere length decreases from 2.24 um to 1.69 um *post mortem*, shear force subsequently increases. Shear force does not increase during *rigor* development when muscle is prevented from shortening (Koohmaraie, 1996).

Other factors that determine toughness are fiber diameter and *post mortem* tenderization. In the cluster analysis of Chikri *et al*., 2012 where the aim was to identify the most important muscle

characteristics for beef tenderness, it was found that fiber diameter plays an important role in predicting tenderness with smaller fibers being more tender.

2.6.2 Tenderising phase in the conversion of muscle to meat

According to Koohmaraie *et al*. (1991a) and Ouali & Talmant (1990) differences in the rate of tenderization via proteolysis is explained by variation in calpastatin activity. In the opinion of Koohmaraie (1996) calpains (and more specifically, µ-calpain) are the only proteases that are directly involved in events leading to meat tenderization. According to Ouali (1990) ageing rate of meat is faster in fast twitch white fibers than in slow twitch red fibers. Ouali (1990) further showed that slow twitch red muscle fibers, which exhibit the lowest ageing rate, have the highest calpain content and suggested that expression of these proteinases is muscle dependent.

2.7 Beef breed types

Our understanding of evolutionary and demographic history of humans, animals and plants came from knowledge obtained by evolutionary anthropologists and archaeobiologists. Recently, our understanding of how these events took place was expanded by use of molecular genetics. It became possible to identify the wild ancestors of modern livestock, thereby expanding our knowledge of the nature of livestock (Bruford *et al*., 2003).

Evolutionary relationships between the two main types of cattle, humped zebu (*Bos indicus*) and humpless taurine (*Bos taurus*), is an active area of research in order to explain genetic differences between the two subspecies. The origin and taxonomic status of domesticated cattle are controversial and there are two theories concerning domestication of cattle. The most widely held view is that both types of cattle derived from a single domestication event 8000-10,000 years ago, but with the use of molecular genetics, phylogenetic analyses and data interpreted in light of data generated from other disciplines such as cytology, archaeology and allozyme studies, estimated divergent times provide the strongest evidence to date for independent domestications of zebu and taurine cattle. The two major mtDNA clades diverged at least 200,000, and possibly as much as 1 million, years ago. This relatively large divergence is interpreted most simply as evidence for two separate domestication events, presumably of different subspecies of the Aurochs, *Bos primigenius* (Loftus *et al*., 1994).

2.7.1 Brahman

Brahman type cattle represented *Bos indicus* in this study. This breed is known for its longevity, mothering ability and efficient beef production. Mature bulls weigh up to between 800 and 1,100 kg and mature cows weigh up to 500 and 700 kg. These animals are large relative to other breeds. Zebu, *Bos indicus*, cattle evolved in the Southern hemisphere and is adapted to muggy and scorching environments. Main characteristic of *Bos indicus* cattle that distinguishes them from *Bos taurus* cattle is their high degree of adaptability to warm climates (Warren, 1984). Unfortunately, cattle with more Brahman breeding are more temperamental compared to those with lower or no Brahman influence (Hammond *et al.*, 1998).

2.7.2 Nguni

Nguni type cattle represented *Sanga* in this study. Nguni probably evolved from crosses between Zebu (indicine) and humpless Hamitic longhorn and shorthorn cattle (taurine) in central and east Africa around 1600 BC (Payne, 1964). This breed developed as a draft animal and to live in close relation with the tribe and in the kraal with other animals. These selection factors resulted in a docile breed with a good temperament (Bester *et al.*, 2005). Furthermore, this breed developed as a result of a long process of natural selection in a climatically challenging environment. It is a small to medium-framed, early maturing breed known for its fertility and resistance to ticks and diseases (Bester *et al*., 2005) and is therefore one of the favourite breeds amongst milk and meat producers of South Africa (Frisch, 1973).

2.7.3 Simmental

Simmental type cattle represented *Bos taurus* in this study. Simmental is an influential breed whose history dates back to the Middle Ages. Early records indicate that this breed originated as a result of a cross between large German cattle and a smaller breed indigenous to Switzerland (Voisinet *et al*., 1997). This breed is known for its high growth rate, docile disposition and good carcass quality (Voisinet *et al*., 1997). The first Simmental cattle were imported to South Africa in 1905 but it was only at the beginning of the 1960's that Simmental actually gained in popularity (Massmann, 1989).

CHAPTER 3 MATERIALS AND METHODS

3.1 Experimental animals

Beef cattle (n = 180) used in this study represented crossbreeds often used in meat production in South Africa (According to Dave Ford of the Feedlot Association of South Africa and committee member of the Red Meat Research and Development Trust (RMRDT)). Three different breed type bulls, Brahman, Nguni and Simmental were purchased from commercial farmers.

Breeds used in this study were breed types and not purebred. "Brahman", "Nguni" and "Simmental" refer to the breed types used in this study.

Brahman (n = 60), (represented *Bos indicus*)
Nguni (n = 60), (represented Sanga) – A cross between *Bos taurus* and *Bos indicus*
Simmental (n = 60), (represented *Bos taurus*)

Live weight, cold carcass weight and warm carcass weight were recorded. Animals (10-12 months, and average weight of 158 kg) were transported to the Agricultural Research Council – Animal Production Institute (ARC-API: Irene), where they were placed under feedlot conditions for a period of about 100 to 120 days until slaughter. The animals received implants (Revalor-S) as applied in South African feedlot practices. The animals were housed in small pens holding ten animals each, with each animal having 10 cm^2 and 50 cm of feed-bunk space. Clean, fresh water was available at all times. A standard type of high concentrate diet was supplied (12 MJ/kg DM, 13,5% protein) to which they were allowed to adapt during a three-week period, from high levels of hay (15%) to low levels of hay (6%). Animals within each breed were blocked for weight and allocated to their respective pens. Within each breed group, animals were weighed at two-weekly intervals and daily health observations on morbidity, consistent breathing and manure consistency were made. All these actions were necessary to comply with rules of the Ethics Committee of the ARC (APIEC 11/025). Animals were slaughtered over a period of 10 weeks, 18 per week when at an A age (10-12 months; 0 incisors) with a fatness class of 2 or 3 (lean-medium fatness) according to current South African Beef Classification System (Government Gazette No. 5092, 1993).

Each breed was subjected to either 24 hours feed withdrawal (Br_{24}, n = 30; Ng_{24}, n = 30 and Sm_{24}, n = 30) or 3 hours feed withdrawal (Br_3, n = 30; Ng_3, n = 30 and Sm_3, n = 30). Animals from the 24 hours feed withdrawal period (FW_{24}) were weighed (9 per slaughter day or 3 from each breed)

and loaded onto a truck. They were transported over 50 km (about 1 h) and offloaded at the abattoir, where they were left to stand overnight until they were slaughtered the next day (start 8:30). Only ten carcasses from each treatment group were used in this study (Br_3, n = 10; Ng_3, n = 10 and Sm_3, n = 10) as the other 20 carcasses were electrically stimulated.

Animals from the 3 hours feed withdrawal period (FW_3) were weighed the day before slaughter (9 per slaughter day or 3 from each breed). Feed withdrawal commenced before transport (6:00 in the morning). Animals were loaded onto a truck and transported over 50 km (about 1 hour). At the abattoir they were offloaded, rested for 2 hours and slaughtered (start 11:00). Only ten carcasses from each treatment group were used in this study (Br_{24}, n = 10; Ng_{24}, n = 10 and Sm_{24}, n = 10) as the other 20 carcasses were electrically stimulated.

Data from electrically stimulated carcasses were not used in this study as it interfered with the catecholamine profile of each animal.

3.2 Slaughter process and physical carcass and meat properties

Animals were slaughtered (heavier animals first) after stunning with a stun gun and by severing the carotid artery. After exsanguination, carcasses were dressed, halved and chilled at 4 °C within 2 hours of slaughter. Warm carcass weight, cold carcass weight and dressing percentage were calculated. *M. longissimus* on the left and right side of each carcass from the first to the last lumbar vertebra (L1 – L6) were used for sampling. Carcasses in the present study were not electrically stimulated as we were interested in inherent energy metabolism of meat and tenderness characteristics without external *post mortem* influences. Position of sampling for each test remained consistent. Samples were either frozen immediately at -20 ˚C or -80 ˚C or vacuum packed using poly-ethylene bags and aged at 2 ± 2 ˚C for 7 or 14 days *post mortem*.

Cranial

	Left	Right
L1 L2 L3	WBSF and colour 1 day post mortem	WBSF 7 days post mortem
L4	Sarcomere length, WBC, energy metabolites	
L5	Calpain system, MFL, Muscle fiber typing	WBSF 14 days post mortem
L6	pH and temperature	

Caudal

Figure 3.1 Schematic representation of *m. longissimus* at L1-L6 indicating approximate anatomical regions for sampling for various analyses

3.2.1 pH decline, ultimate pH and temperature measurement

As indicated in figure 3.1, pH and temperature decline was measured in *m. longissimus*, at the last lumbar vertebrae on the left side of each carcass at 1, 2, 3, 6, 9 and 24 (pHu) hours after slaughter with a digital handheld meat pH meter (Unitemp) fitted with a polypropylene spear type gel electrode.

3.2.2 Meat colour

Researchers have successfully used CIE colour expressions for detection of fresh meat colour differences. They are lightness (L*), redness (a*) and yellowness (b*) (Hunt *et al*., 1999). These colour expressions can be interpreted on a three dimentional model where a* and b* represent the X and Y axises and L* represents the third dimention (Z) (figure 3.2)

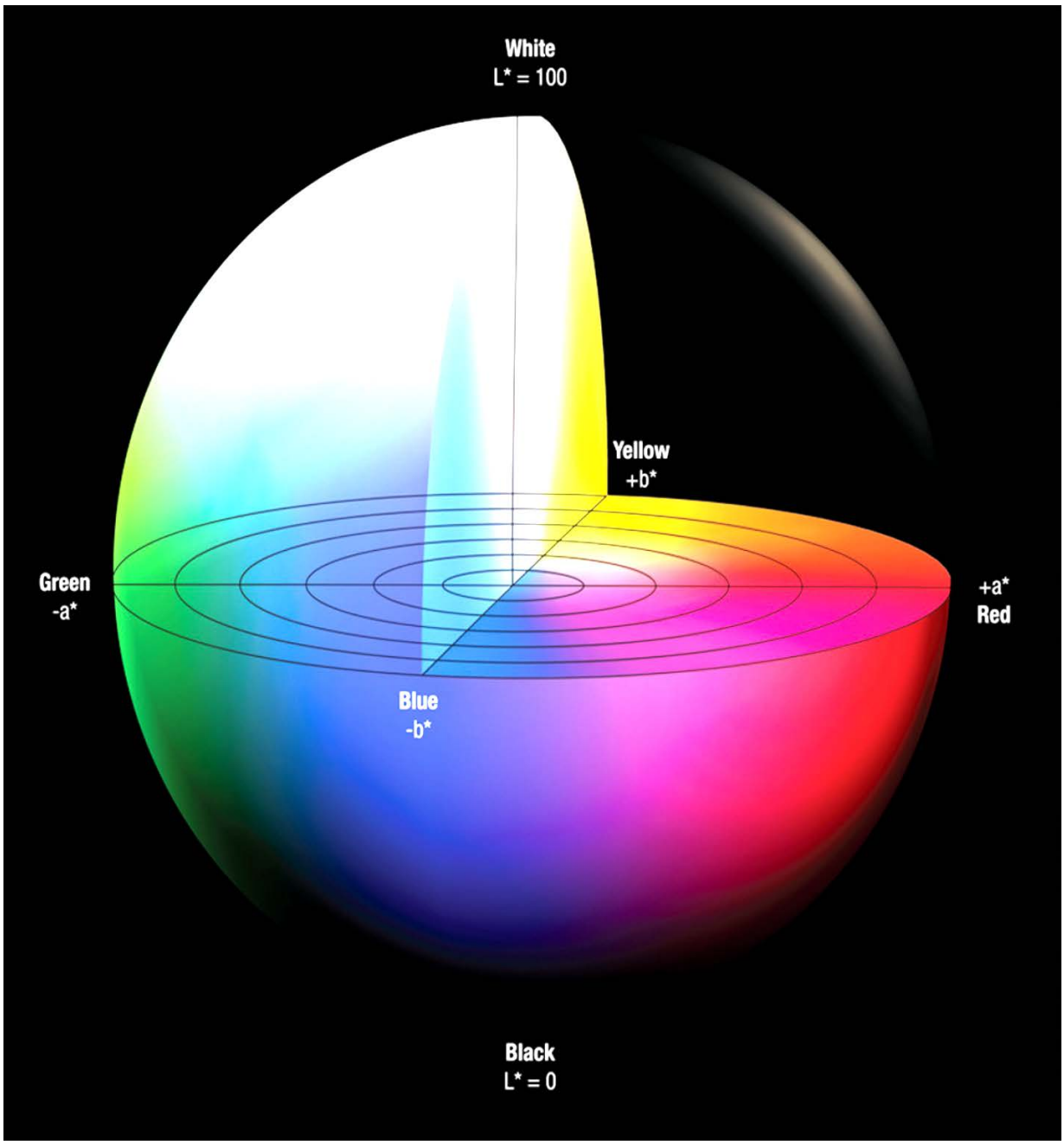

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Figure 3.2 Three dimentional representation of CIE L*a*b* colour expressions. (From Hunt *et al.*, 2012) Image courtesy of Konica Minolta Sensing Americas.

Lightness, L*, indicates black-whiteness of a substance. Its values range from 0 (all light absorbed) to 100 (all light reflected). Redness, a*, ranges from -60 (green) to +60 (red). Yellowness, b*, spans from -60 (blue) to +60 (yellow).

The following calculations were done using the CIE L*a*b* values: Physiological attributes of chroma (intensity of red colour) also known as saturation index [Si =(a*2+b*2)1/2] (MacDougall, 1982); hue angle indicating discolouration [HA = tan−1 (b*/a*)] (Young *et al*., 1999) and redness index (Ri = a*/b*) where larger ratios of a*/b*indicate more redness and less discoloration (Hunt *et al*., 2012). Steaks were removed from *m. longissimus* at the approximate region indicated in figure 3.1, as soon as ultimate pH (pHu) was reached (at approximately 24 hours *post mortem*). Two freshly cut steaks of 15 mm thickness each were allowed to bloom at room temperature for 60 minutes. Thereafter, CIE colour expressions were determined on freshly exposed surfaces with a Minolta chroma meter (Minolta CR200, Minolta, Japan). Some technical specifications of this instrument include: Type - Hand-held dual-function reflected-light colorimeter; Illumination type - diffuse illumination; observer angle - 0° viewing angle; measuring area - 8mm; measuring range - 1.5 to 100% reflectance ratio.

The chroma meter was calibrated against a white calibration tile. Three replicate measurements were done on each steak and special caution were made in order avoid areas with connective tissue and intramuscular fat. Mean values were used for statistical analysis.

3.2.3 Water binding capacity

Determination of water binding capacity was performed on fresh samples at 1 day *post mortem*. 400 to 600 mg meat sample were removed from *m. longissimus* at the approximate region indicated in figure 3.1. Meat area as well as liquid area were obtained after pressing the meat sample on a filter paper (Whatman 4) sandwiched between two perspex plates and pressed at constant pressure for 5 minutes as described by Grau & Hamm (1953). Areas were measured by means of a video image analyser (Soft Imaging System, Olympus, Japan) described by Irie *et al*. (1996). Water binding capacity was expressed as area of meat divided by area of moisture.

3.2.4 Muscle fiber composition

One hundred gram sample was removed from *m. longissimus* (see figure 3.1) directly after exsanguination and frozen in liquid nitrogen. For histochemical demonstration of succinic dehydrogenase situated in mitochondria, the nitro-blue tetrazolium technique of Bourne & Malaty (1953) was used. Fibers were classified under 100 x magnification by means of a video image analyser (Soft Imaging System, Olympus, Japan) into red, intermediate and white fibers according to

the intensity of the staining reaction. Fibre cross-sectional areas were also determined with a video image analyser.

3.2.5 Warner-Bratzler shear force

Three 20-25 cm thick whole-loin cuts were removed from *m. longissimus* on the right and left side (See figure 3.1) for Warner-Bratzler shear force evaluations. Each of the three cuts were vacuum packed, and aged for 1, 7, and 14 days *post mortem* at 4 °C, frozen, and stored (-25 °C) for later determination of Warner-Bratzler shear force evaluation. The vacuum bag thickness used was 70microns of various sizes. The vacuum packer: A Boss twin-chamber vacuum machine; type: Titan-X 630; no. 080842; id no. 063-Z3-0-DN-0-4-0-00 (Meistertech Z3000). Vacuum pump: 100m3/h, Vacuumed at 99% at 1°C. Blocks of approximately 6 x 6 x 2.5 cm were cut from *m. longissimus* at 24 hours, 7 days and 14 days *post mortem*. Frozen samples (-20 °C) were processed into 30 mm steaks by means of a band saw. The frozen steaks were thawed at 4 °C for 24 hours after which they were cooked using an oven-broiling (Mielé, model H217, Mielé & Cie, Gűtersloh, Germany) method with direct radiant heat (American Meat Science Association (AMSA), 1995). The steaks were broiled at 260 °C (pre-set) to 70 °C internal temperature and cooled down to room temperature (±18 °C). Eight round cores (12.7 mm diameter) were removed from the steaks parallel to the long axis of the fibers (AMSA, 1995). Each core was sheared once through the centre, perpendicular to the fiber direction, by a Warner-Bratzler shear device mounted on a Universal Instron apparatus (Model 4301, Instron Ltd, Buckinghamshire, UK; crosshead speed = 200 mm/min). Warner-Bratzler shear force was measured as the peak force (kg/12.5 mm Θ) average for eight cores per sample.

3.3 Urinary catecholamines and energy metabolites

3.3.1 Urinary catecholamines

In order to measure urinary catecholamines (norepinephrine, epinephrine and dopamine), urine was collected from the bladder of each animal with a syringe and needle, immediately after evisceration (approximately 12 minutes *post mortem*). Four mℓ urine was mixed with 50 µℓ concentrated HCℓ to stabilise and preserve catecholamines. After HCℓ was added, all samples were transported on ice to the laboratory and stored at –70 ˚C until analysis. At the time of analysis, urine samples were sent to a local pathology laboratory - Ampath (Ampath facilities are accredited and

ISO certified by the South African National Accreditation Authority). Catecholamines were extracted from urine samples by cation-exchange solid phase extraction and determined by using a high performance liquid chromatography method, as described by Gouarne *et al*. (2004). Catecholamine turnover rate was calculated by taking the ratios of norepinephrine to dopamine (NORDOP) and epinephrine to dopamine (EPIDOP).

3.3.2 Energy metabolites

For determination of energy metabolites, 20 g muscle was removed from *m. longissimus* (see figure 3.1) 1, 3, 6 and 24 hours *post mortem.* Samples were covered with aluminium foil, frozen by means of liquid nitrogen and then ultra-frozen at –70 °C until analyses. Samples from the four specified time intervals were used for biochemical analyses (*post mortem* energy metabolites) using perchloric acid as described by Dalrymple & Hamm (1973). Glycogen concentration was determined as glycosyl units after hydrolysis with α-amyloglucosidase and correction for glucose concentration in the extract to the method of Keppler & Decker (1981). ATP, glucose-6-phosphate and creatine phosphate were determined in perchloric acid extracts according to Lamprecht & Stein (1965) and L-lactate concentration according to Gutmann & Wahlefield (1974). Glycolytic potential of treatment groups was calculated as follows: 2x [(glycogen) + (glucose-6-phosphate)] + (L-lactate) and statistically analysed (Monin & Sellier, 1985).

3.4 Toughening phase

3.4.1 Sarcomere length

Samples for sarcomere length (µm) determination were removed from *m. longissimus* (see figure 3.1) at 1 and 3 days *post mortem* and prepared according to Hegarty & Naudé (1970). Samples were homogenized in approximately 15 mℓ distilled water using an ultra Turrax blender at low speed. A few droplets of homogenate were mounted on a slide, covered with a cover slip and immediately viewed under an Olympus B340 system microscope at 31,000 magnification, equipped with a CC12 video camera (Olympus, Tokyo, Japan) and attached to a video image analyser. Fifty sarcomeres per sample were measured.

3.5 Tenderising phase

3.5.1 Myofibrillar fragmentation length

Myofibrillar fragmentation length (µm) (MFL) was used as an indicator of myofibrillar structure weakening as a result of *post mortem* proteolysis. Extraction procedure was done as described by Culler *et al*. (1978) and adapted by Heinze & Bruggemann (1994). Frozen *m. longissimus* samples from days 1, 7 and 14 were cut with a knife and all visible fat and connective tissue were removed. Samples were then finely minced by means of a scissor. Three grams of minced sample was weighed into a 50 mℓ Böhler glass. 30 mℓ of the MFL extraction buffer (0,02 M potassium phosphate buffer containing: 100 mM KCℓ, 1 mM $MgC\ell_2$, 1 mM EDTA, 1 mM $NaNO_3$, pH 7) (4 °C) was added. The sample was allowed to thaw for 60 seconds and homogenised for exactly 30 seconds in the Böhler HO4 homogeniser, at 20,000 rpm. The sample was chilled with ice water in the homogeniser and the blade was turned around in order to fragment the myofibrils with the blunt side of the blade. After homogenisation, the samples were transferred into centrifuge tubes and centrifuged at 4 °C, 3000 rpm for 15 minutes. The supernatant was discarded and pellet suspended in 30 mℓ MFL extraction buffer. The sample was again centrifuged at 4 °C, 3000 rpm for 15 minutes. The supernatant was discarded and pellet suspended in 10 mℓ MFL extraction buffer. The suspension was then filtered under vacuum through a 1000 µm polyethylene strainer. An additional 5 mℓ MFL extraction buffer was used to facilitate the passage of the myofibrils through the strainer. The filtrate was subsequently filtered under vacuum through a 250 µm polyethylene strainer. The samples were transferred on to a slide and covered with a slip. The excess water is dried and the slide was cleaned. 100 Single myofibril fragments were measured by means of a video image analyser (Kontron, Germany).

3.5.2 Proteolytic enzymes

Samples were removed from *m. longissimus* (see figure 3.1) at 1 and 24 hours *post mortem*, snap frozen in liquid nitrogen and kept at -70 ˚C until analysis. Five grams of the frozen *m. longissimus* sample was used to determine the proteinase activity and the method used was as described by Dransfield (1996). Calpastatin in combination with µ-calpain and m-calpain were extractable from and separated by means of the two-step gradient ion-exchange chromatography-method according to Geesink & Koohmaraie (1999). Eluates containing both calpain and calpastatin

were estimated from calpastatin measurements before and after heating the eluates. Calpain assays were done using azo-casein as substrate according to Dransfield (1996). One unit of calpain activity was defined as an increase in absorbance at 366 nm of 1.0 per hour at 25 °C. One unit of calpastatin activity was defined as the amount that inhibited one unit of m-calpain activity. For the present study data were expressed as units per milligram extracTable protein (specific activity). Protein concentration of *m. longissimus* frozen samples was determined by the biuret method of Cornall *et al*. (1949) as sited by Bailey (1967).

3.6 Statistical analyses

There were three breeds and two feed withdrwal periods and a 3 x 2 experimental design were used for six groups. Repeated measures over time (pH, temperature, MFL, Warner-Bratzler shear force, sarcomere length, glycogen-, glucose-6-phosphate-, glucose-, L-lactate-, creatine phosphate, ATP concentration and glycolytic potential) were analysed by analysis of variance of contrast variables by means of the GLM procedure of SAS (2008) at a significance level of $P<0.05$. Interactions between breed type and feed withdrawal were taken into account. Single observations (carcass weight, L*, a*, b*, HA, Ri, Si, catecholamine concentration and catecholamine conversion rate) were analysed by analysis of least square means by means of the GLM procedure of SAS (2008) at a significance level of $P<0.05$. Interactions between breed type and feed withdrawal were taken into account. Correlations between parameters were done using IBM SPSS Statistics version 21 (2013). Bivariate correlation analysis was performed with a two-tailed test of significance and Pearson correlation coefficients.

3.7 Representation of results

Results will be presented by providing multifactorial analysis of variance to indicate the effects of the main factors, breed type, feed withdrawal period and first order interactions on dependent variables that were investigated in this study. Significant effects of the main factors and first order interactions on dependent variables will be highlighted in MANOVA Tables. Least square means for main factors ($\bar{x}$ ± SEM) will follow MANOVA Tables followed by least square means of interactions between main factors ($\bar{x}$ ± SEM). Significant differences will be indicated with superscripts and explained where necessary. Where applicable, figures will be given of differences in dependent variables over time for interacations between main factors, to indicate tendencies. Main

factors include breed type (Brahman, Nguni and Simmental) and feed withdrawal period (3 or 24 hours feed withdrawal). Interaction between main factors is the interaction between breed type and feed withdrawal period.

CHAPTER 4 RESULTS

4.1 Carcass characteristics

4.1.1 Multifactorial analysis of variance for carcass characteristics

Table 4.1 MANOVA results for the effects of breed type, feed withdrawal period and first order interactions on carcass characteristics (data pooled for breed type and feed withdrawal period; degrees of freedom parenthesized)

	Warm carcass weight		Cold carcass weight		Dressing percentage	
	F ratio	Significance	F ratio	Significance	F ratio	Significance
Main factor						
B	**169.80**	**P<0.01**	**173.56**	**P<0.01**	**4.49**	**P=0.02**
FW	0.69	P=0.41	0.75	P=0.39	0.76	P=0.39
Interaction						
B x FW	**68.21**	**P<0.01**	**69.76**	**P<0.01**	0.07	P=0.07

B = Breed type (Brahman, Nguni and Simmental)
FW = Feed withdrawal period (24 hours and 3 hours feed withdrawal)

4.1.2 Effect of breed type, feed withdrawal period and first order interactions on carcass characteristics

Table 4.2 Least square means for the effect of breed type on warm- and cold carcass weight and dressing percentage ($\bar{x} \pm SEM$)

Carcass characteristics	Breed type			SEM
	Brahman	Nguni	Simmental	
Warm carcass weight	282.35[a]	206.88[b]	275.35[a]	3.2
Cold carcass weight	277.28[a]	202.61[b]	270.01[a]	3.13
Dressing percentage	58.19[a]	56.53[b]	56.93[b]	0.41

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Table 4.3 Least square means for the effect of feed withdrawal period on warm- and cold carcass weight and dressing percentage ($\bar{x} \pm SEM$)

Carcass characteristics	Feed withdrawal period		SEM
	3 hours	24 hours	
Warm carcass weight	253.33	256.39	2.61
Cold carcass weight	248.41	251.53	2.55
Dressing percentage	57.42	57.01	0.33

The South African feedlot industry produces approximately 70 to 80% of beef. Animals often enter the feedlot system weighing between 200 and 220kg. During this time the animal adds approximately 100kg withing 100 days which realises a carcass weighing between 220 to 225 kg on average for all breeds (The value chain for red meat, 2003).

In the present study the highest average carcass weight was obtained for Brahman type cattle (277.28 kg). The Nguni breed type is a small frame indigenous breed. As expected, warm and cold carcass weights of Nguni were lower *(P<0.05)* than Brahman and Simmental type carcasses in this study. Dressing percentage for Brahman type cattle was significantly higher (*P<0.05*) than that of Nguni and Simmental type cattle. In the South African beef industry, the mean dressing percentage for carcasses with a fat score of 2 to 3 varies from 54 to 56% (KwaZulu-Natal Department of Agriculture and Environmental Affairs, 2008).

Feed withdrawal period had no significant effect on warm carcass weight, cold carcass weight or dressing percentage.

Table 4.4 Least square means for the effect of interactions between breed type and feed withdrawal period on warm- and cold carcass weight and dressing percentage ($\bar{x}$ ± SEM)

Carcass characteristics	Feed withdrawal	Breed type			SEM
		Brahman	Nguni	Simmental	
Warm carcass weight	3 hours	278.54[a]	206.26[b]	275.18[a]	4.53
	24 hours	286.16[a]	207.50[b]	275.52[a]	
Cold carcass weight	3 hours	273.26[a]	201.94[b]	270.02[a]	4.42
	24 hours	281.30[a]	203.28[b]	270.00[a]	
Dressing percentage	3 hours	58.02[*]	56.95	57.30	0.58
	24 hours	58.36[a**]	56.12[b]	56.56[b]	

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*
[*, **]Different superscripts within the same column differ significantly *(P<0.05)*

As expected, warm and cold carcass weights for Ng_{24} were lower *(P<0.05)* compared to Br_{24} and Sm_{24}. Similarly, warm and cold carcass weights of Ng_3 were lower *(P<0.05)* compared to Br_3 and Sm_3. Dressing percentage for Br_{24} was higher *(P<0.05)* compared to dressing percentage for Ng_{24} and Sm_{24}. Dressing percentage, calculated as (Carcass Weight / Live Weight) x 100 varies between an average of 62% and 56%. Brahman type cattle had the most favourable dressing percentage in the current study.

4.2 pH and temperature (ºC) in *m. longissimus* from 1 to 24 hours *post mortem*

4.2.1 Multifactorial analysis of variance for pH and temperature (ºC) in *m. longissimus* from 1 to 24 hours *post mortem*

Table 4.5 MANOVA results for the effects of breed type, feed withdrawal period and first order interactions on pH and temperature (ºC) in *m. longissimus* from 1 to 24 hours *post mortem* (data pooled for breed type and feed withdrawal period; degrees of freedom parenthesized)

Time		pH		Temperature	
		F ratio	Significance	F ratio	Significance
	Main factor				
	B	1.26	P=0.29	0.29	P=0.40
1 hour	FW	0.26	P=0.61	**4.10**	**P=0.04**
	Interaction				
	B x FW	1.03	P=0.36	0.89	P=0.41
	Main factor				
	B	1.72	P=0.18	-	-
2 hours	FW	**4.03**	**P=0.05**	-	-
	Interaction				
	B x FW	0.99	P=0.37	-	-
	Main factor				
	B	**3.90**	**P=0.02**	**3.51**	**P=0.03**
3 hours	FW	**5.13**	**P=0.02**	**7.55**	**P=0.01**
	Interaction				
	B x FW	0.20	P=0.82	0.22	P=0.81
	Main factor				
	B	**3.13**	**P=0.05**	**4.18**	**P=0.02**
6 hours	FW	**7.16**	**P=0.01**	0.16	P=0.69
	Interaction				
	B x FW	0.78	P=0.46	0.25	P=0.78
	Main factor				
	B	**4.04**	**P=0.02**	**13.88**	**P<0.01**
9 hours	FW	**6.39**	**P=0.01**	**58.69**	**P<0.01**
	Interaction				
	B x FW	0.26	P=0.77	0.96	P=0.39
	Main factor				
	B	**5.21**	**P=0.01**	**18.83**	**P<0.01**
24 hours	FW	**7.06**	**P=0.01**	**9.39**	**P=0.01**
	Interaction				
	B x FW	**4.42**	**P=0.01**	0.79	P=0.46

B = Breed type (Brahman, Nguni and Simmental)
FW = Feed withdrawal period (24 hours and 3 hours feed withdrawal)

4.2.2 Effect of breed type, feed withdrawal period and first order interactions on *post mortem* pH and temperature (ºC)

Table 4.6 Least square means for the effect of breed type on pH and temperature (ºC) in *m. longissimus* from 1 to 24 hours ($\bar{x}$ ± SEM)

pH	Breed type			SEM
	Brahman	Nguni	Simmental	
At 1 hour	6.57	6.61	6.64	0.044
At 2 hours	6.35	6.40	6.45	0.042
At 3 hours	6.21	6.29	6.32	0.049
At 6 hours	5.96	6.04	6.04	0.039
At 9 hours	5.78[a]	5.90[b]	5.88[ab]	0.041
At 24 hours	5.70[a]	5.81[b]	5.72[ab]	0.035
Temperature				
At 1 hour	38.31	36.90	38.71	1.026
At 3 hours	28.32[a]	25.88[b]	28.84[a]	0.501
At 6 hours	18.34[a]	15.93[b]	18.31[a]	0.493
At 9 hours	15.27[a]	12.92[b]	14.84[a]	0.531
At 24 hours	4.04	3.73	3.90	0.397

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Table 4.7 Least square means for the effect of feed withdrawal period on pH and temperature (ºC) in *m. longissimus* from 1 to 24 hours *post mortem* ($\bar{x}$ ± SEM)

pH	Feed withdrawal		SEM
	3 hours	24 hours	
At 1 hour	6.57	6.63	0.036
At 2 hours	6.34[a]	6.45[b]	0.034
At 3 hours	6.20[a]	6.34[b]	0.040
At 6 hours	5.94[a]	6.08[b]	0.032
At 9 hours	5.79[a]	5.91[b]	0.033
At 24 hours	5.70	5.78	0.028
Temperature			
At 1 hour	37.48	38.45	0.838
At 3 hours	27.69	27.67	0.409
At 6 hours	16.64[a]	18.40[b]	0.402
At 9 hours	13.45[a]	15.23[b]	0.433
At 24 hours	4.04	3.73	0.324

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Temperature and were measured from *m. longissimus*. There were no significant differences in pH between breeds from 1 to 6 hours *post mortem*, but at 9 and 24 hours *post mortem*, pH for Nguni was higher *(P<0.05)* compared to Brahman type cattle. Although pH for Nguni was higher (*P<0.05*) at 9 and 24 hours compared to Brahman, ultimate pH was below 6 for Nguni. As a consequence of smaller carcasses, muscle temperature of Nguni was lower *(P<0.05)* compared to Brahman and Simmental type cattle at 3, 6 and 9 hours *post mortem.*

As glycogen stores were depleted, pH values were higher (*P<0.05*) at 2, 3, 6 and 9 hours *post mortem* for the groups that were submitted to 24 hours feed withdrawal in comparison to the groups that were submitted to 3 hours feed withdrawal. FW_{24} showed higher *(P<0.05)* temperature than FW_3 at 3 and 9 hours *post mortem*.

Table 4.8 Least square means for the effect of the interactions between breed type and feed withdrawal period on pH and temperature (ºC) in *m. longissimus* from 1 to 24 hours *post mortem* ($\bar{x}$ ± SEM)

pH	Feed withdrawal	Breed type			SEM
		Brahman	Nguni	Simmental	
At 1 hour	3 hours	6.5	6.62	6.60	0.06
	24 hours	6.64	6.55	6.68	0.06
At 2 hours	3 hours	6.24^{a*}	6.40^{ab}	6.40^{b}	0.07
	24 hours	6.45^{**}	5.42	6.48	0.06
At 3 hours	3 hours	6.11^{*}	6.23	6.26	0.07
	24 hours	6.32^{**}	6.31	6.39	0.07
At 6 hours	3 hours	5.87^{*}	5.94^{*}	6.00	0.05
	24 hours	6.03^{**}	6.16^{**}	6.06	0.05
At 9 hours	3 hours	5.73^{*}	5.78^{*}	5.86	0.06
	24 hours	5.89^{**}	5.92^{**}	5.94	0.05
At 24 hours	3 hours	5.77	5.60^{*}	5.75	0.06
	24 hours	5.79	5.77^{**}	5.80	0.06
Temperature					
At 1 hour	3 hours	38.74	34.89^{*}	38.84	0.42
	24 hours	37.87	38.91^{**}	38.57	0.36
At 3 hours	3 hours	27.79^{ab}	26.60^{a}	28.69^{b}	1.04
	24 hours	28.85^{a}	25.16^{b}	28.99^{a}	0.86
At 6 hours	3 hours	17.28^{*}	15.57	17.08^{*}	1.29
	24 hours	19.41^{a**}	16.28^{b}	19.53^{a**}	1.33
At 9 hours	3 hours	13.76^{*}	12.45	14.15	0.76
	24 hours	16.77^{a**}	13.39^{b}	15.53^{a}	0.66
At 24 hours	3 hours	4.23	3.84	4.05	1.05
	24 hours	3.84	3.61	3.74	0.85

$^{a, b}$Different superscripts within the same row differ significantly *(P<0.05)*
$^{*, **}$Different superscripts within the same column differ significantly *(P<0.05)*

pH did not differ between treatment groups at any of the *post mortem* time intervals when feed was withdrawn for 24 hours. When feed was withdrawn for only 3 hours, pH for Br_3 was lower *(P<0.05)* compared to Sm_3 at 2 hours *post mortem*. There were no other differences between breeds when feed was withdrawn for 3 hours.

Differences within breeds were observed between feed withdrawal treatments. Br_{24} showed higher *(P<0.05)* pH at 2, 3 and 9 hours *post mortem* compared to Br_3. pH at 24 hours, did not differ between Br_{24} and Br_3. pH at 6 and 24 hours *post mortem* were higher *(P<0.05)* for Ng_{24} than for Ng_3. There were no differences in pH between Sm_{24} and Sm_3 at any of the *post mortem* time intervals. Table 4.8 shows a continuing pH decline for all breeds with 24 hours feed withdrawal from 6 to 24 hours *post mortem*.

Carcass temperature between breeds and within feed withdrawal periods differed significantly as a result of different carcass weights between breeds. Carcass temperature was lower *(P<0.05)* for Ng_{24} than for Br_{24} and Sm_{24} at 3, 6 and 9 hours *post mortem*. Carcass temperature was lower *(P<0.05)* for Ng_3 than for Sm_3 at 3 hours *post mortem*.

Carcass temperature differed significantly in some instances between feed withdrawal treatments and within breed types. Carcass temperature was higher *(P<0.05)* for Br_{24} compared to Br_3 at 6 and 9 hours *post mortem*. Carcass temperature was higher *(P<0.05)* for Ng_{24} than for Ng_3 at 1 hour *post mortem*. Carcass temperature was higher *(P<0.05)* for Sm_{24} than for Sm_3 at 6 hours *post mortem*.

4.2.3 pH measured from 1 to 24 hours *post mortem* in *m. longissimus*

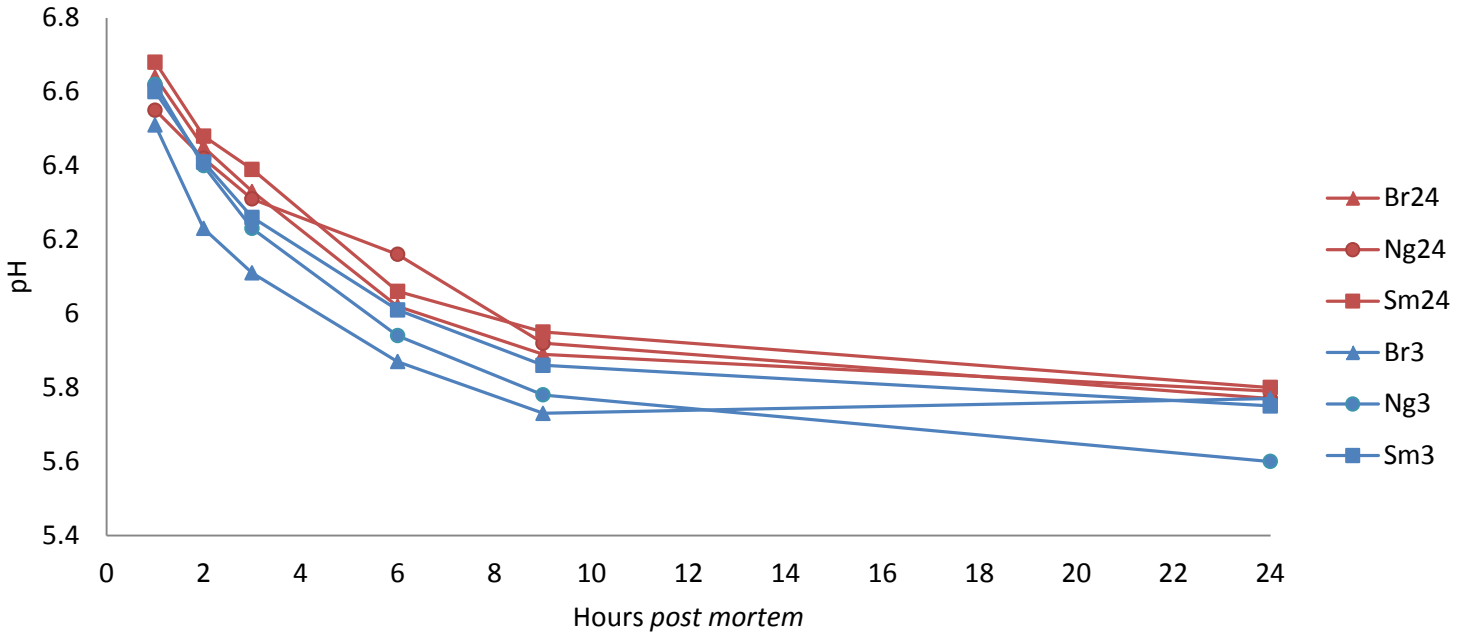

Figure 4.1 pH measured from 1 to 24 hours *post mortem* in *m. longissimus* of Brahman, Nguni and Simmental type cattle subjected to 3 or 24 hours feed withdrawal

As observed from the graph it seems as if pH decline was faster for Ng_3 between 6 and 24 hours compared to Br_3 and Sm_3 and pH decline for Br_{24} was faster between 6 and 24 compared to for Br_3.

4.2.4 Temperature and pH relationship between breed type and feed withdrawal period

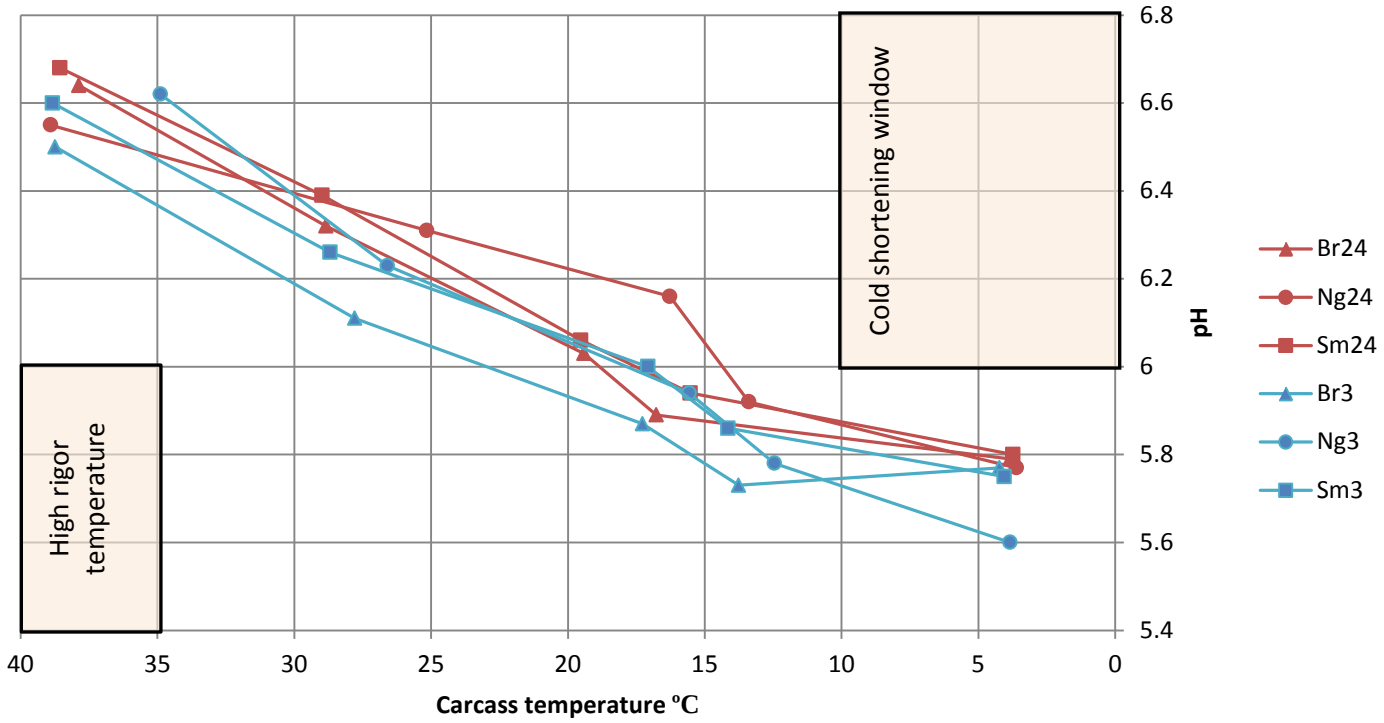

Figure 4.2 Temperature and pH relationship in *m. longissimus* of Brahman, Nguni and Simmental type cattle subjected to 24 or 3 hours feed withdrawal [cold shortening and high *rigor* temperature windows according to Pearson and Young (1989)]

No treatment group fell under the high *rigor* temperature or cold shortening windows

4.3 Biochemical observations: urinary catecholamines, catecholamines turnover rates and muscle energy metabolites

Urinary catecholamine concentration did not correlate with Warner-Bratzler shear force in the present study.

4.3.1 Multifactorial analysis of variance for urinary catecholamine concentrations and catecholamine turnover

Table 4.9 MANOVA results for the effects of breed type, feed withdrawal period and first order interactions on urinary catecholamine concentration and catecholamine turnover (data pooled for breed type and feed withdrawal period; degrees of freedom parenthesized)

	Norepinephrine		Epinephrine		Dopamine		NORDOP		EPIDOP	
	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance
Main factor										
B	**4.52**	**P<0.01**	**5.12**	**P=0.01**	**6.18**	**P=0.00**	**2.76**	**P=0.05**	**2.64**	**P=0.05**
FW	0.56	P=0.45	0.00	P=0.96	**13.13**	**P<0.01**	**6.98**	**P=0.01**	**4.47**	**P=0.04**
Interaction										
B x FW	0.66	P=0.52	1.12	P=0.33	2.55	P=0.08	1.54	P=0.22	1.82	P=0.17

B = Breed type (Brahman, Nguni and Simmental)
FW = Feed withdrawal period (24 hours and 3 hours feed withdrawal)
NORDOP = Norepinephrine: Dopamine
EPIDOP = Epinephrine: Dopamine

4.3.2 Effect of breed type, feed withdrawal period and first order interactions on catecholamine concentration and catecholamine turnover

Table 4.10 Least square means for the effect of breed type on urinary catecholamine concentration (ng / µmol creatinine) and catecholamine turnover ($\bar{x}$ ± SEM)

Catecholamine	Breed type			SEM
	Brahman	Nguni	Simmental	
Norepinephrine	2.56[a]	4.67[b]	2.48[a]	0.48
Epinephrine	1.16[a]	3.24[b]	1.40[a]	0.44
Dopamine	4.06[a]	4.18[a]	6.14[b]	1.14
Norepinephrine: Dopamine	3.12[a]	1.60[ab]	0.61[b]	0.71
Epinephrine: Dopamine	1.52[a]	1.14[ab]	0.37[b]	0.55

[a, b]Different superscripts within the same row differ significantly (*P<0.05*)

Table 4.11 Least square means for the effect of feed withdrawal period on urinary catecholamine concentration (ng / µmol creatinine) and catecholamine turnover ($\bar{x}$ ± SEM)

Catecholamine	Feed withdrawal		SEM
	3 hours	24 hours	
Norepinephrine	3.28	3.19	0.39
Epinephrine	1.92	1.95	0.36
Dopamine	6.40[a]	3.19[b]	0.93
Norepinephrine: Dopamine	0.76[a]	2.79[b]	0.58
Epinephrine: Dopamine	0.49[a]	1.53[b]	0.45

[a, b]Different superscripts within the same row differ significantly (*P<0.05*)

Results from this study indicated that urinary norepinephrine and epinephrine concentrations for Nguni were higher *(P<0.05)* compared to values observed for Brahman and Simmental. Simmental had higher *(P<0.05)* dopamine concentrations than Brahman and Nguni type cattle.

Significant differences were found between breed types for the conversion rate of dopamine to norepinephrine and dopamine to epinephrine. For Brahman type cattle, NORDOP and EPIDOP were higher *(P<0.05)* compared to Simmental type cattle. Therefore, dopamine was converted to norepinephrine and epinephrine at a faster rate for Brahman compared to Simmental cattle. The conversion rate of dopamine to norepinephrine and epinephrine for Nguni was intermediate between Brahman and Simmental.

Feed withdrawal period had no effect on urinary norepinephrine and epinephrine concentrations. However, dopamine concentration was lower (*P<0.05*) when feed was withdrawn for 24 hours compared to 3 hours.

The ratios of norepinephrine to dopamine and epinephrine and dopamine differed significantly between feed withdrawal treatment groups. When feed was withdrawn for 24 hours, the ratio of NORDOP as well as EPIDOP was higher (*P<0.05*) compared to when feed was withdrawn for 3 hours.

Table 4.12 Least square means for the effect of the interactions between breed type and feed withdrawal period on urinary catecholamine concentration (ng / µmol creatinine) and catecholamine turnover (x ± SEM)

Catecholamine	Feed withdrawal	Breed			SEM
		Brahman	Nguni	Simmental	
Norepinephrine	3 hours	2.47^{a}	4.72^{b}	2.65^{a}	0.68
	24 hours	2.66^{a}	4.62^{b}	2.30^{a}	
Epinephrine	3 hours	1.36^{a}	2.89^{b}	1.50^{a}	0.62
	24 hours	0.97^{a}	3.58^{b}	1.30^{a}	
Dopamine	3 hours	5.71^{a*}	5.61^{a}	7.89^{b*}	1.61
	24 hours	2.40^{a**}	2.75^{a}	4.40^{b**}	
Norepinephrine: Dopamine	3 hours	0.53^{*}	1.23	0.52	1.00
	24 hours	5.70^{a**}	1.97^{b}	0.68^{b}	
Epinephrine: Dopamine	3 hours	0.29^{*}	0.85	0.33	0.78
	24 hours	2.75^{a**}	1.43^{b}	0.40^{b}	

$^{a, b}$Different superscripts within the same row differ significantly *(P<0.05)*
$^{*, **}$Different superscripts within the same column differ significantly *(P<0.05)*

Urinary norepinephrine and epinephrine concentrations for Nguni type cattle were higher (*P<0.05*) compared to that of Brahman and Simmental type cattle, irrespective of feed withdrawal period. In other words, Ng_3 as well as Ng_{24} had higher *(P<0.05)* norepinephrine and epinephrine concentrations than Br_3, Sm_3; and Br_{24}, Sm_{24}. Within each cattle breed type, there were no significant differences for norepinephrine and epinephrine concentrations between 3 and 24 hours feed withdrawal groups. Dopamine concentration for Simmental type cattle was higher (*P<0.05*) compared to Brahman and Nguni cattle, irrespective of feed withdrawal period. This means that Sm_3 and Sm_{24} had higher *(P<0.05)* urinary dopamine concentration than Br_3, Ng_3; and Br_{24}, $Ng_{24;}$ respectively. Dopamine concentration for Br_3 and Sm_3 was higher *(P<0.05)* compared to Br_{24} and Sm_{24}.

Significant differences were found between breed types and feed withdrawal groups for NORDOP and EPIDOP. In the case where feed was withdrawn for 24 hours before slaughter, NORDOP for Sm_{24} was lower *(P<0.05)* compared to Br_{24}, but only marginally lower *(P>0.05)* compared to Ng_{24}. Ng_{24} had intermediate NORDOP compared to Br_{24} and Sm_{24}. When feed was withdrawn for only 3 hours, there were no significant differences between breeds. For Brahman type cattle, NORDOP was higher *(P<0.05)* for Br_{24} compared to Br_3. NORDOP for Ng_{24} was marginally higher *(P>0.05)* compared to Ng_3. For Simmental type cattle NORDOP remained unchanged between different feed withdrawal groups.

4.3.3 Multifactorial analysis of variance for *post mortem* glycogen, glucose-6-phosphate, glucose. L-lactate, creatine phosphate, ATP (µmol glycosyl units / g muscle) and glycolytic potential

Table 4.13 MANOVA results for the effects of breed type, feed withdrawal period and first order interactions on glycogen, glucose-6-phosphate, glucose, L-lactate, creatine phosphate, ATP (µmol glycosyl units / g muscle) and glycolytic potential in *m. longissimus* (data pooled for breed type and feed withdrawal period; degrees of freedom parenthesized)

Time		Glycogen		glucose-6-phosphate		Glucose		L-lactate		Creatine phosphate		ATP		Glycolytic potential	
		F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance
	Main factor														
	B	**5.95**	**P=0.01**	0.04	P=0.96	1.05	P=0.35	0.06	P=0.95	2.52	P=0.09	0.81	P=0.17	2.12	P=0.13
1 hour	FW	**22.19**	**P<0.01**	1.19	P=0.28	3.05	P=0.08	0.16	P=0.69	0.06	P=0.80	0.26	P=0.61	**10.55**	**P=0.002**
	Interaction														
	B x FW	1.19	P=0.31	**3.19**	**P=0.04**	1.04	P=0.36	1.72	P=0.18	1.13	P=0.36	1.01	P=0.37	**5.08**	**P=0.001**
	Main factor														
	B	**3.40**	**P=0.04**	0.26	P=0.77	1.55	P=0.22	0.23	P=0.80	2.35	P=011	**4.64**	**P=0.01**	1.12	P=0.33
3 hours	FW	**21.37**	**P<0.01**	**5.83**	**P=0.02**	**5.51**	**P=0.02**	1.67	P=0.20	0.00	P=0.99	0.43	P=0.51	**10.77**	**P=0.002**
	Interaction														
	B x FW	0.60	P=0.66	1.84	P=0.16	1.34	P=0.26	2.07	P=0.09	0.94	P=0.46	0.84	P=0.43	**3.03**	**P=0.018**
	Main factor														
	B	**3.95**	**P=0.02**	1.31	P=0.27	1.81	P=0.17	1.39	P=0.25	**2.96**	**P=0.05**	0.39	P=0.68	1.65	P=0.20
6 hours	FW	**11.14**	**P=0.01**	**5.27**	**P=0.02**	**6.93**	**P=0.01**	**6.05**	**P=0.02**	2.09	P=0.15	1.04	P=0.31	**12.55**	**P=0.001**
	Interaction														
	B x FW	0.05	P=0.95	**3.24**	**P=0.04**	1.27	P=0.28	0.27	P=0.76	1.65	P=0.16	0.10	P=0.90	**3.41**	**P=0.01**
	Main factor														
	B	**3.94**	**P=0.02**	**4.46**	**P=0.01**	2.79	P=0.06	0.37	P=0.70	**6.73**	**P=0.01**	**7.16**	**P=0.01**	0.99	P=0.38
24 hours	FW	**4.91**	**P=0.03**	**10.06**	**P=0.01**	**5.18**	**P=0.02**	2.92	P=0.09	0.89	P=0.35	0.00	P=0.95	2.20	P=0.14
	Interaction														
	B x FW	0.19	P=0.82	0.65	P=0.52	1.75	P=0.18	0.86	P=0.43	**3.14**	**P=0.01**	0.60	P=0.55	1.02	P=0.42

B = Breed type (Brahman, Nguni and Simmental)
FW = Feed withdrawal period (24 hours and 3 hours feed withdrawal)

4.3.4 Effect of breed type, feed withdrawal period and first order interactions on *post mortem* glycogen concentration (µmol glycosyl units / g muscle)

Table 4.14 Least square means for the effect of breed type on glycogen concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glycogen	Breed type		
	Brahman	Nguni	Simmental
At 1 hour	54.00±3.78[a]	26.87±4.65[b]	49.93±3.51[a]
At 3 hours	44.31±3.70[a]	24.01±4.55[b]	40.11±3.43[a]
At 6 hours	40.91±3.71[a]	21.59±4.57[b]	33.55±3.44[ab]
At 24 hours	21.44±3.64[a]	2.05±4.48[b]	15.89±3.38[a]

[a, b]Different superscripts within the same row differ significantly (*P<0.05*)

Table 4.15 Least square means for the effect of feed withdrawal period on glycogen concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ±SEM)

Glycogen	Feed withdrawal	
	3 hours	24 hours
At 1 hour	46.81±2.54[a]	40.39±2.55[b]
At 3 hours	38.38±2.48	33.94±2.49
At 6 hours	34.44±2.50	29.60±2.50
At 24 hours	13.22±2.44	13.04±2.45

[a, b]Different superscripts within the same row differ significantly (*P<0.05*)

Glycogen concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. In the present study, glycogen concentration for Nguni was lower *(P<0.05)* at 1, 3 and 24 hours *post mortem* compared to both Brahman and Simmental type cattle. Glycogen concentration was also lower (*P<0.05*) for Nguni cattle at 6 hours *post mortem* compared to Brahman type cattle. From here on one can predict a difference in glycolytic potential for Nguni type cattle.

As expected muscle glycogen was lower *(P<0.05)* at 1 hour *post mortem* when feed was withdrawn for 24 hours compared to when feed was withdrawn for only 3 hours before slaughter. Over time there was no difference in glycogen concentration for cattle between FW_{24} and FW_3.

Table 4.16 Least square means for the effect of the interactions between breed type and feed withdrawal period on glycogen concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glycogen	Feed withdrawal	Breed type		
		Brahman	Nguni	Simmental
at 1 hour	3 hours	59.31±4.77^{a*}	34.67±5.62^{b*}	46.45±4.68^{b}
	24 hours	48.68±5.02^{a**}	19.08±5.57^{b**}	53.41±4.69^{a}
at 3 hours	3 hours	45.44±4.66^{a}	28.79±5.49^{b}	40.81±4.57^{ab}
	24 hours	43.17±4.91^{a}	19.23±5.44^{b}	39.40±4.58^{a}
at 6 hours	3 hours	42.81±4.69^{a}	25.55±5.52^{b}	34.95±4.6^{ab}
	24 hours	39.00±4.93^{a}	17.63±5.46^{b}	32.16±4.6^{a}
at 24 hours	3 hours	24.05±4.61^{a}	1.73±5.42^{b}	13.88±4.51^{a}
	24 hours	18.83±4.84^{a}	2.38±5.36^{b}	17.91±4.52^{a}

$^{a, b}$Different superscripts within the same row differ significantly *(P<0.05)*
$^{*, **}$Different superscripts within the same column differ significantly *(P<0.05)*

Glycogen concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. Evidently, glycogen concentration was depleted in Nguni type cattle that were submitted to 24 hours feed withdrawal. Ng_{24} had lower *(P<0.05)* glycogen concentration at 1, 3, 6 and 24 hours than Br_{24} and Sm_{24}. Glycogen concentration at 1 hour *post mortem* in Br_3 was higher *(P<0.05)* compared to Ng_3 and Sm_3. Br_3 had higher *(P<0.05)* glycogen concentration at 3 and 6 hours *post mortem* compared to Ng_3. Ng_3 had lower *(P<0.05)* glycogen concentration at 24 hours *post mortem* than Br_3 and Sm_3.

Glycogen concentration at 1 hour *post mortem* was higher *(P>0.05)* for Br_3 than Br_{24}. On average Br_3 had higher *(P>0.05)* glycogen concentrations at 3, 6 and 24 hours *post mortem* than Br_{24}. Glycogen concentration was higher *(P<0.05)* in Ng_3 muscle at 1 hour *post mortem* compared to Ng_{24}. Sm_{24} had higher *(P>0.05)* glycogen concentration at 1, 6 and 24 hours *post mortem* than Sm_3. Glycogen concentration at 3 hours *post mortem* did not differ between Sm_{24} and Sm_3. Non-significant values for glycogen concentration in Simmental are reported as these results were not as expected and may be referred to “out of trend” observations.

4.3.5 Glycogen concentrations (µmol glycosyl units / g muscle) as measured in *m. longissimus* from 1 to 24 hours *post mortem*

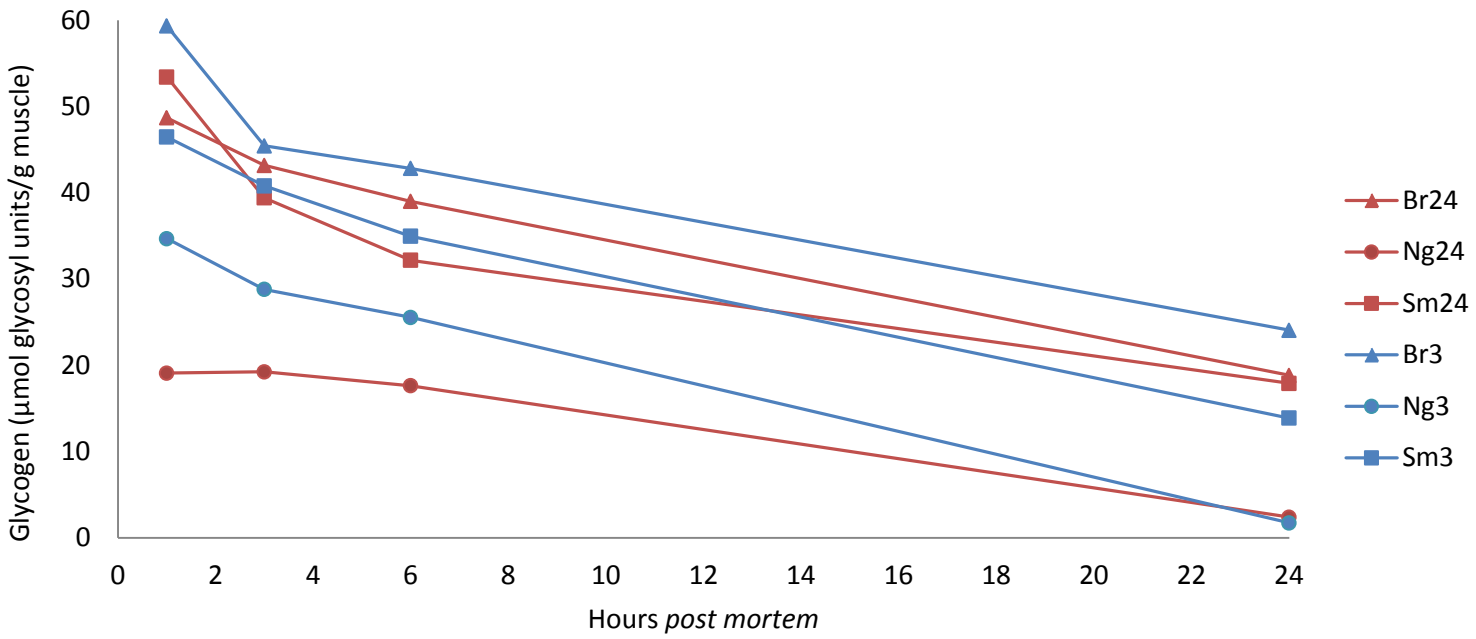

Figure 4.3 Glycogen concentrations (µmol glycosyl units / g muscle) measured from 1 to 24 hours *post mortem* in *m. longissimus* of Brahman, Nguni and Simmental type cattle subjected to 3 or 24 hours feed withdrawal

Between 1 and 6 hours *post mortem*: The rate of glycogen decline was higher *(P<0.05)* for Sm_{24} compared to Sm_3. The rate of glycogen decline was lower *(P<0.05)* for Ng_{24} compared to Ng_3. From Figure 4.3 it seems as if the rate of glycogen decline was lower for Ng_{24} compared to all other treatment groups.

Between 6 and 24 hours *post mortem*: There were no differences between the main effects or any of the treatment groups for the rate of glycogen metabolism late *post mortem*.

4.3.6 Effect of breed type, feed withdrawal period and first order interactions on *post mortem* glucose-6-phosphate concentration (µmol glycosyl units / g muscle)

Table 4.17 Least square means for the effect of breed type on glucose-6-phosphate concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glucose-6-phosphate	Breed type			SEM
	Brahman	Nguni	Simmental	
At 1 hour	2.62[a]	2.52[ab]	3.46[b]	0.36
At 3 hours	2.24	2.08	2.69	0.40
At 6 hours	2.01	2.12	2.88	0.51
At 24 hours	9.44[a]	7.04[b]	8.22[ab]	0.67

[a, b]Different superscripts within the same row differ significantly (*P<0.05*)

Table 4.18 Least square means for the effect of feed withdrawal period on glucose-6-phosphate concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glucose-6-phosphate	Feed withdrawal		SEM
	3 hours	24 hours	
At 1 hour	3.06	2.67	0.30
At 3 hours	2.62	2.05	0.32
At 6 hours	2.69	1.98	0.42
At 24 hours	8.50	7.97	0.55

Glucose-6-phosphate concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups Glucose-6-phosphate concentration was higher *(P<0.05)* for Simmental compared to Brahman type cattle at 1 hour *post mortem*. Glucose-6-phosphate concentration was higher *(P<0.05)* for Brahman compared to Nguni type cattle at 24 hours *post mortem*.

There were no differences in glucose-6-phosphate concentrations between 3 and 24 hours feed withdrawal at any of the *post mortem* time intervals.

Table 4.19 Least square means for the effect of the interactions between breed type and feed withdrawal period on glucose-6-phosphate concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glucose-6-phosphate	Feed withdrawal	Breed type			SEM
		Brahman	Nguni	Simmental	
at 1 hour	3 hours	2.18[a]	2.69[a]	4.31[b*]	0.51
	24 hours	3.06	2.35	2.61[**]	
at 3 hours	3 hours	2.21	2.57	3.09	0.56
	24 hours	2.28	1.59	2.28	
at 6 hours	3 hours	2.06	2.64	3.35	0.72
	24 hours	1.97	1.57	2.41	
at 24 hours	3 hours	9.60	7.93	7.98	0.95
	24 hours	9.28[a]	6.17[b]	8.45[b]	

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*
[*, **]Different superscripts within the same column differ significantly *(P<0.05)*

Glucose-6-phosphate concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. Glucose-6-phosphate concentration in muscle is an indication of the amount of glycogenolysis that took place and from the results in Table 4.19, glucose-6-phosphate concentration in at 1 hour *post mortem* was higher *(P<0.05)* for Sm_3 compared to Br_3 and Ng_3. There were no significant differences in glucose-6-phosphate concentration between breeds that were submitted to 3 hours feed withdrawal at any of the other time intervals. For treatments groups submitted to 24 hours feed withdrawal, glucose-6-phosphate concentration at 24 hours *post mortem* was higher *(P<0.05)* for Br_{24} compared to Ng_{24} and Sm_{24}.

Glucose-6-phosphate concentration at 1 hour *post mortem* was lower *(P<0.05)* for Sm_{24} compared to Sm_3.

4.3.7 Glucose-6-phosphate concentrations (µmol glycosyl units / g muscle) as measured in *m. longissimus* from 1 to 24 hours *post mortem*

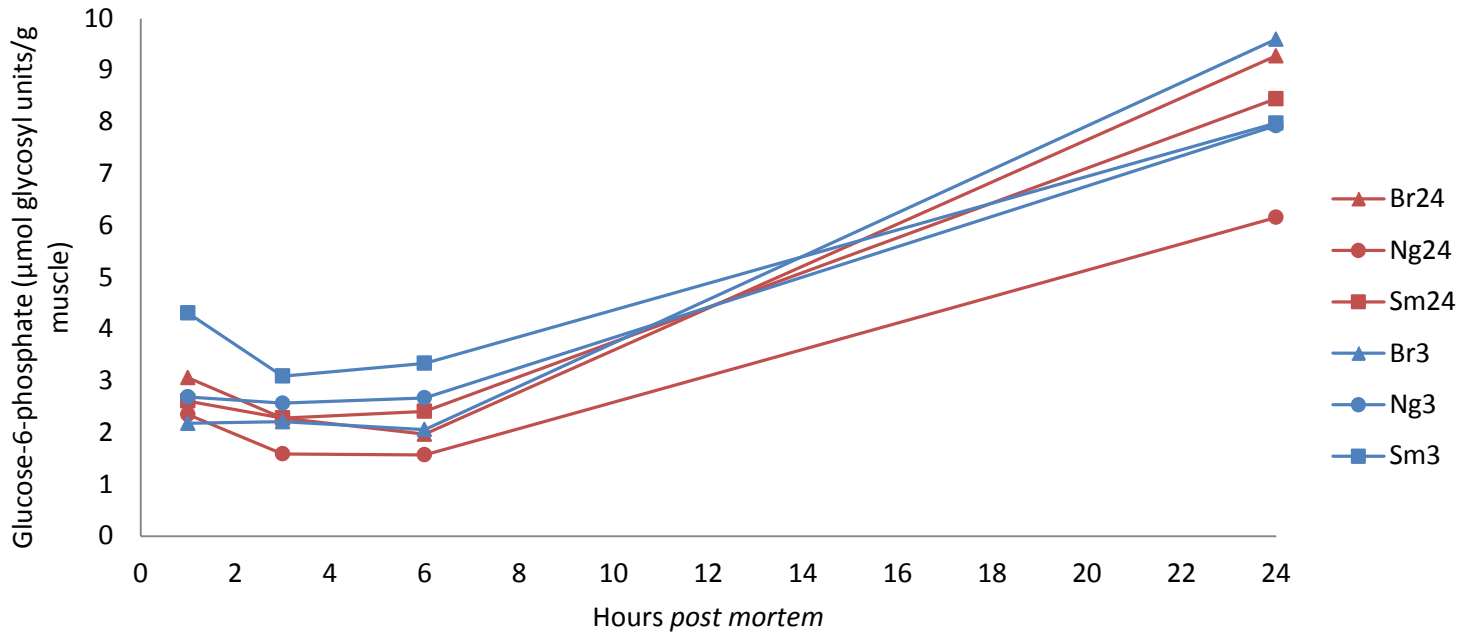

Figure 4.4 Glucose-6-phosphate concentrations (µmol glycosyl units / g muscle) measured from 1 to 24 hours *post mortem* in *m. longissimus* of Brahman, Nguni and Simmental type cattle subjected to 3 or 24 hours feed withdrawal

Between 1 and 6 hours *post mortem*, there was a decline in glucose-6-phosphate concentration in *m. longissimus* for all treatment groups. Between 6 and 24 hours *post mortem*, glucose-6-phosphate concentration inclined for all treatment groups. There were no significant differences between groups.

4.3.8 Effect of breed type, feed withdrawal period and first order interactions on *post mortem* glucose concentration (µmol glycosyl units / g muscle)

Table 4.20 Least square means for the effect of breed type on glucose concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glucose	Breed type			SEM
	Brahman	Nguni	Simmental	
At 1 hour	1.51	1.56	1.58	0.19
At 3 hours	1.86	1.54	1.73	0.23
At 6 hours	2.09	1.73	2.05	0.25
At 24 hours	4.93[a]	4.49[b]	4.59[ab]	0.32

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Table 4.21 Least square means for the effect of feed withdrawal period on glucose concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glucose	Feed withdrawal		SEM
	3 hours	24 hours	
At 1 hour	1.75[a]	1.35[b]	0.15
At 3 hours	1.99[a]	1.43[b]	0.19
At 6 hours	2.41[a]	1.51[b]	0.20
At 24 hours	4.91[a]	3.99[b]	0.26

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Glucose concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. Glucose concentration at 24 hours *post mortem* was higher *(P<0.05)* for Brahman compared to Nguni type cattle.

Similar to muscle glycogen concentration, muscle glucose concentration was lower (*P<0.05*) at 1 hour *post mortem* when feed was withdrawn for 24 hours but remained lower (*P<0.05*) at 3, 6 and 24 hours *post mortem* compared to when feed was withdrawn for only 3 hours *ante mortem*.

Table 4.22 Least square means for the effect of the interactions between breed type and feed withdrawal period on glucose concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glucose	Feed withdrawal	Breed type			SEM
		Brahman	Nguni	Simmental	
at 1 hour	3 hours	1.54	1.93*	1.77	0.27
	24 hours	1.48	1.18**	1.39	
at 3 hours	3 hours	2.13	1.90	1.94	0.32
	24 hours	1.59	1.17	1.52	
at 6 hours	3 hours	2.48	3.29*	2.45	0.35
	24 hours	1.70	1.17**	1.65	
at 24 hours	3 hours	5.50*	4.58*	4.64	0.45
	24 hours	4.36**	3.07**	4.52	

*, **Different superscripts within the same column differ significantly *(P<0.05)*

Glucose concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. There was no difference in glucose concentration in *m. longissimus* between breeds when feed was withdrawn for 3 hours *ante mortem*. Glucose concentration at 24 hours *post mortem* was lower *(P<0.05)* for Ng_{24} compared to Br_{24} and Sm_{24}. Glucose concentration was higher *(P<0.05)* for Ng_3 at 1, 6 and 24 hours *post mortem* compared to Ng_{24} and higher *(P<0.05)* for Br_3 at 24 hours *post mortem* compared to Br_{24}.

4.3.9 Glucose concentrations (µmol glycosyl units / g muscle) as measured in *m. longissimus* from 1 to 24 hours *post mortem*

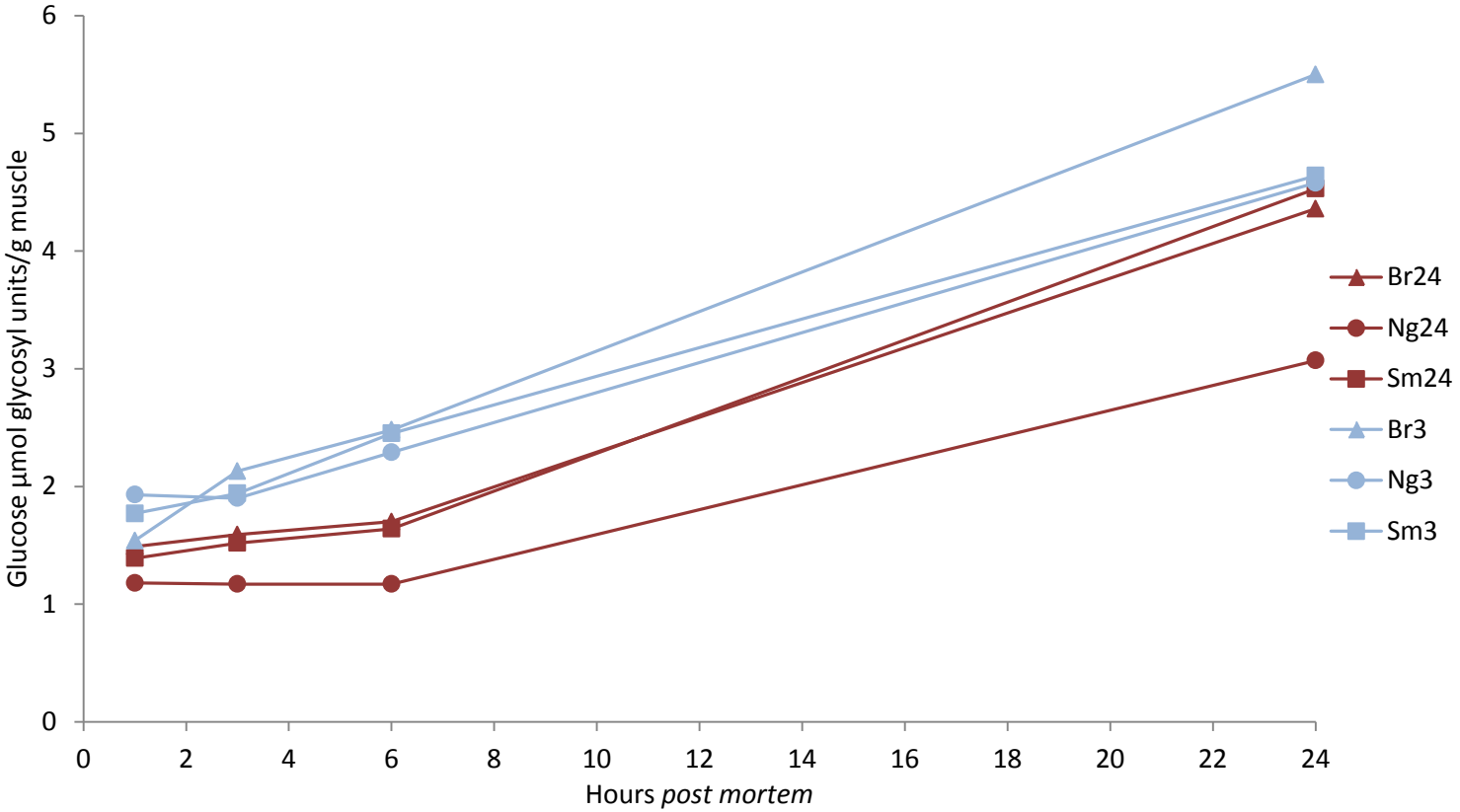

Figure 4.5 Glucose concentrations (µmol glycosyl units / g muscle) measured from 1 to 24 hours *post mortem* in *m. longissimus* of Brahman, Nguni and Simmental type cattle subjected to 3 or 24 hours feed withdrawal

Figure 4.5 shows that glucose concentration in *m. longissimus* increased at a steady rate between 1 and 6 hours for Br_{24} and Sm_{24}; as well as for Br_3 and Sm_3. On the other hand, there was little or no increase in glucose for Ng_{24} and Ng_3 between 1 and 6 hours *post mortem*.

4.3.10 Effect of breed type, feed withdrawal period and first order interactions on *post mortem* L-lactate concentration (µmol glycosyl units / g muscle)

Table 4.23 Least square means for the effect of breed type on L-lactate concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

L-lactate	Breed type			SEM
	Brahman	Nguni	Simmental	
At 1 hour	25.64	29.26	22.65	3.03
At 3 hours	36.53	39.59	31.90	3.65
At 6 hours	44.74	44.84	44.00	3.65
At 24 hours	74.34	73.92	71.58	4.03

Table 4.24 Least square means for the effect of feed withdrawal period on L-lactate concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

L-lactate	Feed withdrawal		SEM
	3 hours	24 hours	
At 1 hour	29.77[a]	21.93[b]	2.48
At 3 hours	41.81[a]	30.20[b]	2.98
At 6 hours	52.95[a]	36.11[b]	2.98
At 24 hours	78.25[a]	68.32[b]	3.29

[a, b] = Different superscripts within the same row differ significantly *(P<0.05)*

L-Lactate concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. There were no differences in L-lactate concentrations between breeds at any of the *post mortem* time intervals.

L-Lactate concentrations were higher *(P<0.05)* for the treatment group that was subjected to 3 hours *ante mortem* feed withdrawal at 1, 3, 6 and 24 hours compared to the treatment group that was subjected to 24 hours feed withdrawal.

Table 4.25 Least square means for the effect of the interactions between breed type and feed withdrawal period on L-lactate concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

L-lactate	Feed withdrawal	Breed type			SEM
		Brahman	Nguni	Simmental	
At 1 hour	3 hours	24.69	35.93*	28.68*	4.29
	24 hours	26.59	22.59**	16.62**	
At 3 hours	3 hours	41.44	46.51*	37.49	5.16
	24 hours	31.61	32.68**	26.31	
At 6 hours	3 hours	51.52*	54.03*	53.29*	5.16
	24 hours	37.96**	35.62**	34.71**	
At 24 hours	3 hours	78.74	78.73	77.26	5.70
	24 hours	69.94	69.11	65.90	

*, **Different superscripts within the same column differ significantly *(P<0.05)*

L-Lactate concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. There were no significant differences in L-lactate concentrations between breed types at 1, 3, 6 and 24 hours *post mortem*. L-Lactate concentration for Br_3 was higher *(P<0.05)* compared to Br_{24} at 6 hours *post mortem*. L-Lactate concentration for Ng_3 was higher *(P<0.05)* compared to Ng_{24} at 1, 3 and 6 hours *post mortem*. L-Lactate concentration in for Sm_3 was higher *(P<0.05)* compared to Sm_{24} at 1 and 6 hours *post mortem*, which is not as expected as glycogen concentration in the same muscle was initially higher *(P<0.05)* for Sm_{24} than Sm_3.

4.3.11 L-Lactate concentrations (µmol glycosyl units / g muscle) as measured in *m. longissimus* from 1 to 24 hours *post mortem*

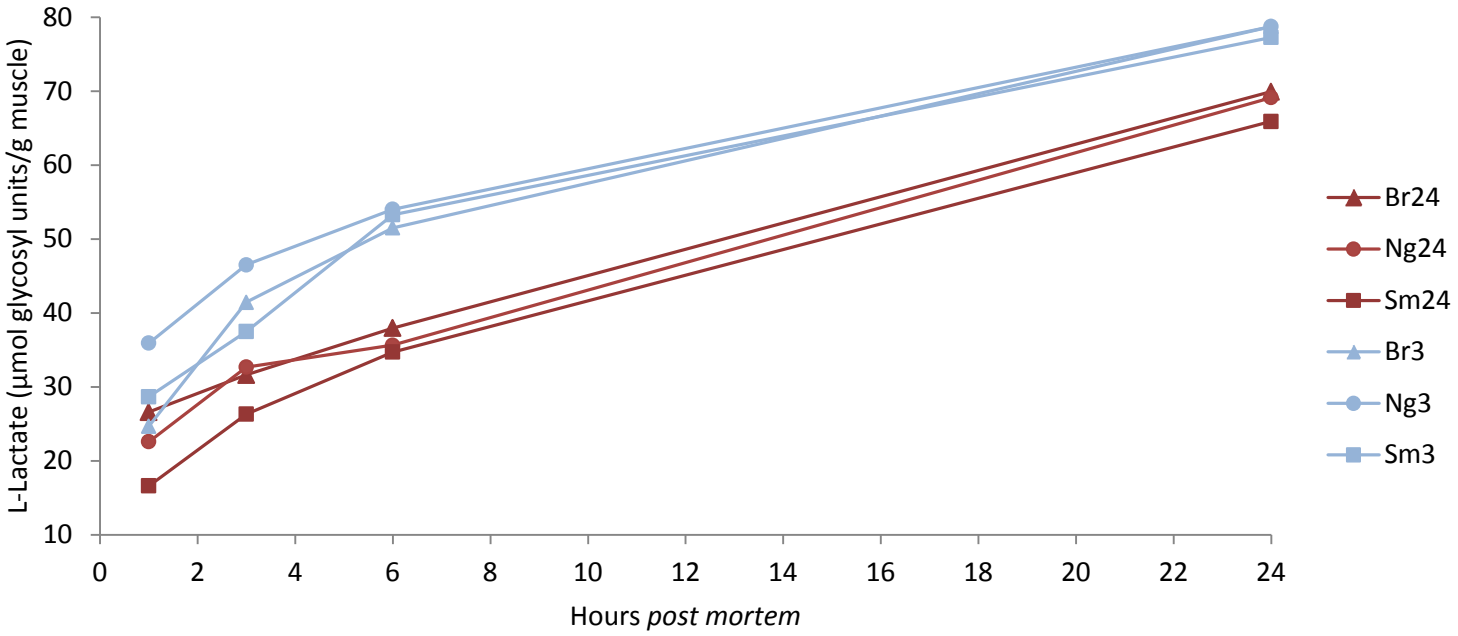

Figure 4.6 L-Lactate concentrations (µmol glycosyl units / g muscle) measured from 1 to 24 hours *post mortem* in *m. longissimus* of Brahman, Nguni and Simmental type cattle subjected to 3 or 24 hours feed withdrawal

From Figure 4.6 it seems as if L-lactate concentrations increased slower between 1 and 6 hours *post mortem* for Sm_{24} compared to Sm_3.

4.3.12 Effect of breed type, feed withdrawal period and first order interactions on *post mortem* creatine phosphate concentration (µmol glycosyl units / g muscle)

Table 4.26 Least square means for the effect of breed type on creatine phosphate concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Creatine phosphate	Breed type			SEM
	Brahman	Nguni	Simmental	
At 1 hour	8.29[a]	10.10[ab]	10.38[b]	1.03
At 3 hours	7.79[a]	9.21[b]	9.02[ab]	0.73
At 6 hours	6.67[a]	8.52[b]	7.49[ab]	0.77
At 24 hours	4.48[a]	7.32[b]	6.12[b]	0.78

[a, b]Different superscripts within the same row differ significantly (*P<0.05*)

Table 4.27 Least square means for the effect of feed withdrawal period on creatine phosphate concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Creatine phosphate	Feed withdrawal		SEM
	3 hours	24 hours	
At 1 hour	9.48	9.70	0.59
At 3 hours	8.68	8.68	0.42
At 6 hours	8.02	7.10	0.43
At 24 hours	6.25	5.60	0.46

Creatine phosphate concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. Creatine phosphate concentration of Brahman type cattle was lower (*P<0.05*) compared to Nguni type cattle at 1, 3, 6 and 24 hours *post mortem* and lower (*P<0.05*) compared to Simmental type cattle at 1 and 24 hours *post mortem*.

There was no difference in creatine phosphate concentration between feed withdrawal periods.

Table 4.28 Least square means for the effect of the interactions between breed type and feed withdrawal period on creatine phosphate concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Creatine phosphate	Feed withdrawal	Breed type			SEM
		Brahman	Nguni	Simmental	
At 1 hour	3 hours	8.56	9.63	10.16	1.03
	24 hours	7.91	10.58	10.61	
At 3 hours	3 hours	7.86	9.01	9.12	0.73
	24 hours	7.68	9.43	8.92	
At 6 hours	3 hours	7.01	8.94	8.16	0.77
	24 hours	6.37	8.10	6.83	
At 24 hours	3 hours	5.23[a]	7.16[b]	6.39[a]	0.78
	24 hours	3.76[a]	7.49[b]	5.55[a]	

[a,b]Different superscripts within the same row differ significantly *(P<0.05)*

Creatine phosphate concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. There were no significant differences in creatine phosphate concentration between any of the treatment groups at 1, 3 or 6 hours *post mortem*. At 24 hours *post mortem*, creatine phosphate concentration of Nguni type cattle was higher *(P<0.05)* than Brahman and Simmental type cattle for both 3 and 24 hours feed withdrawal periods.

4.3.13 Creatine phosphate concentrations (µmol glycosyl units / g muscle) as measured in *m. longissimus* from 1 to 24 hours *post mortem*

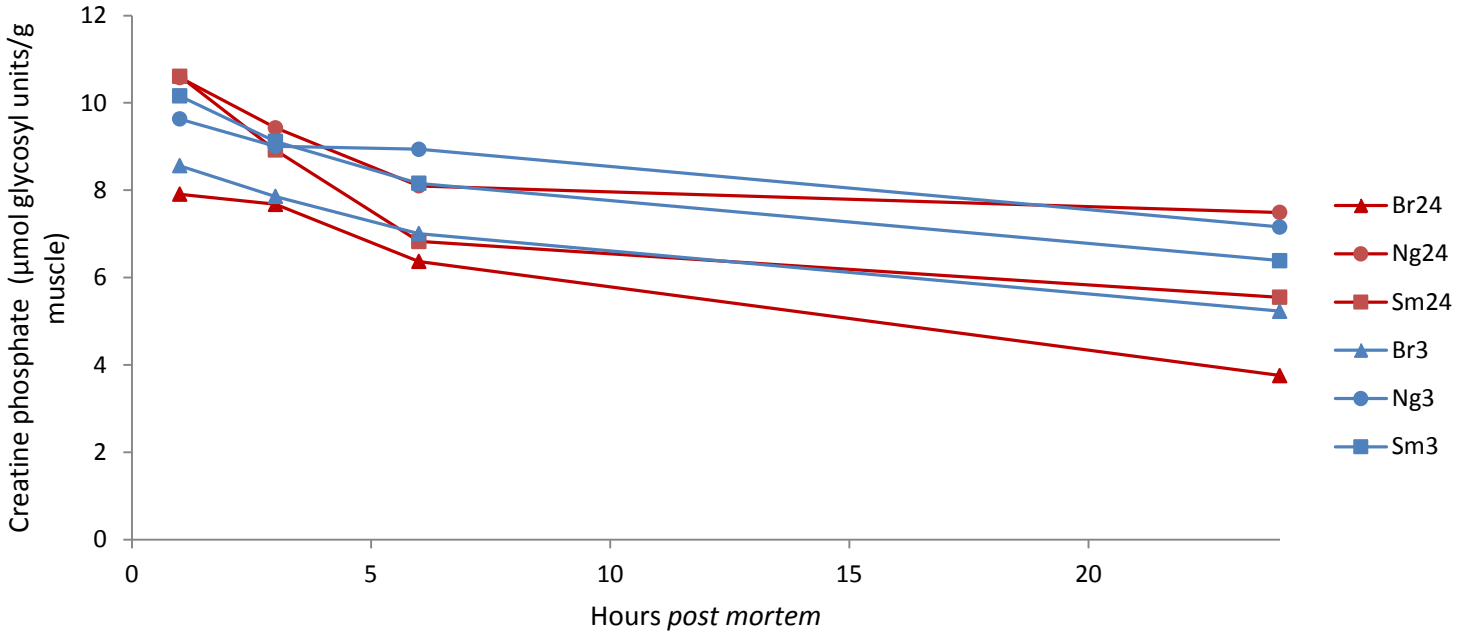

Figure 4.7 Creatine phosphate concentrations (µmol glycosyl units / g muscle) measured from 1 to 24 hours *post mortem* in *m. longissimus* of Brahman, Nguni and Simmental type cattle subjected to 3 or 24 hours feed withdrawal

From Figure 4.7 it seems as if the rate of decline for creatine phosphate concentrations in *m. longissimus* was faster *(P<0.05)* between 1 to 6 hours *post mortem* for Sm_{24} compared to Sm_3.

4.3.14 Effect of breed type, feed withdrawal period and first order interactions on *post mortem* ATP concentration (µmol glycosyl units / g muscle)

Table 4.29 Least square means for the effect of breed type on ATP concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

ATP	Breed type			SEM
	Brahman	Nguni	Simmental	
At 1 hour	6.92	8.30	6.61	0.51
At 3 hours	6.13[a]	8.52[b]	6.19[a]	0.55
At 6 hours	5.70[a]	8.21[b]	5.82[a]	0.49
At 24 hours	2.15[a]	5.55[b]	2.47[a]	0.36

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Table 4.30 Least square means for the effect of feed withdrawal period on ATP concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

ATP	Feed withdrawal		SEM
	3 hours	24 hours	
At 1 hour	7.16	7.39	0.37
At 3 hours	6.78	7.11	0.37
At 6 hours	6.33	6.83	0.36
At 24 hours	3.55	3.24	3.29

ATP concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. Concentrations of muscle ATP for Nguni were higher *(P>0.05)* at 3, 6 and 24 hours *post mortem* compared to Brahman and Simmental type cattle.

ATP concentrations did not differ between 3 and 24 hours feed withdrawal period groups.

Table 4.31 Least square means for the effect of the interactions between breed type and feed withdrawal period on ATP concentration (µmol glycosyl units / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

ATP	Feed withdrawal	Breed type			SEM
		Brahman	Nguni	Simmental	
at 1 hour	3 hours	7.07	7.62	6.80	0.69
	24 hours	6.77[a]	8.99[b]	6.41[a]	
at 3 hours	3 hours	6.48	7.56[*]	6.30	0.68
	24 hours	5.77[a]	9.50[b**]	6.08[a]	
at 6 hours	3 hours	5.54	7.52	5.94	0.66
	24 hours	5.86[a]	8.91[b]	5.71[a]	
at 24 hours	3 hours	2.37[a]	5.60[b]	2.68[a]	0.45
	24 hours	1.93[a]	5.51[b]	2.27[a]	

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*
[*, **]Different superscripts within the same column differ significantly *(P<0.05)*

ATP concentration (µmol glycosyl units / g muscle) was measured from *m. longissimus* for all groups. For treatment groups submitted to 3 hours feed withdrawal, ATP concentration was higher *(P<0.05*) for Nguni cattle compared to Brahman and Simmental type cattle at 24 hours *post mortem* only. For treatment groups submitted to 24 hours feed withdrawal, Nguni had higher *(P<0.05)* ATP concentrations than Brahman and Simmental type cattle at 1, 3, 6 and 24 hours *post mortem*. Muscle ATP concentration for Ng_{24} was higher *(P<0.05)* compared to Ng_3 at 3 hours *post mortem*.

4.3.15 ATP concentrations (µmol glycosyl units / g muscle) as measured in *m. longissimus* from 1 to 24 hours *post mortem*

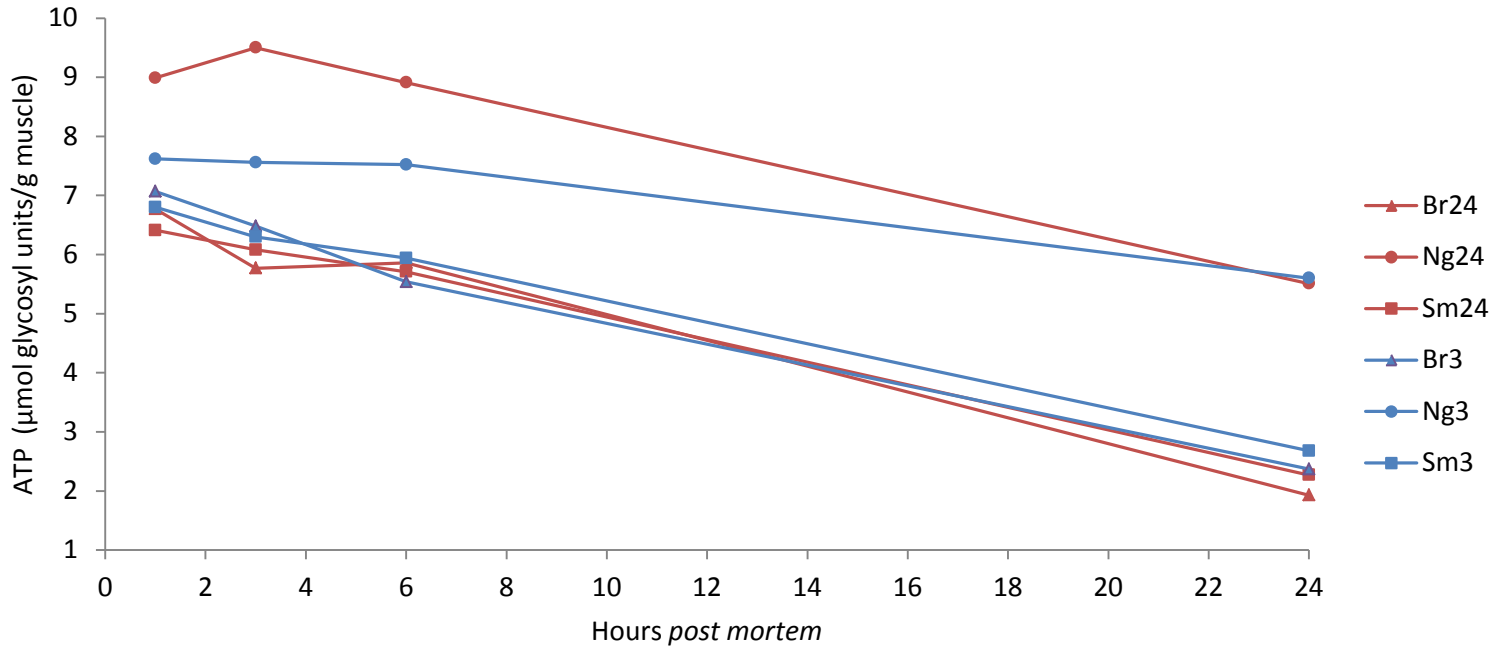

Figure 4.8 ATP concentrations (µmol glycosyl units / g muscle) measured from 1 to 24 hours *post mortem* in *m. longissimus* of Brahman, Nguni and Simmental type cattle subjected to 3 or 24 hours feed withdrawal

Although Ng_{24} and Ng_3 showed more ATP concentration in *m. longissimus* early *post mortem* as seen from Figure 4.8 it seems as if ATP hydrolysis was slower for Nguni compared to Brahman and Simmental type cattle. There was no significant difference in the rate of ATP metabolism early and late *post mortem* time intervals.

4.3.16 Effect of breed type, feed withdrawal period and first order interactions on glycolytic potential as calculated from *m. longissimus* between 1 and 24 hours *post mortem*

Table 4.32 Least square means for the effect of breed type on glycolytic potential (µmol / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glycolytic potential	Breed type			SEM
	Brahman	Nguni	Simmental	
At 1 hour	126.46[a]	108.03[b]	120.03[ab]	5.64
At 3 hours	120.12	107.10	110.29	5.95
At 6 hours	123.30[a]	104.00[b]	111.35[ab]	7.00
At 24 hours	123.99	111.66	110.61	7.46

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Table 4.33 Least square means for the effect of feed withdrawal period on glycolytic potential (µmol / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glycolytic potential	Feed withdrawal		SEM
	3 hours	24 hours	
At 1 hour	129.56[a]	106.79[b]	4.61
At 3 hours	123.79[a]	101.21[b]	4.85
At 6 hours	127.24[a]	98.53[b]	5.71
At 24 hours	121.75	109.09	6.09

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Glycolytic potential (µmol / g muscle) was measured from *m. longissimus* of all treatment groups. Glycolytic potential in *m. longissimus* of Brahman cattle was higher *(P<0.05)* than for Nguni type cattle at 1 and 6 hours *post mortem* as was also found by Frylinck *et al*. (2013).

Glycolytic potential for FW_3 was higher *(P<0.05)* compared to FW_{24} at 1, 3 and 6 hours *post mortem*.

Table 4.34 Least square means for the effect of the interactions between breed type and feed withdrawal period on glycolytic potential (µmol / g muscle) in *m. longissimus* at 1, 3, 6 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Glycolytic potential	Feed withdrawal	Breed type			SEM
		Brahman	Nguni	Simmental	
at 1 hour	3 hours	136.90	130.90*	120.87	7.98
	24 hours	116.03^{a}	85.17^{b**}	119.19^{a}	
at 3 hours	3 hours	128.48	124.75*	118.15	8.41
	24 hours	111.76^{a}	89.44^{b**}	102.42^{ab}	
at 6 hours	3 hours	134.94	122.36*	124.41*	9.89
	24 hours	111.64^{a}	85.63^{b**}	98.28^{ab**}	
at 24 hours	3 hours	135.62	117.85	111.87	10.55
	24 hours	112.54	105.47	109.34	

$^{a, b}$Different superscripts within the same row differ significantly *(P<0.05)*
$^{*, **}$Different superscripts within the same column differ significantly *(P<0.05)*

Glycolytic potential (µmol / g muscle) was measured from *m. longissimus* of all treatment groups. As expected and due to lower initial glycogen, glycolytic potential in *m. longissimus* of Ng_{24} was lower *(P<0.05)* than Br_{24} at 1, 3 and 6 hours *post mortem* and also lower *(P<0.05)* than Sm_{24} at 1 hour *post mortem*. Glycolytic potential of Ng_{24} was lower *(P<0.05)* than Ng_{3} at 1, 3 and 6 hours *post mortem*. Glycolytic potential for Sm_{24}, was lower *(P<0.05)* than Sm_{3} at 6 hours *post mortem*.

4.3.17 Glycolytic potential (µmol / g muscle) as measured in *m. longissimus* from 1 to 24 hours *post mortem*

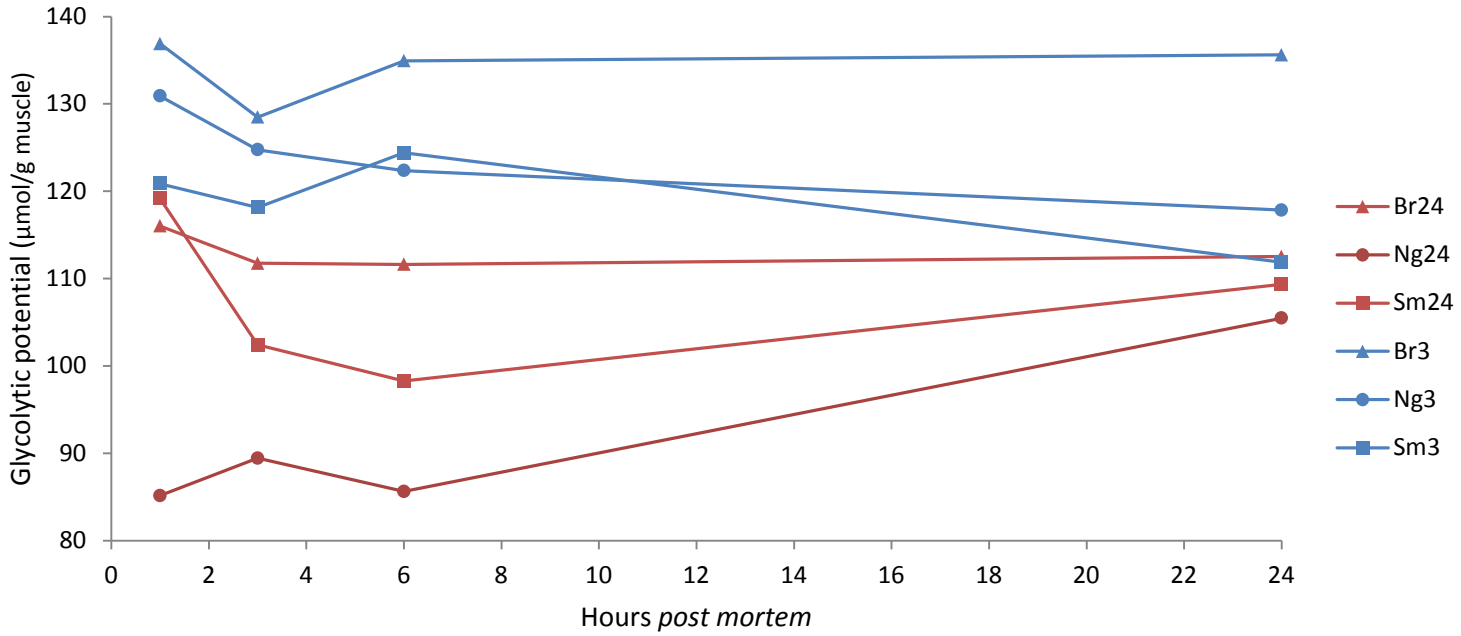

Figure 4.9 Glycolytic potential (µmol / g muscle) measured from 1 to 24 hours *post mortem* in *m. longissimus* of Brahman, Nguni and Simmental type cattle subjected to 3 or 24 hours feed withdrawal

Glycolytic potential measured in *m. longissimus* of Br_{24}, Sm_{24}, Br_3, Ng_3 and Sm_3 decreased from 1 and 3 hours *post mortem* and even more dramatically for Sm_{24}. the glycolytic potential in *m. longissimus* of Br_3 and Sm_3 increased. Glycolytic potential in *m. longissimus* of Br_3 and Sm_3 increased between 3 and 6 hours *post mortem*, Glycolytic potential in *m. longissimus* of Br_{24} remained constant between 3 and 6 hours *post mortem*. Between 3 and 6 hours *post mortem*, glycolytic potential of Ng_3, Ng_{24} and Sm_{24} decreased where Sm_{24} showed the most dramatic decrease. Between 6 and 24 hours *post mortem*, glycolytic potential of Br_3, Ng_3 and Br_{24} remained constant. Between 6 and 24 hours *post mortem*, glycolytic potential of Sm3 decreased. Between 6 and 24 hours *post mortem*, glycolytic potential of Sm_{24} and Ng_{24} increased where Sm_{24} showed the most dramatic increase.

4.4 Physical meat properties: Meat colour, water binding capacity, muscle fiber typing and Warner-Bratzler shear force

4.4.1 Multifactorial analysis of variance for *post mortem* colour coordinates (L*, a*, b*, Si, HA and Ri) and water binding capacity

Table 4.35 MANOVA results for the effects of breed type, feed withdrawal period and first order interactions on colour coordinates and water binding capacity of *m. longissimus* (data pooled for breed type and feed withdrawal period; degrees of freedom parenthesized)

Effect	L*		a*		b*		Si		HA		Ri		WBC	
	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance
Main factor														
B	**3.52**	**P= 0.04**	**4.54**	**P=0.02**	**6.68**	**P<0.01**	**5.18**	**P=0.01**	**3.68**	**P=0.03**	**3.67**	**P=0.03**	0.15	P=0.43
FW	**3.91**	**P= 0.05**	3.15	P=0.08	2.01	P=0.16	2.99	P=0.09	0.18	P=0.68	0.18	P=0.67	0.23	P=0.23
Interaction														
B x FW	**2.46**	**P=0.04**	**2.60**	**P=0.04**	**3.19**	**P=0.01**	**2.83**	**P=0.02**	1.47	P=0.22	1.47	P=0.22	1.43	P=0.32

B = Breed type (Brahman, Nguni and Simmental)
FW = Feed withdrawal period (24 hours and 3 hours feed withdrawal)
WBC = Water binding capacity

4.4.2 Effect of breed type, feed withdrawal period and first order interactions on meat colour coordinates (L*, a*, b*, Si, HA and Ri) and water binding capacity

Table 4.36 Least square means for the effect of breed type on meat colour coordinates (L*, a*, b*, Si, HA and Ri) and waterbinding capacity of *m. longissimus* ($\bar{x}$ ± SEM)

Colour	Breed type			SEM
	Brahman	Nguni	Simmental	
Lightness (L*)	36.92[a]	35.04[b]	36.61[a]	0.56
Redness (a*)	14.77[a]	13.20[b]	14.33[a]	0.38
Yellowness (b*)	6.44[a]	6.72[b]	6.19[a]	0.30
Chroma (Si)	16.34[a]	14.45[b]	15.79[a]	0.43
Hue angle (HA)	1.97[a]	2.12[b]	2.03[ab]	0.04
Redness index (Ri)	2.13[a]	2.27[b]	2.19[ab]	0.04
Water binding capacity	0.45	0.46	0.45	0.01

[a,b]Different superscripts within the same row differ significantly *(P<0.05)*

Table 4.37 Least square means for the effect of feed withdrawal period on meat colour coordinates (L*, a*, b*, Si, HA and Ri) and waterbinding capacity of *m. longissimus* ($\bar{x}$ ± SEM)

Colour	Feed withdrawal		SEM
	3 Hours	24 Hours	
Lightness (L*)	36.81[a]	35.56[b]	0.46
Redness (a*)	14.51	13.70	0.31
Yellowness (b*)	6.67	6.23	0.20
Chroma (Si)	15.98	15.08	0.35
Hue angle (HA)	2.03	2.05	0.03
Redness Index (Ri)	2.19	2.21	0.03
Water binding capacity	0.45	0.46	0.01

[a,b]Different superscripts within the same row differ significantly *(P<0.05)*

Meat colour coordinates (L*, a*, b*, Si, HA and Ri) as well as water binding capacity were measured from *m. longissimus* of all treatment groups. L* and a* of Nguni cattle were lower *(P<0.05)* than for Brahman and Simmental type cattle and appeared darker and more red. b* of Nguni was higher *(P<0.05)* compared to Brahman and Simmental type cattle. Si as measured from

m. longissimus was lower for Nguni type cattle than for Brahman and Simmental type cattle. Hue angle (HA) and Ri was higher *(P<0.05)* for Nguni type cattle than for Brahman type cattle.

Surface colour as measured from *m. longissimus* from groups subjected to 24 hours feed withdrawal appeared darker (L* was lower) *(P<0.05)* compared to meat from those animals subjected to 3 hours feed withdrawal.

Table 4.38 Least square means for the effect of interactions between breed type and feed withdrawal period on meat colour coordinates (L*, a*, b*, Si, HA and Ri) and water binding capacity of *m. longissimus* ($\bar{x}$ ± SEM)

Colour	Feed withdrawal	Breed			SEM
		Brahman	Nguni	Simmental	
Lightness (L*)	3 hours	37.18	35.97*	37.3	0.75
	24 hours	36.67^{a}	34.11^{b**}	35.91^{a}	
Redness (a*)	3 hours	15.07	13.78	14.67	0.53
	24 hours	14.47^{a}	12.62^{b}	14.00^{ab}	
Yellowness (b*)	3 hours	6.62	7.01	6.40	0.31
	24 hours	6.26^{a}	6.42^{b}	5.99^{a}	
Chroma (Si)	3 hours	16.66^{a}	15.09^{b}	16.17^{ab}	0.60
	24 hours	16.03^{a}	13.80^{b}	15.40^{a}	
Hue angle (HA)	3 hours	1.98^{a}	2.11^{b}	2.02^{ab}	0.06
	24 hours	1.97^{a}	2.14^{b}	2.05^{ab}	
Redness Index (Ri)	3 hours	2.14	2.26	2.17	0.05
	24 hours	2.13	2.28	2.21	
Water binding capacity	3 hours	0.44	0.47	0.44	0.01
	24 hours	0.45	0.46	0.45	

$^{a, b}$Different superscripts within the same row differ significantly *(P<0.05)*
$^{*, **}$Different superscripts within the same column differ significantly *(P<0.05)*

Meat colour coordinates (L*, a*, b*, Si, HA and Ri) as well as water binding capacity was measured from *m. longissimus* of all treatment groups. Nguni cattle subjected to 24 hours feed withdrawal had the darkest meat surface, L* was lower *(P<0.05)* for Ng_{24} compared to Br_{24} and Sm_{24}. In addition, significant difference in lightness within a breed but between feed withdrawal periods was only observed for Nguni type cattle where L* was lower *(P<0.05)* for Ng_{24} compared to Ng_{3}. Ng_{24} had lower *(P<0.05)* a* compared to Br_{24} and higher *(P<0.05)* b* compared to Br_{24} and Sm_{24}.

The saturation index (Si) for Ng_{24} was lower *(P<0.05)* compared to Br_{24} and Sm_{24} and Ng_{3} showed lower *(P<0.05)* Si compared to Br_{3}. Hue angle for Brahman type cattle was lower *(P<0.05)* compared to Nguni type cattle, irrespective of feed withdrawal period.

When feed was withdrawn for 3 hours before slaughter, there were no significant differences for L*, a* or b* as measured from *m. longissimus* between breed types

4.4.3 Multifactorial analysis of variance for muscle fiber types

Table 4.39 MANOVA results for the effects of breed type, feed withdrawal period and first order interactions on muscle fiber types of *m. longissimus* (data pooled for breed type and feed withdrawal period; degrees of freedom parenthesized)

Effect	% Type I		% Type IIA		% Type IIB	
	F ratio	Significance	F ratio	Significance	F ratio	Significance
Main factor						
B	2.96	P=0.06	0.54	P=0.58	0.69	P=0.51
FW	1.02	P=0.32	0.00	P=0.95	0.66	P=0.42
Interaction						
B x FW	0.49	P=0.61	2.63	P=0.08	**3.63**	**P=0.03**

B = Breed type (Brahman, Nguni and Simmental)
FW = Feed withdrawal period (24 hours and 3 hours feed withdrawal)

4.4.4 Effect of breed type, feed withdrawal period and first order interactions on muscle fiber type

Table 4.40 Least square means for the effect of breed type on muscle fiber type of *m. longissimus* ($\bar{x}$ ± SEM)

Muscle fiber type	Breed type			SEM
	Brahman	Nguni	Simmental	
% Type I	35.97^{ab}	37.08^{a}	34.69^{b}	0.70
% Type IIA	27.43	27.53	28.50	0.81
% Type IIB	36.61	35.40	36.81	0.82

$^{a, b}$Different superscripts within the same row differ significantly *(P<0.05)*

Table 4.41 Least square means for the effect of feed withdrawal period on muscle fiber type of *m. longissimus* ($\bar{x}$ ± SEM)

Muscle fiber type	Feed withdrawal		SEM
	3 Hours	24 Hours	
% Type I	35.50	36.32	0.60
% Type IIA	27.79	27.85	0.65
% Type IIB	36.71	35.84	0.67

Muscle fiber type was measured from *m. longissimus* of all treatment groups. Results in this study indicated that *m. longissimus* from the Nguni breed type contained more *(P<0.05)* Type I or oxidative muscle fibers in comparison to Simmental type cattle.

Feed withdrawal period had no significant effect on muscle fiber types between groups.

Table 4.42 Least square means for the effect of interactions between breed type and feed withdrawal period on muscle fiber type of *m. longissimus* ($\bar{x}$ ± SEM)

Muscle fiber type	Feed withdrawal	Breed			SEM
		Brahman	Nguni	Simmental	
% Type I	3 hours	35.29^{ab}	37.28^{a}	33.99^{b}	0.99
	24 hours	36.65	36.92	35.38	
% Type IIA	3 hours	27.3	28.85	27.22	1.14
	24 hours	27.56^{ab}	26.20^{a}	29.78^{b}	
% Type IIB	3 hours	37.41^{ab}	33.91^{a}	38.79^{b*}	1.31
	24 hours	35.80	36.89	34.84**	

$^{a, b}$Different superscripts within the same row differ significantly *(P<0.05)*
$^{*, **}$Different superscripts within the same column differ significantly *(P<0.05)*

Muscle fiber type was measured from *m. longissimus* of all treatment groups. Ng_3 had more *(P<0.05)* Type I fibers compared to Sm_3. Sm_{24} showed more *(P<0.05)* Type IIA fibers compared to Ng_{24}. Sm_{24} showed more *(P<0.05)* Type IIB fibers compared to Sm_3. Sm_3 showed more *(P<0.05)* Type IIB fibers compared to Ng_3.

4.4.5 Multifactorial analysis of variance for Warner-Bratzler shear force (kg/12.5 mm Ɵ)

Table 4.43 MANOVA results for the effects of breed type, feed withdrawal period and first order interactions on Warner-Bratzler shear force (kg/12.5 mm Ɵ) of *m. longissimus* (data pooled for breed type and feed withdrawal period; degrees of freedom parenthesized)

Effect	Shear force (kg/12.5 mm Ɵ) 1 day *post mortem*		Shear force (kg/12.5 mm Ɵ) 7 days *post mortem*		Shear force (kg/12.5 mm Ɵ) 14 days *post mortem*	
	F ratio	Significance	F ratio	Significance	F ratio	Significance
Main factor						
B	2.82	P=0.06	0.99	P=0.37	**3.59**	**P=0.03**
FW	**4.86**	**P=0.03**	0.44	P=0.51	**6.12**	**P=0.01**
Interaction						
B x FW	0.77	P=0.46	0.06	P=0.94	1.19	P=0.31

B = Breed type (Brahman, Nguni and Simmental)
FW = Feed withdrawal period (24 hours and 3 hours feed withdrawal)

4.4.6 Effect of breed type, feed withdrawal period and first order interactions on Warner-Bratzler shear force (kg/12.5 mm Θ)

Table 4.44 Least square means for the effect of breed type on Warner-Bratzler shear force (kg/12.5 mm Θ) of *m. longissimus* at 1, 7 and 14 days *post mortem* ($\bar{x}$ ± SEM)

Warner-Bratzler shear force (kg/12.5 mm Θ)	Breed type			SEM
	Brahman	Nguni	Simmental	
1 day	8.30[a]	8.63[a]	9.52[b]	0.32
7 days	5.91	5.86	6.50	0.28
14 days	4.77[a]	5.19[a]	6.01[b]	0.23

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Table 4.45 Least square means for the effect of feed withdrawal period on Warner-Bratzler shear force (kg/12.5 mm Θ) of *m. longissimus* at 1, 7 and 14 days *post mortem* ($\bar{x}$ ± SEM)

Warner-Bratzler shear force (kg/12.5 mm Θ)	Feed withdrawal		SEM
	3 Hours	24 Hours	
1 day	8.34[a]	9.29[b]	0.26
7 days	5.93	6.25	0.23
14 days	5.01[a]	5.64[b]	0.19

[a, b] = Different superscripts within the same row differ significantly *(P<0.05)*

Warner-Bratzler shear force was measured from *m. longissimus* of all treatment groups. At 1 and 14 days *post mortem* muscle samples from Simmental type cattle were more tough and had higher *(P<0.05)* Warner-Bratzler shear force values compared to Brahman and Nguni type cattle.

Warner-Bratzler shear force values from muscle samples of groups that were subjected to 24 hours feed withdrawal were higher *(P<0.05)* in comparison to those groups that were subjected to 3 hours feed withdrawal.

Table 4.46 Least square means for the effect of interactions between breed type and feed withdrawal period on Warner-Bratzler shear force (kg/12.5 mm Θ) of *m. longissimus* at 1, 7 and 14 days *post mortem* ($\bar{x} \pm$ SEM)

Warner-Bratzler shear force (kg/12.5 mm Θ)	Feed withdrawal	Breed			SEM
		Brahman	Nguni	Simmental	
1 day	3 hours	8.09	8.23	8.68*	0.45
	24 hours	8.50^{a}	9.00^{a}	10.36^{b**}	
7 days	3 hours	5.88	5.83	6.06*	0.4
	24 hours	5.93^{a}	5.89^{a}	6.93^{b**}	
14 days	3 hours	4.86	4.89	5.28*	0.32
	24 hours	4.69^{a}	5.50^{b}	6.70^{c**}	

$^{a, b, c}$Different superscripts within the same row differ significantly *(P<0.05)*
$^{*, **}$Different superscripts within the same column differ significantly *(P<0.05)*

Warner-Bratzler shear force was measured from *m. longissimus* of all treatment groups. Feed withdrawal period had significant negative effects on Warner-Bratzler shear force of Simmental type cattle. Warner-Bratzler shear force for Sm_{24} was higher *(P<0.05)* compared to Br_{24} and Ng_{24} at 1 and 7 days *post mortem*. Warner-Bratzler shear force for Br_{24} was lower *(P<0.05)* compared to Ng_{24} and Sm_{24} and Warner-Bratzler shear force for Ng_{24} was lower than Sm_{24} at 14 days *post mortem*. Warner-Bratzler shear force for Sm_{24} was higher *(P<0.05)* compared to Sm_{3} at 1, 7 and 14 days *post mortem*. There were no significant differences between breeds when feed was withdrawn for 3 hours before slaughter.

4.5 The toughening phase of the conversion of muscle to meat: sarcomere lengths

4.5.1 Multifactorial analysis of variance for sarcomere lengths (µm)

Table 4.47 MANOVA results for the effects of breed type, feed withdrawal period and first order interactions on sarcomere lengths (µm) of *m. longissimus* (data pooled for breed type and feed withdrawal period; degrees of freedom parenthesized)

Effect	Sarcomere lengths (µm) 1 day *post mortem*		Sarcomere lengths (µm) 3 days *post mortem*	
	F ratio	Significance	F ratio	Significance
Main factor				
B	**6.91**	**P=0.001**	**5.26**	**P=0.006**
FW	**8.71**	**P=0.004**	**8.34**	**P=0.004**
Interaction				
B x FW	1.11	P=0.333	0.64	P=0.527

B = Breed type (Brahman, Nguni and Simmental)
FW = Feed withdrawal period (24 hours and 3 hours feed withdrawal)

4.5.2 Effect of breed type, feed withdrawal period and first order interactions on sarcomere length (µm)

Table 4.48 Least square means for the effect of breed type on average sarcomere length (µm) of *m. longissimus* at 1 and 3 days *post mortem* ($\bar{x}$ ± SEM)

Average Sarcomere length (µm)	Breed type			SEM
	Brahman	Nguni	Simmental	
Day 1	1.70[a]	1.61[b]	1.66[ab]	0.03
Day 3	1.70[a]	1.62[b]	1.68[ab]	0.02

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Table 4.49 Least square means for the effect of feed withdrawal period on average sarcomere length (µm) of *m. longissimus* at 1 and 3 days *post mortem* ($\bar{x}$ ± SEM)

Average Sarcomere length (µm)	Feed withdrawal		SEM
	3 Hours	24 Hours	
Day 1	1.69[a]	1.63[b]	0.02
Day 3	1.69	1.65	0.02

[a, b]Different superscripts within the same row differ significantly *(P<0.05)*

Sarcomere length (µm) was measured from *m. longissimus* of all treatment groups. Sarcomere lengths as measured from *m. longissimus* of Nguni type cattle were shorter *(P<0.05)* than sarcomere lengths of Brahman type cattle at 1 and 3 days *post mortem*. There were no differences in sarcomere lengths between Simmental and Nguni as well as between Simmental and Brahman type cattle.

Sarcomere lengths (µm) of groups of cattle that were subjected to 24 hours feed withdrawal were shorter *(P<0.05)* in comparison to groups that were subjected to 3 hours feed withdrawal at 1 day *post mortem*.

Table 4.50 Least square means for the effect of interactions between breed type and feed withdrawal period on average sarcomere length (µm) of *m. longissimus* at 1 and 3 days *post mortem* ($\bar{x}$ ± SEM)

Average Sarcomere length (µm)	Feed withdrawal	Breed			SEM
		Brahman	Nguni	Simmental	
1 day	3 hours	1.72[a]	1.62[b]	1.72[a**]	0.04
	24 hours	1.70[a]	1.60[b]	1.59[b*]	
3 days	3 hours	1.72[a]	1.63[b]	1.73[a**]	0.03
	24 hours	1.68	1.62	1.64[*]	

[a, b] = Different superscripts within the same row differ significantly *(P<0.05)*
[*, **] = Different superscripts within the same column differ significantly *(P<0.05)*

Sarcomere length (µm) was measured from *m. longissimus* of all treatment groups. Average sarcomere lengths were longer *(P<0.05)* for Br_{24} compared to Ng_{24} and Sm_{24} at 1 day *post mortem*. Average sarcomere lengths were shorter *(P<0.05)* for Ng_3 than for Br_3 and Sm_3 at 1 and 3 days *post mortem*. Average sarcomere lengths were shorter for Sm_{24} than for Sm_3 at 1 and 3 days *post mortem*. Feed withdrawal period had no effect on sarcomere length for Brahman and Nguni.

4.6 The tenderising phase of the conversion of muscle to meat: calcium activated proteolytic enzyme activity and myofibrillar fragmentation lengths

4.6.1 Multifactorial analysis of variance for calcium activated enzyme activity and myofibrillar fragmentation lengths (µm)

Table 4.51 MANOVA results for the effects of breed type, feed withdrawal period and first order interactions on calcium activated enzymes of *m. longissimus* (data pooled for breed type and feed withdrawal period; degrees of freedom parenthesized)

Effect	Calpastatin activity/ extracTable protein		µ-calpain activity/ extracTable protein		m-calpain activity/extracTable protein		Calpastatin / µ-calpain		Calpastatin/(µ-calpain + m-calpain)	
	1 hr	24 hrs	1 hr	24 hrs	1 hr	24 hrs	1 hr	24 hrs	1 hr	24 hrs
	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance	F ratio	Significance
Main factor										
B	0.05	P=0.95	**10.15**	**P=0.00**	1.73	P=0.18	1.73	P=0.18	**9.88**	**P<0.001**
FW	0.07	P=0.79	1.79	P=0.18	0.86	P=0.35	0.86	P=0.35	**4.57**	**P=0.03**
Interaction										
B x FW	**3.37**	**P=0.04**	1.96	P=0.14	0.59	P=0.23	0.59	P=0.23	**3.03**	**P=0.05**

B = Breed type (Brahman, Nguni and Simmental)
FW = Feed withdrawal period (24 hours and 3 hours feed withdrawal)

Table 4.52 MANOVA results for the effects of breed type, feed withdrawal period and first order interactions on myofibrillar fragmentation lengths (µm) of *m. longissimus* (data pooled for breed type and feed withdrawal period; degrees of freedom parenthesized)

Effect	Myofibrillar fragmentation length (µm) 1 day *post mortem*		Myofibrillar fragmentation length 7 (µm) days *post mortem*		Myofibrillar fragmentation length (µm) 14 days *post mortem*	
	F ratio	Significance	F ratio	Significance	F ratio	Significance
Main factor						
B	1.52	P=0.22	**5.73**	**P=0.001**	2.23	P=0.11
FW	1.97	P=0.16	0.93	P=0.34	0.59	P=0.44
Interaction						
B x FW	0.38	P=0.69	0.05	P=0.95	0.05	P=0.95

B = Breed type (Brahman, Nguni and Simmental)
FW = Feed withdrawal period (24 hours and 3 hours feed withdrawal)

4.6.2 Effect of breed type, feed withdrawal period and first order interactions on proteolytic enzyme activity and myofibrillar fragmentation lengths (µm)

Table 4.53 Least square means for the effect of breed type proteolytic enzymes of *m. longissimus* at 1 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Calcium activated enzymes	Time	Breed			SEM
		Brahman	Nguni	Simmental	
Total calpastatin activity/total extracTable protein	1 hr	2.71	2.64	2.82	0.09
	24 hrs	2.65	2.58	2.67	0.11
Total µ-calpain activity/total extracTable protein	1 hr	0.03^{a}	0.04^{b}	0.04^{b}	0.001
	24 hrs	0.02^{a}	0.03^{b}	0.03^{b}	0.001
Total m-calpain activity/total extracTable protein	1 hr	0.02	0.01	0.01	0.001
	24 hrs	0.02	0.02	0.02	0.001
Calpastatin/µ-calpain	1 hr	1.72^{a}	1.28^{b}	1.33^{b}	0.09
	24 hrs	2.26^{a}	1.42^{b}	1.59^{b}	0.19
Calpastatin/(µ-calpain + m-calpain)	1 hr	1.08^{a}	0.89^{b}	0.94^{b}	0.04
	24 hrs	1.06^{a}	0.86^{b}	0.94^{ab}	0.06

$^{a, b}$Different superscripts within the same row differ significantly *(P<0.05)*

Calcium activated proteolytic enzyme activity was extractable from *m. longissimus* of all treatment groups. µ-Calpain activity/total extracTable protein at 1 and 24 hours *post mortem* was lower *(P<0.05)* for Brahman compared to Nguni and Simmental type animals in the present study. Brahman also had higher *(P<0.05)* calpastatin/µ-calpain at 1 and 24 hours *post mortem* than Nguni and Simmental, which is as expected for Brahman type cattle. Brahman had higher *(P<0.05)* calpastatin/(µ-calpain + m-calpain) at 1 hour *post mortem* than Nguni and Simmental. Brahman had higher *(P<0.05)* calpastatin/(µ-calpain + m-calpain) at 24 hours *post mortem* than Nguni.

Table 4.54 Least square means for the effect of feed withdrawal period on proteolytic enzymes of *m. longissimus* at 1 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Calcium activated enzymes	Time	Feed withdrawal		SEM
		3 Hours	24 Hours	
Total calpastatin activity/total extracTable protein	1 hr	2.78	2.66	0.08
	24 hrs	2.74	2.53	0.09
Total µ-calpain activity/total extracTable protein	1 hr	0.04	0.04	0.001
	24 hrs	0.03	0.03	0.001
Total m-calpain activity/total extracTable protein	1 hr	0.01	0.02	0.001
	24 hrs	0.02	0.02	0.001
Calpastatin/µ-calpain	1 hr	1.84	1.44	0.07
	24 hrs	1.67	1.44	0.16
Calpastatin/(µ-calpain + m-calpain)	1 hr	0.98	0.96	0.03
	24 hrs	0.96	0.94	0.05

Calcium activated proteolytic enzyme activity was measured from *m. longissimus* of all treatment groups. Feed withdrawal period had no significant effect on proteolytic enzyme activity of any of the treatment groups.

Table 4.55 Least square means for the effect of interactions between breed type and feed withdrawal period on proteolytic enzymes of *m. longissimus* at 1 and 24 hours *post mortem* ($\bar{x}$ ± SEM)

Calcium activated proteinase	Time	Feed withdrawal	Breed			SEM
			Brahman	Nguni	Simmental	
Total calpastatin activity/total extracTable protein	1 hr	3 hours	2.71	2.72	2.91	0.14
		24 hours	2.71	2.55	2.72	
	24 hrs	3 hours	2.81	2.64	2.78	0.16
		24 hours	2.50	2.53	2.57	
Total µ-calpain activity/total extracTable protein	1 hr	3 hours	0.03^{a}	0.04^{b}	0.04^{b}	0.003
		24 hours	0.03^{a}	0.04^{b}	0.04^{b}	
	24 hrs	3 hours	0.03^{a}	0.03^{b}	0.03^{b}	0.002
		24 hours	0.02^{a}	0.03^{b}	0.03^{b}	
Total m-calpain activity/total extracTable protein	1 hr	3 hours	0.02	0.01	0.02	0.0001
		24 hours	0.02	0.01	0.02	
	24 hrs	3 hours	0.02	0.02	0.02	0.0001
		24 hours	0.02	0.02	0.02	
Calpastatin/µ-calpain	1 hr	3 hours	1.69^{a}	1.30^{b}	1.33^{b}	0.12
		24 hours	1.74^{a}	1.25^{b}	1.33^{b}	
	24 hrs	3 hours	1.97	1.40	1.62	0.28
		24 hours	2.54^{a}	1.43^{b}	1.55^{b}	
Calpastatin/(µ-calpain + m-calpain)	1 hr	3 hours	1.08^{a}	0.91^{b}	0.96^{ab}	0.06
		24 hours	1.08^{a}	0.87^{b}	0.92^{b}	
	24 hrs	3 hours	1.07	0.87	0.96	0.09
		24 hours	1.05	0.86	0.92	

$^{a, b}$ = Different superscripts within the same row differ significantly *(P<0.05)*

Calcium activated proteolytic enzyme activity was measured from *m. longissimus* of all treatment groups. Irrespective of feed withdrawal period, total µ-calpain activity/total extracTable protein at 1 and 24 hours *post mortem* for Brahman (Br_{24} and Br_3) was less *(P<0.05)* compared to Nguni (Ng_{24}; Ng_3) and Simmental breed types (Sm_{24}; Sm_3). Similarly, irrespective of feed withdrawal period calpastatin/µ-calpain protein at 1 hours *post mortem* was higher *(P<0.05)* for Brahman (Br_{24} and Br_3) compared to Nguni (Ng_{24}; Ng_3) and Simmental breed types (Sm_{24}; Sm_3). Calpastatin/µ-calpain protein at 24 hours *post mortem* was higher *(P<0.05)* for Br_{24} compared to Ng_{24} and Sm_{24}. Br_{24} had higher *(P<0.05)* calpastatin/(µ-calpain + m-calpain) at 1 and 24 hours *post mortem* compared to Ng_{24} and Sm_{24}. Br_3 had higher *(P<0.05)* calpastatin/(µ-calpain + m-calpain) at 24 hours *post mortem* compared to Ng_3.

Table 4.56 Least square means for the effect of breed type on myofibrillar fragmentation lengths (µm) of *m. longissimus* at 1, 7 and 14 days *post mortem* ($\bar{x}$ ± SEM)

Myofibrillar fragmentation length (µm)	Breed type			SEM
	Brahman	Nguni	Simmental	
1 day	44.78	39.19	43.44	1.88
7 days	35.83[a]	26.07[b]	33.60[a]	1.74
14 days	26.79	22.74	25.92	1.65

[a, b] = Different superscripts within the same row differ significantly *(P<0.05)*

Myofibrillar fragmentation lengths were measured from *m. longissimus* of all treatment groups. Myofibrillar fragmentation lengths, which is an indication of proteolytic enzyme activity, of Nguni was shorter *(P<0.05)* at 7 days *post mortem* compared to Brahman and Simmental.

Table 4.57 Least square means for the effect of feed withdrawal period on myofibrillar fragmentation lengths (µm) of *m. longissimus* at 1, 7 and 14 days *post mortem* ($\bar{x}$ ± SEM)

Myofibrillar fragmentation length (µm)	Feed withdrawal		SEM
	3 Hours	24 Hours	
1 day	41.95	42.99	1.26
7 days	31.79	32.18	1.17
14 days	25.28	25.02	0.87

Feed withdrawal period had no significant effect on MFL of any of the treatment groups.

Table 4.58 Least square means for the effect of interactions between breed type and feed withdrawal period on myofibrillar fragmentation lengths (µm) of *m. longissimus* at 1, 7 and 14 days *post mortem* ($\bar{x}$ ± SEM)

Myofibrillar fragmentation length (µm)	Feed withdrawal	Breed type			SEM
		Brahman	Nguni	Simmental	
1 day	3 hours	44.75	38.37	42.72	2.49
	24 hours	44.81	40.01	44.16	
7 days	3 hours	35.34[a]	25.53[b]	33.60[a]	2.31
	24 hours	36.32[a]	26.61[b]	33.60[a]	
14 days	3 hours	27.23	22.71	25.89	1.72
	24 hours	26.35	22.77	25.94	

[a, b] = Different superscripts within the same row differ significantly *(P<0.05)*

Myofibrillar fragmentation lengths (µm) as measured from *m. longissimus* at 7 days *post mortem* was shorter *(P<0.05)* for Nguni muscle compared to Brahman and Simmental type muscle for both 3 and 24 hours feed withdrawal groups.

4.10 Correlation Tables

Significant differences were found between breeds for Warner-Bratzler shear force as well as between feed withdrawal periods. Correlations were drawn for each breed and each feed withdrawal period, between urinary catecholamine concentrations, catecholamine turnover rate, energy metabolites, calcium activated proteolytic enzymes, myofibrillar fragmentation lengths and Warner-Bratzler shear force in order to explain the factors influencing variation in Warner-Bratzler shear force for each breed and each feed withdrawal period. Only factors that showed significant correlations with Warner-Bratzler shear force were tabulated.

Table 4.59 Factors that correlate with Warner-Bratzler shear force for Brahman type cattle subjected to 3 hours feed withdrawal

Parameter	Time *post mortem* (hrs)	Shear force		
		24 hrs	7 days	14 days
pH 1 hr *post mortem*	1	0.362*	-	-
Glucose-6-phosphate (µmol/g)	6	-0.600**	-	-
Glucose (µmol/g)	1	-0.594**	-	-
	3	-0.556**	-	-
	6	-0.549**	-	-
Lactate (µmol/g)	1	-0.545**	-	-
	3	-0.466**	.	-
	6	-0.540**	-	-
ATP (µmol/g)	6	0.484**	-	0.480**
Calpastatin / µ-calpain	1	-	0.515**	-
Total calpastatin activity/total extracTable protein	24	0.433*	0.423*	0.439*
Calpastatin / (µ-calpain + m-calpain)	24	-0.383*	-	-

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 4.60 Factors that correlate with Warner-Bratzler shear force for Nguni type cattle subjected to 3 hours feed withdrawal

Parameter	Time *post mortem* (hrs)	Shear force		
		24 hrs	7 days	14 days
NORDOP		0.444*	0.542**	0.497**
Glucose (µmol/g)	6	-0.381*	-	-
Sarcomere length (µm)	72	-0.380*	-0.404*	-
Myofibrillar fragmentation length (µm)	14 days	0.420*	0.474**	0.615**

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 4.61 Factors that correlate with Warner-Bratzler shear force for Simmental type cattle subjected to 3 hrs feed withdrawal

Parameter	Time *post mortem* (hrs)	Shear force		
		24 hrs	7 days	14 days
pH	1	0.657**	-	-
	2	0.598**	-	-
Glucose-6-phosphate (µmol/g)	1	0.488**	-	-
Glucose (µmol/g)	1	-	-0.375*	-0.465**
	3	-0.404*	-0.569**	-0.508**
	6	-0.373*	-0.432*	-0.414*
L-Lactate (µmol/g)	1	-0.452*	-	-
	3	-0.485**	-	-
	6	-0.482**	-	-
	24	-0.395*	-	-
ATP (µmol/g)	24	-0.371*	-	-
Calpastatin / extracTable protein	1	0.430*	-	0.368*

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 4.62 Factors that correlate with Warner-Bratzler shear force for Brahman type cattle subjected to 24 hours feed withdrawal

Parameter	Time *post mortem* (hrs)	Shear force		
		24 hrs	7 days	14 days
pH	1	0.591**	-	-
	2	0.621**	0.380*	-
	3	0.401*	-	-
Glycogen (µmol/g)	1	0.489**	-	-
	3	0.492**	-	-
	6	0.436*	-	-
Glucose-6-phosphate (µmol/g)	6	-0.471**	-	-
Glucose (µmol/g)	1	-0.535**	-	-
	3	-0.543**	-	-
	6	-0.590**	-	-
L-Lactate (µmol/g)	1	-0.553**	-	-
	3	-0.517**	-	-
	6	-0.613**	-	-
ATP (µmol/g)	6	0.407*	-	-
Sarcomere length (µm)	**24**	-0.406*	-	-
	72	-0.400*	-	-

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 4.63 Factors that correlate with Warner-Bratzler shear force for Nguni type cattle subjected to 3 hours feed withdrawal

Parameter	Time *post mortem* (hrs)	Shear force		
		24 hrs	7 days	14 days
pH	1	0.343**	-	-
Glucose (µmol/g)	3	-0.308*	-	-
	6	-0.358**	-	-
Lactate (µmol/g)	1	-0.268*	-	-0.257*
	3	-0.285*	-	-0.301*
Myofibrillar fragmentation length (µm)	7 days	-	0.403**	0.303*
	14 day	-	0.408**	0.452**

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 4.64 Factors that correlate with Warner-Bratzler shear force for Simmental type cattle subjected to 24 hours feed withdrawal

Parameter	Time *post mortem* (hrs)	Shear force		
		24 hrs	7 days	14 days
pH	1	0.737**	0.657**	0.684**
	2	0.397*	-	-
	3	0.362*	-	-
Glycogen (µmol/g)	1	0.549**	0.504**	0.441*
	3	0.523**	0.437*	0.400*
	6	0.445*	0.390*	0.420*
Glucose-6-phosphate (µmol/g)	1	0.414*	-	-
	24	0.408*	-	-
Glucose (µmol/g)	1	-0.607**	-0.478**	-0.567**
	3	-0.745**	-0.583**	-0.582**
	6	-0.652**	-0.446*	-0.513**
Lactate (µmol/g)	1	-0.666**	-0.569**	-0.632**
	3	-0.679**	-0.542**	-0.596**
	6	-0.597**	-0.473**	-0.572**
ATP (µmol/g)	6	0.420*	-	-

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

CHAPTER 5 DISCUSSION

5.1 Warm carcass weight, cold carcass weight, dressing percentage, pH and temperature profiles between Brahman, Nguni and Simmental type cattle subject to 24 or 3 hours feed withdrawal

Average carcass weights obtained in the South African beef industry ranges between 220kg and 250 kg. Nguni has been classified and referred to as an indigenous, small frame breed by Lepen (1996), Bester *et al*. (2005) and Strydom *et al*. (2008) and in the present study Nguni carcasses were the smallest with an average cold carcass weight of 202.61 kg, followed by Simmental (270.01 kg) and Brahman (277.28 kg). Table 4.6 shows that carcass temperature was lower for Nguni compared to Brahman and Simmental type carcasses at 3, 6 and 9 hours *post mortem* as a result of Nguni carcasses being significantly smaller than Brahman and Simmental type carcasses (Table 4.2).

Between feed withdrawal periods, temperature decline was slower for FW_{24} compared to FW_3 and could be as a result of *post mortem* calorocity. *Post mortem* calorocity is a term applied in forensic science as a condition where there is actually a rise in muscle temperature due to *post mortem* glycogenolysis (Bardale, 2011). There was no difference in temperature decline between Ng_{24} and Ng_3. Glycogen was depleted to less than 30 µmol glycosyl units/g at slaughter for Ng_{24} (Table 4.16) and *post mortem* calorocity was possibly less.

The dressing percentage for Brahman was higher than for Nguni and Simmental (Table 4.2) making Brahman a favourable beef breed in terms of economic return from results in this study.

By plotting the temperature and pH relationship of all treatment groups on a graph, Figure 4.2 shows that none of the treatment groups fell within the high *rigor* temperature or cold shortening window as defined by Pearson and Young (1989). None of the treatment groups were susceptible to sarcomere shortening by this definition. The term “high *rigor* temperature carcasses” are currently used and refers to beef carcasses that undergoes rapid pH fall while muscle temperature is still high. High *rigor* temperatures will cause denaturation of proteins which causes higher drip loss, paler colour and less ageing (Warner *et al*., 2014) , which – according to definition – was not a problem in the current study.

5.2 Differences in urinary catecholamines and catecholamine turnover rates between Brahman, Nguni and Simmental type cattle subjected to 24 or 3 hours feed withdrawal

5.2.1 Differences in urinary catecholamines between Brahman, Nguni and Simmental type cattle subjected to 24 or 3 hours feed withdrawal

It was clear that there are genetic differences in catecholamine synthesis between breeds. Nguni showed significantly higher levels of urinary norepinephrine and epinephrine than Brahman and Simmental types. It is known that catecholamine synthesis and catecholamine in urine at a specific given time is genetically inherited as polymorphisms occur in the dopamine-β-hydroxylase gene (Kopeckova *et al*., 2006). Since dopamine-β-hydroxylase is responsible for conversion of dopamine into norepinephrine and subsequently epinephrine, it may explain differences in catecholamine synthesis between Brahman, Nguni and Simmental.

Practical implications of differences in catecholamine synthesis include animal domestication, animal behaviour and temperament. Catecholamines have been shown to be involved in behaviour of animal species such as foxes and rats. From classic domestication studies with silver foxes and rats, animals with a possible longer history of domestication showed higher levels of catecholamines in the central and peripheral nervous system and were more tame (Nikulina, 1990; Price, 1999). In human studies, attention deficit hyperactivity disorder and over reactivity has been described as a result of catecholamine shortages, with subsequent lower blood flow to the brain coupled with lower gluconeogenesis (Todd & Botteron, 2001). Human subjects with this disorder were unable to cope with challenging environments. Clearly catecholamines are responsible for providing the brain with sufficient glucose in order to cope with environmental stimuli. Lacourt & Tarrant (1985) explained that when animals experience acute stress through transport and handling, a quick release of catecholamines will lead to changes in carbohydrate metabolism in order to provide glucose for important functions. Two central integrated processes namely the autonomic nervous system and hypothalamic-pituitary-adrenal axis are activated as neuroendocrinal responses to fear-eliciting stimuli. Acute stressors, such as human contact, activate an autonomic response in order to initiate reaction that requires a rapid response. By activating the autonomic nervous system, tachycardia, increased respiration rate, elevated body temperature and redistribution of visceral blood volume towards skeletal muscle and the brain are some of the physiological changes that incur.

It has been shown that *Bos indicus* breed types (such as Brahman) were more temperamental than *Bos taurus* types by Voisinet *et al.* (1997). Hammond (1996) from Turner (1980) explained that several traits of Brahman have a negative impact on their acceptability to some cattle of which temperament is one. The involvement of catecholamines in cattle temperament should be considered as Nguni and Simmental showed higher levels of urinary catecholamines than the typically temperamental Brahman.

Simmental showed significantly higher dopamine levels compared to Nguni and Brahman types (Table 4.12), but only as long as feed was not restricted i.e. 3 hours feed withdrawal. It is known that aversive conditions (such as transport and handling at an abattoir) increase urinary dopamine as described for other species by Abercrombie *et al*. (2006). Dopamine has been shown to be involved in gluconeogenesis (Matsumura *et al*., 1980) in order to make glucose available to an animal to cope with its environment. According to Parkinson *et al*. (2002) dopamine is an important neurotransmitter which is involved in an animal's attraction to a stimulus that predicts reward. This adaptive value helps to guide an animal towards objects in the environment that will provide food and warmth and away from dangerous objects or environments. Simmental (as long as feed is not restricted) may be more adaptive in the way that it seeks rewarding stimuli.

Feed withdrawal period had no effect on norepinephrine or epinephrine but dopamine was significantly less when feed was withdrawn for 24 hours (Table 4.11). Schaefer *et al*. (2001) explained that exposure to longer feed withdrawal increases the utilization and synthesis of dopamine as the availability of tyrosine for catecholamine synthesis becomes rate-limiting. Schaefer *et al*. (2001) connects tyrosine availability for catecholamine synthesis with restlessness in cattle subjected to longer feed withdrawal periods.

Although norepinephrine and epinephrine did not differ significantly between FW_{24} and FW_3 within each breed type (Table 4.12), norepinephrine and epinephrine for Sm_{24} was on average lower *(P>0.05)* than for Sm_3, which is in contrast with Br_{24} and Ng_{24} where norepinephrine was higher for Br_3 and Ng_3. This may implicate Simmental's sensitivity towards feed withdrawal.

5.2.3 Differences in catecholamine turnover rates between Brahman, Nguni and Simmental type cattle subjected to 24 or 3 hours feed withdrawal

Longer feed withdrawal with consequent depletion of available tyrosine could possible explain lower observed dopamine values but had no effect on norepinephrine or epinephrine. There are several metabolic end products secreted in urine from tyrosine metabolism into norepinephrine

and epinephrine production (Eisenhofer, 2001; Eisenhofer *et al*., 2004). These metabolic end products indicate amine turnover. No metabolic end products for catecholamine metabolism were measured in this study. For this reason, the rate of catecholamine turnover was used as a parameter to indicate a possible response to feed withdrawal between breeds. As norepinephrine and epinephrine are "metabolites" of dopamine (Eisenhofer *et al*., 2004) NORDOP and EPIDOP were calculated and analysed statistically in an attempt to determine catecholamine turnover rates. While longer feed withdrawal resulted in an increase in the turnover rate of catecholamines for Brahman and (not significantly) Nguni it had no effect on the turnover rate of catecholamine for Simmental. Catecholamines are involved in changes in energy metabolism including lipolysis, glycogenolysis in muscle and gluconeogenesis in the live animals (Ferguson & Warner, 2008). As the average urinary NORDOP and EPIDOP at slaughter did not differ between Sm_{24} and Sm_{3}, it seems as if longer feed withdrawal did not change the rate of energy metabolism of Simmental in the live animal. This may be possible for Simmental as some mammals have the ability to save their energy during periods of fasting (Malatesta *et al*., 2007).

5.3 Differences in *post mortem* energy metabolites from *m. longissimus* between Brahman, Nguni and Simmental type cattle subjected to 24 or 3 hours feed withdrawal

5.3.1 Glycogen:

Breed type had significant effects on glycogen at slaughter due to differences in muscle fiber types between breeds as seen from Table 4.40. (Judge *et al*., 1989; From: Immonen & Puolanne, 2000) confirmed that oxidative muscle fiber types contain less glycogen. Nguni showed lower glycogen at all *post mortem* times compared to Brahman and Simmental breed types (Table 4.14). This stands well in comparison with results obtained for muscle fiber typing where Nguni contained on average more Type I fibers compared to Brahman and significantly more Type I fibers compared to Simmental breed types (Table 4.40).

Numerous researches have shown the involvement of glycogen at slaughter with pH decrease. pH of muscle that contains less glycogen declines at a slower rate or remains high (above pH 6.0) (Bate-Smith & Bendall, 1949; Lawrie, 1953; Howard & Lawrie, 1956; Tarrant *et al*., 1972; Hamm 1974). Similar effects for the involvement of glycogen in pH decrease were found for Nguni.

In combination with lower glycogen, Nguni showed significantly but not meaningful higher pH values compared to Brahman at 9 hours *post mortem* and compared to Brahman and Simmental at 24 hours *post mortem* (Table 4.6).

As expected, longer feed withdrawal had significant effects on glycogen at slaughter as hunger triggers the glycogenolytic action of norepinephrine and epinephrine (Voet & Voet, 1990). For Br_{24} and Ng_{24}, glycogen at slaughter (1 hr *post mortem*) was less compared to Br_3 and Ng_3 (Table 4.16). It seems therefore as if the rate of *ante mortem* glycogenolysis was higher for Br_{24} and Ng_{24} compared to Br_3 and Ng_3. For Br_{24} and Ng_{24} pH remained higher at 3, 6 and 9 hours *post mortem* compared to Br_3 and Ng_3 (Table 4.8) as muscle with less glycogen undergo less acidification. For Br_{24} and Ng_{24}, hunger, as a result of longer feed withdrawal in combination with higher catecholamine turnover was associated with lower glycogen which resulted in less muscle acidification. This effect of longer feed withdrawal on catecholamine turnover rate, glycogen depletion and pH was not seen with Sm_{24} when compared to Sm_3. In sharp contrast with Brahman and Nguni, glycogen was higher *(P>0.05)* at 1 and 24 hours *post mortem* for Sm_{24} compared to Sm_3. This could have been the result of an energy sparing effect for Simmental after periods of fasting as explained by Malatesta *et al*. (2007).

Six hours *post mortem* was the turning point for the incline or decline of metabolite concentrations as well as the rate at which metabolites incline or decline. The rate of glycogenolysis between 6 and 24 hours slowed down for all treatment groups. This could be due to inactivation of the enzymes that are responsible for glycogenolysis that is found to be temperature and pH sensitive, (Ylä-Ajos, 2006). As these enzymes became deactivated, glycogenolysis continued at a slower rate and the rate of glycolysis slowed down.

Sm_{24} showed more *(P>0.05)* glycogen than Sm_3 at slaughter and the rate of glycogenolysis and glycogen decline was higher for Sm_{24} up to 3 hour pm compared to Sm_3. The rate of *post mortem* glycogenolysis were higher for Sm_{24} compared to Sm_3 as a possible result of higher available substrate (glycogen) concentration.

5.3.2 Glucose-6-phosphate:

Glucose-6-phosphate is an indicator of the extent of glycogenolysis. Between breeds Simmental showed higher *(P<0.05)* glucose-6-phosphate than Brahman at 1 hour *post mortem* and Brahman showed higher *(P<0.05)* glucose-6-phosphate than Nguni at 24 hours *post mortem* (Table 4.17). This means that the overall rate of glycogenolysis for Simmental was higher from the *ante*

mortem period up until 1 hour *post mortem* compared to Brahman. Overall the rate of glycogenolysis was higher for Brahman before 24 hours *post mortem* compared to Nguni.

Feed withdrawal period had no effect, but in combination with breed type had significant effects on glucose-6-phosphate. At 1 hour *post mortem* glucose-6-phosphate was higher for Sm_3 compared to Br_3 and Ng_3. To recollect, Sm_3 showed significantly higher dopamine than Br_3 and Ng_3 (Table 4.12). Dopamine is involved in an animal's attraction to a stimulus that predicts reward Parkinson *et al*. (2002) and dopamine is also involved in gluconeogenesis (Matsumura *et al*., 1980). Gluconeogenesis makes glucose available to enable an animal to cope physically to its challenging environment. Simmental type cattle may be more adapted to cope with physically challenging environments. As dopamine concentration increases during aversive conditions (Abercrombie *et al*., 2006), high dopamine (Table 4.12) in combination with high glucose-6-phosphate at 1 hour *post mortem* (Table 4.19) indicated that Simmental possibly reacted to transport and handling in order to increase *ante mortem* gluconeogenesis (for as long as feed was not restricted).

As soon as feed was restricted for 24 hours, higher glycogen *($P>0.05$)* for Sm_{24} compared to Sm_3 at 1 hour *post mortem* was associated with lower *($P<0.05$)* glucose-6-phosphate for Sm_{24} compared to Sm_3 at 1 hour *post mortem* (Table 4.19). This observation should be highlighted as it is in contrast with what was expected from longer feed withdrawal. The reader is reminded that the catecholamine turnover rate did not differ between Sm_{24} and Sm_3. High glycogen and low glucose-6-phosphate as well as no difference in catecholamine turnover for Simmental subjected to 24 hours feed withdrawal indicates a lowering in metabolic rate for this treatment group.

Glucose-6-phosphate was significantly less for Ng_{24} than for Br_{24} and Sm_{24} at 24 hours *post mortem* as there was initially less available glycogen due to genetic muscle fiber type differences.

Between 1 and 6 hours *post mortem*, there was a decline in glucose-6-phosphate levels for all treatment groups. After slaughter oxidative phosphorylase comes to an end. In order to maintain cellular homeostasis and as a result of ATP depletion, glycolysis is activated (Scheffler *et al*., 2011). Glucose-6-phosphate declined as it served as a form of easy metabolisable energy substrate that was "pulled into" the glycolytic pathway to produce energy (either as ATP or to produce L-lactate).

5.3.3 Glucose:

Scheffler *et al*. (2011) explained that glycogenolysis continues *post mortem*, with a subsequent increase in glucose levels. Between breeds, Nguni produced less glucose (*$P<0.05$*) than Brahman and Simmental after completion of *post mortem* glycogenolysis (Table 4.20) as there was

less available glycogen initially (Table 4.14). Between feed withdrawal groups FW_{24} showed lower *(P<0.05)* glucose than FW_3 at 1, 3, 6 and 24 hours *post mortem* (Table 4.21) as glycogen at slaughter was also lower for FW_{24} compared to FW_3 (Table 4.15) as a result of longer feed withdrawal.

Feed withdrawal period in combination with breed type had significant effects on glucose *post mortem*. Ng_{24} showed less glucose at 24 hours *post mortem* compared to Br_{24} and Sm_{24} (Table 4.22) because of the combined effects of low initial glycogen and / or possible lower glycogen debranching enzyme and phosphorylase activity as described for oxidative fibers (Ylä-Ajos, 2006). Glucose was lower at 1, 3 and 6 hours *post mortem* for Ng_{24} than for Ng_3, (Table 4.22) as was initial glycogen levels at for Ng_{24}. Br_{24} showed less glucose than Br_3 at 24 hours *post mortem* as glycogen levels were also lower at slaughter for Br_{24}. Glucose levels did not differ significantly between Sm_{24} and Sm_3 at any of the *post mortem* time intervals (Table 4.22) however from Figure 4.3, appeared to follow the same trend as Br_{24} vs. Br_3 and Ng_{24} vs. Ng_3 *post mortem.*

The rate of glucose metabolism and increased glucose levels for all breed types was steady between 1 and 6 hours *post mortem* but increased at an accelerated rate between 6 and 24 hours *post mortem* (Figure 4.5). Literature states that as pH drops below 6 between 6 and 9 hours *post mortem* glycolytic enzymes (phosphofructokinase in particular Fidelman *et al*. (1982) From: Jerrett & Holland (1998)) become deactivated. Glucose was still produced through glycogenolysis, but not "consumed" further through glycolysis.

5.3.4 L-lactate:

As long as all enzymes are still active and there is no shortage of energy substrates, L-lactate levels in *post mortem* muscle will increase (Scheffler *et al*., 20011). There were no differences between breeds for L-lactate levels at any of the *post mortem* intervals (Table 4.23). FW_{24} showed lower *(P<0.05)* L-lactate levels at all the time intervals, which is self-explainable as the rate of glycolysis is a function of available glycogen (Vetharaniam *et al*., 2010)]. As soon as glycogen was used as an energy source *ante mortem*, less L-lactate was synthesised in the *post mortem* period and was evident from these results.

For feed withdrawal in combination with breed type, as glycogen levels at slaughter was lower for Br_{24} compared to Br_3 (Table 4.16) L-lactate showed a tendency to be lower for Br_{24} compared to Br_3 (Table 4.25). As glycogen was close to depletion for Ng_{24}, L-lactate was significantly less for Ng_{24} compared to Ng_3.

L-lactate accumulated at an increased rate for all breeds up until 6 hours *post mortem* after which it accumulated at a decreased rate (Figure 4.6). This could be due to the deactivation of some pH sensitive enzymes such as phosphofructokinase (Fidelman *et al*., 1982 From: Jerrett & Holland, 1998) and subsequent slower glycolytic rate.

5.3.5 Creatine phosphate:

Between breeds, creatine phosphate was higher *(P>0.05)* for Nguni compared to Brahman and Simmental breed types which indicated that *post mortem* glycolysis was slower for Nguni which correspond with the results from Frylinck *et al*. (2013). There were no differences in creatine phosphate between feed withdrawal treatments (Table 4.27).

For feed withdrawal period in combination with breed type there were no significant differences between any of the treatment groups at 1, 3 or 6 hours *post mortem*. Nguni showed higher *(P<0.05)* creatine phosphate than Brahman and Simmental breed types for both FW_{24} and FW_3 at 24 hours *post mortem* and these observations was due to slower rates of glycolysis for Nguni treatment groups (Table 4.28).

The rate of creatine phosphate decline was faster *(P<0.05)* for Sm_{24} compared to Sm_3 between 1 and 6 hours *post mortem* (Figure 4.7). This shows that carbohydrate metabolism was higher for Sm_{24} compared to Sm_3 as was also seen for glycogen between Sm_{24} and Sm_3 (but not the rate of complete glycolysis from L-lactate data). Depletion of creatine phosphate indicates the onset of *rigor mortis* as described and reviewed by Savell *et al*. (2005). The rate of creatine phosphate depletion was higher for Sm_{24} before 6 hours *post mortem*. Although the rate of creatine phosphate depletion was the same for all treatment groups after 6 hours *post mortem*, it seems as if Sm_{24} experiences an earlier onset of *rigor* before 6 hours *post mortem* and while the carcass was still relatively warm for this treatment group.

5.3.6 ATP:

ATP is replenished by the breakdown of creatine phosphate and degradation of carbohydrates via anaerobic pathways (Bendall, 1973).The initial splitting of ATP to ADP plus inorganic phosphate (Pi) and H^+ (during the first biochemical steps of glycolysis), determines the rate and magnitude of carbohydrate catabolism. Without this reaction glycolysis and acidification can come to a halt very rapidly (Bendall, 1973). Nguni showed more ATP than Brahman and Simmental at all the time

intervals (Table 4.33). Firstly, oxidative or slow contracting fibers have the ability to generate more ATP in the mitochondria aerobically (Pösö & Puolanne, 2005) and therefore Nguni showed higher ATP early *post mortem*. Similarly, as discussed with creatine phosphate, *post mortem* glycolysis was slower for Nguni [as was also found by Frylinck *et al*. (2013)] and therefore ATP remained higher for Nguni.

Feed withdrawal period had no effect on ATP (Table 4.30). In combination with breed type, feed withdrawal period had significant effects on ATP for Nguni only where Ng_{24} showed more ATP at 6 hours *post mortem* compared to Ng_3 (Table 4.31).

There were no significant differences in the rate of ATP metabolism between all the treatment groups (Figure 4.8). Although the rate of creatine phosphate metabolism was higher for Sm_{24} compared Sm_3 there was no significant difference in the rate of ATP metabolism between Sm_{24} and Sm_3 (Figure 4.8). There were no observations between 6 and 24 hours *post mortem*. More frequent observations in this time window could have shown differences in the rate of ATP hydrolysis (as carcasses cooled down more rapidly) between treatment groups.

5.3.7 Glycolytic potential:

Wulf *et al*. (2002) stated that higher glycolytic potential is associated with increased tenderness - low glycolytic potential (less than ~100 µmol/g muscle) at slaughter is associated higher shear force. In the present study, glycolytic potential of Ng_{24} was less than ~100 µmol/g muscle; however, Warner-Bratzler shear force was not higher for this treatment group. This indicated that other meat quality factors play important roles in ensuring meat tenderness for the Nguni breed type. As glycolytic potential is a factor of glycogen, glucose-6-phophate and L-lactate (2x [(glycogen) + (glucose-6-phosphate)] + (L-lactate) (Monin & Sellier, 1985) it explains why glycolytic potential of Ng_{24} was lower *($P<0.05$)* than Br_{24} at 1, 3 and 6 hours *post mortem*, lower *($P<0.05$)* than Sm_{24} at 1 hour *post mortem* and lower *($P<0.05$)* than Ng_3 at 1, 3 and 6 hours *post mortem*, as glycogen was also lower. Sm_{24} differed from the other breed treatments where glycolytic potential was lower *($P<0.05$)* than Sm_3 at 6 hours *post mortem* which indicated that glycogen and glucose-6-phosphate was less for Sm_{24} at 6 hours *post mortem* compared to Sm_3.

From the following observations, the rate of *post mortem* glycogenolysis but not glycolysis was faster for Sm_{24} compared to other treatment groups: Glycogen declined faster for Sm_{24}; neither pH drop nor L-lactate concentrations for Sm_{24} differed significantly between 1 and 6 hours *post mortem*; the rate of glycolytic potential decline was faster between 1 and 6 hours for Sm_{24}. As

glycolytic potential is a factor of 2x [(glycogen) + (glucose-6-phosphate)] + (L-lactate) (Monin & Sellier, 1985) it indicated that glycogenolysis, but not complete glycolysis, was higher for Sm_{24} between 1 and 6 hours compared to all other treatment groups. Glycogenolysis in skeletal muscle is regulated by enzymatic activity of glycogen phosphorylase. Activation of this enzyme is triggered by muscle contraction and Ca^{2+} in the cytosol (Tarrant, 1989).

5.4 The toughening phase of the conversion of muscle to meat

5.4.1 Differences in sarcomere length (µm) from *m. longissimus* between Brahman, Nguni and Simmental type cattle subjected to 24 or 3 hours feed withdrawal

None of the treatment groups fell within the cold shortening window or high *rigor* temperature window as by definition, which emphasises the importance of temperature control in an abattoir. Nguni breed type showed shorter *(P<0.05)* sarcomere length than Brahman at 1 and 3 days *post mortem* (Table 4.48). Although temperature and pH relationships at the moment of onset of *rigor* can be considered a decisive factor in the degree of cold shortening (Hannula & Puolanne, 2004), none of the treatment groups fell within the "cold shortening zone" (Figure 4.2) as described by Pearson and Young (1989). Nevertheless, Nguni carcasses in this study were smaller and chilled faster compared to Brahman and Simmental and for this reason some degree of cold shortening was expected as described for smaller carcasses (Savell *et al.*, 2005). Low muscle temperature before the onset phase of *rigor* renders the sarcoplasmic reticulum unable to sequester Ca^{2+} back into the sarcoplasmic reticulum. Furthermore, as Nguni contained more oxidative fibers than Brahman and Simmental, sarcomere shortening was also expected due to the slower rate of Ca^{2+} sequestration described for oxidative fibers (Hopkins, 2006). Shorter sarcomere length for Nguni breed type could also be due to genetic differences.

Longer feed withdrawal period in combination with breed type had a significant negative impact on sarcomere length for Sm_{24}. Although Sm_{24} was far from the warm *rigor* temperature danger zone, the rate of creatine phosphate depletion was higher (Figure 4.7), and glycogenolysis, but not glycolysis was faster for Sm_{24} compared to $Sm_{3.}$ within 6 hours *post mortem*. Clearly muscle from Sm_{24} contracted and underwent the process of *rigor* before 6 hours *post mortem*. Thus, *rigor* set

in earlier for this treatment group at ~ 1 to 6 hours *post mortem* and carcass temperature of 38.57 to 19.53 ˚C.

5.5 The tenderising phase of the conversion of muscle to meat

5.5.1 Differences in myofibrillar fragmentation length from *m. longissimus* between Brahman, Nguni and Simmental type cattle subjected to 24 or 3 hours feed withdrawal

Breed type had a significant effect on myofibrillar fragmentation length whereas feed withdrawal period had no significant effect on myofibrillar fragmentation length which indicated that breed type was the determining factor in proteolytic enzyme activity in *m. longissimus* of Brahman, Nguni and Simmental breed types. Ng_{24} and Ng_3 showed the shortest myofibrillar fragmentation lengths (Table 4.58) at 7 days *post mortem*. According to Frylinck *et al*. (2009) shorter myofibrillar fragmentation lengths indicates higher calpain proteinase action that contributes to more tender meat. Koohmaraie & Geesink (2006) explained that the proteolytic degradation of cytoskeletal proteins such a nebulin, titin and vinculin, during *post mortem* storage are important aspects of meat tenderness. Therefore, as myofibrillar fragmentation length is highly related to meat tenderness (Koohmaraie, 1994) more tender meat could be predicted for Nguni irrespective of its shorter sarcomere length.

5.5.2 Differences in the calpain and calpastatin systems from *m. longissimus* between Brahman, Nguni and Simmental type cattle subjected to 24 or 3 hours feed withdrawal

Breed type had significant effects on the calpain-calpastatin system (Brahman showed lower *($P<0.05$)* µ-calpain activity at 1 and 24 hours *post mortem* than Nguni and Simmental; higher *($P<0.05$)* calpastatin/µ-calpain at 1 and 24 hours *post mortem* than Nguni and Simmental and higher *($P<0.05$)* calpastatin/(µ-calpain + m-calpain) at 1 hour *post mortem* than Nguni and Simmental. Brahman showed higher *($P<0.05$)* calpastatin/(µ-calpain + m-calpain) at 24 hours *post mortem* than Nguni (Table 4.53)) but feed withdrawal period had no effect (Table 4.54). Similar to results from myofibrillar fragmentation length, breed type was the determining factor in *post mortem* proteolysis.

Irrespective of feed withdrawal period, Brahman measured the highest ratio of calpastatin to µ-calpain as well as the lowest µ-calpain activity compared to Simmental and Nguni. This corresponds with the typically less favourable calpain system characteristics of *Bos indicus* breed types explained by Koohmaraie *et al*. (1995) and Shackelford *et al*. (1994).

5.6 Physical properties of meat

5.6.1 Differences in meat colour, muscle fiber typing and water binding capacity from *m. longissimus* between Brahman, Nguni and Simmental type cattle subjected to 24 or 3 hours feed withdrawal

Breed type had significant effects on muscle fiber types (Table 4.40) and colour coordinates (Table 4.36) of fresh meat. Breed type had no effect water binding capacity (Table 4.36). Nguni meat was more red and darker in colour compared to Brahman and Simmental (Table 4.36) and contained on average more *(P>0.05)* oxidative fibers than Brahman and significantly more oxidative fibers than Simmental (Table 4.40).

Feed withdrawal had significant effects on L* where FW_{24} showed darker meat than FW_3 (Table 4.37). Darker meat in association with high pH values (Table 4.7) and lower glycogen levels (Table 4.19) for FW_{24} compared to FW_3 corresponds with the findings of Purchas *et al*. (1999) where *ante mortem* glycogen depletion in muscle resulted in meat with higher pHu values. Generally beef with higher pHu values is undesirable because of its dark colour (Bartoš *et al*., 1993; Mounier *et al*., 2006).

Feed withdrawal of 3 hours in combination with breed type had no significant effects on L*, a* or b*. Ng_3 showed higher *(P<0.05)* HA compared to Br_3 but no differences were found for Ri between any of the treatments groups (although Ri was on average higher for Ng_{24} and Ng_3 compared to Br_{24}, Br_3, Sm_{24} and Sm_3). Differences in colour coordinates was observed between breed types as soon as feed was withdrawn for 24 hours (Ng_{24} showed lower L* *(P<0.05)* compared to Br_{24} and Sm_{24}; lower *(P<0.05)* a* compared to Br_{24}; higher *(P<0.05)* b* compared to Br_{24} and Sm_{24}; lower *(P<0.05)* Si compared to Br_{24} and Sm_{24}; lower *(P<0.05)* Si compared to Br_{24} and higher *(P<0.05)* HA compared to Br_{24}). This emphasises the large impact of glycogen depletion in oxidative muscle fibers on meat colour and indicated that feed withdrawal had greater effects on meat colour than breed type. Similarly, Ng_{24} showed lower *(P<0.05)* L* as well as less available glycogen

compared to Ng_3 and indicated that there was less glycolysis in the glycogen depleted Nguni muscle as described by (Immonen & Puolanne, 2000). Meat colour results corresponds with lower glycolytic potential *(P<0.05)* for Ng_{24} than Br_{24} at 1, 3 and 6 hours *post mortem* and lower glycolytic potential *(P<0.05)* for Ng_{24} than Sm_{24} at 1 hour *post mortem* (Table 4.34). The glycolytic potential of Ng_{24} was lower *(P<0.05)* than Ng_3 at 1, 3 and 6 hours *post mortem* as was also found by Frylinck *et al*. (2013) for this breed type. Feed withdrawal period in combination with breed type had no effect on water binding capacity.

5.6.2 Differences in and correlations with Warner-Bratzler shear force from *m. longissimus* between Brahman, Nguni and Simmental type cattle subjected to 24 or 3 hours feed withdrawal

Breed type had significant effects on Warner-Bratzler shear force where Simmental showed higher *(P<0.05)* Warner-Bratzler shear force values compared to Brahman and Nguni at 1 and 14 days *post mortem* (Table 4.48). This is in direct contrast with previous research where meat from *Bos taurus* cattle was more tender than meat from *Bos indicus* cattle (Shackelford *et al*., 1991; Pringle *et al*., 1997; Voisinet *et al*., 1997 and Wheeler *et al*., 2010)

Feed withdrawal period had significant effects on Warner-Bratzler shear force where FW_{24} showed higher Warner-Bratzler shear force than FW_3. This corresponds with the results from sarcomere length where FW_{24} showed shorter sarcomere length than FW_3 and emphasises the importance of glycogen depletion in muscle in terms of the conversion of muscle to meat. Glycogen depletion results in meat with high variation in tenderness (Silva *et al*., 1999). Researchers such as Hartschuh *et al*. (2002) reported that glycolytic potential is sensitive to *ante mortem* nutrition, as nutrition alters glycogen in the muscle.

For breed type in combination with feed withdrawal, there were no significant differences in Warner-Bratzler shear force between breeds with FW_3 at any of the *post mortem* time intervals. This emphasises the importance of sufficient available energy in muscle for optimum meat tenderness. Warner-Bratzler shear force for Sm_{24} was higher (*P<0.05*) compared to Br_{24} and Ng_{24} at 1 and 7 days *post mortem*. This shows that some breeds are more negatively impacted by longer feed withdrawal than others.

There were no correlations between catecholamines and catecholamine conversion rates with myofibrillar length or the proteases. Catecholamine had no effect on *post mortem* tenderisation of meat in the present study.

Although pH did not differ meaningfully between treatment groups, it correlated with Warner-Bratzler shear force within each group and was therefore predicting factor in the variation of Warner-Bratzler shear force. High pH at 1 and / or 2 hours correlated with high Warner-Bratzler shear force for all treatments groups emphasizing the importance of *post mortem* acidification which is prerequisite to turn muscle into high quality meat. Decades ago Howard & Lawrie (1956) suggested that the rate of pH decline has an inverse relationship to tenderness.

For all treatment groups high L-lactate and glucose correlated with low Warner-Bratzler shear force. Although L-lactate synthesis retards *post mortem* muscle acidification (Bendall, 1973; Honikel & Hamm, 1974; Robergs *et al*., 2004; Scheffler *et al*., 2011), it proves to be an indicator of the extent of fibre energy metabolism that induces increased H^+ release and subsequent acidification. L-Lactate maintains the rate of anaerobic glycolysis by maintaining the redox equilibrium between NAD^+ and NADH (Nelson & Cox, 2005). High glucose at 3 and / or 6 hours correlated with low Warner-Bratzler shear force for all treatment groups. Glucose is an indicator of glycogenolysis and may be an activator for apoptosis and necrosis (Donnini *et al*., 1996). Non-enzymatic degradation of cytoskeletal proteins specifically necrosis, apoptosis and the caspases, as described by Ouali *et al*. (2006), may be glucose dependent.

Correlations from Table 4.59:

The relationship between Warner-Bratzler shear force and *Bos indicus* inheritance in this study is contradictory. In the study of Voisinet *et al*. (1997), using breeds with similar marbling scores, 3/8 *Bos indicus* composite beef cattle measured higher 24 hour calpastatin activity compared to *Bos taurus*. In a number of studies beef tenderness (at least in *m. longissimus*) decreases as percentage *Bos indicus* inheritance increases (Shackelford *et al*., 1991b; Pringle *et al*., 1997; Shackelford *et al*., 1995; Strydom *et al*., 2000). In this study there were no significant differences for Warner-Bratzler shear force between breed types when feed was not restricted for longer than 3 hours. For Br_3, Warner-Bratzler shear force correlated most with calpastatin. Similarly, 3/8 *Bos indicus* composite beef cattle measured higher 24 hours calpastatin activity compared with the *Bos taurus* breeds and aged slower in the study by Whipple *et al*. (1990). The effect of breed type on reduced ageing potential of *Bos indicus* beef was brought about by increased calcium dependent protease inhibitor, calpastatin activity (O'Connor *et al*., 1997). From results in this study it is clear that most variation in Warner-Bratzler shear force for Br_3 between individuals within this treatment group could be explained by calpastatin activity. In living muscle, elevation of calpastatin suppresses

µ-calpain (and perhaps m-calpain), which in turn reduces the rate of protein degradation resulting in an increase in muscle growth (Koohmaraie *et al*., 2002). Although there were no significant breed differences for calpastatin, Brahman showed the highest ratio of calpastatin to µ-calpain as well as calpastatin to µ- + m-calpain (Table 4.53). Despite Brahman showing the highest ratio of calpastatin to µ-calpain as well as calpastatin to µ- + m-calpain there were two reasons for Brahman not having higher Warner-Bratzler shear force compared to Nguni and Simmental and are listed below:

The glycolytic potential for Brahman was higher and thus followed a more positive *post mortem* glycolysis profile (Table 4.34) in order to ensure tender beef.

With regards to sarcomere length, Marsh & Leet (1966) stated that *post mortem* muscle shortening of less than 20% in sarcomere length produced almost negligible effects on beef tenderness. If resting sarcomere length of longissimus muscle is taken as 2.1 µm (Marsh & Carse, 1974), then Brahman sarcomere lengths fall within the negligible range. (Table 4.50) which indicated that Brahman was not susceptible factors that cause sarcomere shortening.

High glucose-6-phosphate at 6 hours *post mortem* indicated that glycogenolysis was not inhibited by other factors and carried on until 6 hours and correlated with low Warner-Bratzler shear force at day 1.

Correlations from Table 4.60:

Sarcomere length for Ng_3 fell within the 20% shortening range stated by Marsh and Leet (1966) in order to have a noticeable effect on beef tenderness (Table 4.50). Variation in tenderness between individuals for Ng_3 could be due to shorter sarcomere lengths

For Ng_3 high MFL at 14 days *post mortem* correlated with high Warner-Bratzler shear force. This indicated that for the present study, after 14 days *post mortem*, a significant amount of variation in Warner-Bratzler shear force between individuals within this treatment group was due to proteolysis.

Sarcomere lengths as well as MFL were two factors that caused variation within Ng_3. Despite the fact the sarcomere length for Ng_3 was shorter compared to Br_3 and Sm_3, Warner-Bratzler shear force for $Ng_{3\ did}$ not differ compared to Br_3 and Sm_3. This indicates that proteolytic enzyme activity played an important role in tenderising meat from Ng_3.

Correlations from Table 4.61:

Sm_3 showed the highest glucose-6-phosphate at 1 hour for all breeds as well as the highest (*P<0.05*) dopamine compared to all treatment groups (Table 4.13). Glucose-6-phosphate is an indicator of the extent of glycogenolysis and dopamine is actively involved in glycogenolysis during stressful and threatening situations (Todd & Botteron, 2001). From the *ante mortem* period up until 1 hour *post mortem*, more glycogenolysis took place in the muscle of Sm_3 compared to Br_3 and Ng_3, possibly due to higher dopamine levels. High glucose-6-phosphate at 1 hour correlated significantly with high Warner-Bratzler shear force. Warner-Bratzler shear force for Sm_3 did not differ significantly compared to Br_3 and Sm_3 but variation in Warner-Bratzler shear force within this treatment group was attributed to high glucose-6-phosphate at 1 hour *post mortem*. A possible explanation for this is that high concentrations of glucose-6-phosphate at 1 hour possibly inhibited the active form of phosphorylase b which inhibited further glycogenolysis and in turn glycolysis as described by Ylä-Ajos (2006).

High ATP at 24 hours correlated with low Warner-Bratzler shear force. A slower rate of anaerobic ATP hydrolysis between 6 and 24 hours with more ATP towards the end of *rigor mortis*, indicated less muscle contraction (Gordon *et al*., 2000; Vetharaniam *et al*., 2010) and therefor lower Warner-Bratzler shear force.

Calpastatin is the endogenous specific inhibitor of µ-calpain (Maki *et al*., 1988). High calpastatin correlated with high Warner-Bratzler shear force for Sm_3, showing that some variations in Warner-Bratzler shear force for Sm_3 could be explained by the inhibition of calpain proteolysis by calpastatin.

Correlations from Table 4.62:

Br_{24} showed the highest NORDOP between all treatment groups (Table 4.12) indicating that there was a higher catecholamine turnover from dopamine to norepinephrine. Br_{24} showed lower glycogen (*P<0.05*) at 1 hour *post mortem* compared to Br_3. As high NORDOP was associated with lower glycogen it seems as if glycogenolysis was higher for this treatment group from the *ante mortem* period up until 1 hour *post mortem* compared to Br_3. Glycogen at slaughter differed most between Br_{24} and Br_3 when compared to the other breed types. Longer feed withdrawal affected the rate of glycogenolysis from the *ante mortem* period up until 1 hour *post mortem* more intensively for

Brahman, but as Brahman contained enough glycogen in *post mortem* muscle, Warner-Bratzler shear force was not affected negatively.

High glycogen at 1, 3 and 6 hours for Br_{24} correlated with high Warner-Bratzler shear force. A slower rate of glycogenolysis (and therefore glycogen remained high) resulted in higher Warner-Bratzler shear force. Glucose-6-phosphate is an allosteric inhibitor of the active form of phosphorylase (Campbell, 1995, Ylä-Ajos, 2006), slowing down the glycogenolytic rate *post mortem*. From Figure 4.3, the rate of glycogen decline was slower for Br_{24} compared to Br_3.

Glucose-6-phosphate at 6 hours indicated the extent of glycogenolysis up until 6 hrs *post mortem*. As high glucose-6-phosphate at 6 hours correlated with low Warner-Bratzler shear force, the importance of a higher rate or more complete glycogenolysis before 6 hours is clear.

Higher ATP at 6 hours *post mortem* is an indication of the rate ATP hydrolysis during the initial phases of *rigor*. ATP decline must preferably be fast at 1 to 6 hrs *post mortem*. ATP at 6 hrs *post mortem*, leaves an abundance of ATP for *rigor mortis* to occur during the final phase *post mortem* and therefore correlated with higher Warner-Bratzler shear force.

Correlations from Table 4.63:

Shorter myofibrillar fragmentation lengths correlated with lower Warner-Bratzler shear force for Ng_{24}. As myofibrillar fragmentation length is highly related to meat tenderness (Koohmaraie, 1994), variation in tenderness for Ng_{24} could be explained by enzymatic degradation.

Although Nguni showed the smallest carcass and shortest sarcomere length its Warner-Bratzler shear force was intermediate between breeds used in this study. Ng_{24} showed lower *($P<0.05$)* Warner-Bratzler shear force compared to Sm_{24} at 14 days *post mortem* (Table 4.46) but both breed types showed additional sarcomere length shortening (Table 4.50). The reason why Warner-Bratzler shear force was lower for Ng_{24} compared to Sm_{24} could be explained by its more positive (shorter) myofibrillar fragmentation lengths, positive proteolytic enzyme profile and thinner fiber diameter. While some researchers found that sarcomere length is correlated with Warner-Bratzler shear force (Bouton *et al*., 1973), others believe that fiber diameter is more indicative of Warner-Bratzler shear force (Chikri *et al*., 2012). As Nguni in this study contained more oxidative fibers compared to Simmental and the diameter of oxidative fibers are smaller this could also explain the reason why Warner-Bratzler shear force for Ng_{24} was lower compared to Sm_{24} at 14 days *post mortem*.

Correlations from Table 4.64

High L-lactate at 1, 3 and 6 hours correlated significantly with low Warner-Bratzler shear force for Sm_{24}. As Warner-Bratzler shear force for Sm_{24} was the highest between all treatment groups, complete glycolysis up until the production of L-lactate, (a measure of muscle acidification) were the most important factors to cause variation in Warner-Bratzler shear force. Similarly high glucose at 1, 3 and 6 hours *post mortem* correlated significantly with lower Warner-Bratzler shear force. Glucose is an indicator of glycogenolysis (Campbell, 1995) and therefore indicates the extent to which energy substrates were available for *post mortem* glycolysis.

Sm_{24} showed the highest ($P>0.05$) glycogen at 1 hour *post mortem* compared to Br_{24} and Ng_{24} as well as compared to Sm_3 (Table 4.16). Although not significant, these results were reported as it was not as expected. There were no difference in turnover rate from dopamine to norepinephrine for Sm_{24} (Table 4.12) compared to Sm_3. This was in contrast with Brahman and Nguni where longer feed withdrawal increased the turnover rate significantly for Brahman and not significantly for Nguni. It seems as if 24 hours feed withdrawal and hunger did not activate the turnover of dopamine to norepinephrine in order to cause the glycogenolytic effect or norepinephrine with less or slower glycogenolysis from the *ante mortem* period up until 1 hour *post mortem*.

High ATP at 6 hours correlated with high Warner-Bratzler shear force. If ATP hydrolysis from 1 to 6 hrs *post mortem* did not occur fast enough, ATP was available during the last phase of *rigor* (6 – 24 hrs *post mortem*) to ensure actomyosin formation.

Sm_{24} showed the highest Warner-Bratzler shear force between all treatment groups. This was not expected as glycogen for this group was higher than Sm_3 and classically the meat from *Bos taurus* cattle were described as being more tender (Voisinet *et al*., 1997). The catecholamine conversion rate of Sm_{24} was unresponsive, glycogen was higher (not significant), glucose-6-phosphate was significantly higher for Sm_3 compared to Sm_{24} and sarcomere length was shorter for Sm_{24} compared to Sm_3. It seems as if *ante mortem* energy metabolism was slower for Sm_{24} compared to Sm_3, but the rate of *post mortem* glycogenolysis and creatine phosphate metabolism was faster for Sm_{24} compared to Sm_3 and in contrast the rate of complete glycolysis for Sm_{24} was slower compared to Sm_3.

Changes in mitochondrial enzyme activity similar to those reported in hibernating have been seen in living, starving animals (Malatesta *et al*., 2007). During starvation when lipid becomes the main energy source, such as Sm_{24}, acetyl-CoA carboxylase (for the conversion of pyruvate to glucose) activity decreases. A change in metabolic fuelling may facilitate a hypometabolic state

(Malatesta *et al*., 2007). A possible hypometabolic state for Sm_{24} could have had an energy sparing effect. Davis *et al.* (2003) found lower metabolic rates in feed restricted *Bos taurus* cattle by measuring the tympanic temperature. Fujita *et al*. (2013) identified a bovine hibernation-specific protein complex in Holstein (*Bos taurus*) non-pregnant cows. This protein complex has up to date only been found in Holstein cows (which belongs to the *Bos taurus* subspecies). Fujita *et al*. (2013) found that bovine HP20 complex started to increase as soon as fasting commenced. Malan *et al.* (1988) explained that through the deactivation of mitochondrial functions (Bronnikov *et al*., 1990) in live hypo-metabolic mammals there is a reduction in ATP production as well as ATP hydrolysis and thus heat production that has been deactivated by Ca^{2+} in the mitochondria of the *ante mortem* hypometabolic cell (Bronnikov *et al*., 1990).

In hypometabolic animals, in addition to the mitochondrial enzyme activity modifications, Ca^{2+} are responsible for the inactivation of mitochondrial ATPase, thereby preventing the exhaustion of cellular ATP in de-energized mitochondria (Bronnikov *et al.*, 1990). A drastic reduction in ATP production is accompanied by a contemporary counterbalancing decrease in ATP consumption (ATP hydrolysis) *ante mortem*. Bruege & Marsh (1975) described the involvement of mitochondrial ATPase with sarcomere shortening as was seen for Sm_{24}.

In this study the rate of creatine phosphate decline for Sm_{24} was faster before 6 hours *post mortem* while ATP was still high (Figure 4.7). This means that muscle contraction (but not relaxation) could take place while the muscle was still warm, and therefore resulted in shorter sarcomere length and higher Warner-Bratzler shear force. Glycogenolysis, but not glycolysis and therefore muscle contraction was triggered before 6 hours *post mortem* for Sm_{24}.

Different conditions for sarcomere shortening have been summarised by Savell *et al* (2005). They are cold shortening, thaw and heat *rigor*. From the results in this study it seems as if another condition for sarcomere shortening can be described such as the depletion of creatine phosphate before 6 hours *post mortem* when the carcass is still warm. As the rate of creatine phosphate depletion between 1 and 6 hours *post mortem* was fast, onset of *rigor* occurred while the muscle was still relatively warm with additional sarcomere shortening for this group. The following summary of the sequence of *ante mortem* events is proposed for bovine that may reduce their metabolic rate during longer feed withdrawal: Malatesta *et al*. (2007) described an energy sparing effect during periods of fasting for hibernating mammals. Mader *et al.* (2008) found lower metabolic rates in feed restricted *Bos taurus* cattle by measuring the tympanic temperature. Fujitu *et al.* (2013) discovered that Bovine HP20 complex starts to increase as soon as fasting commences in order to lower metabolic rate in *Bos taurus* type cattle. Bronnikov *et al*. (1990) explained the deactivation of

mitochondrial functions in hibernating or fasting animals. Bronnikov *et al*. (1990) described that Ca^{2+} concentration increases in the mitochondria which deactivates mitochondrial ATPase with subsequent decrease in ATP production and ATP hydrolysis.

Mitochondria are involved in Ca^{2+} regulation in the cytosol. According to England *et al*. (2013) the ability of mitochondria to buffer Ca^{2+} influences Ca^{2+} oscillation within the cytosol. Ca^{2+} handling properties of mitochondria and its tolerance for atypical Ca^{2+} levels may differ according to fiber type. With increased Ca^{2+} from the sarcoplasmic reticulum mitochondria may play a significant role in accumulating Ca^{2+} and restraining glycolysis. The precise mechanism and involvement of mitochondrial functions of fasting animals is still an open area of research, but it seems as if early *post mortem* muscle contraction due to elevated cytosolic concentration as a result of lowered mitochondrial buffering capacity may play a significant role.

CHAPTER 6 CONCLUSION AND RECOMMENDATIONS

In this study significant differences in urinary catecholamine concentrations were found between breeds that were handled similarly through transport and *ante mortem* slaughter procedures. The main role of norepinephrine release is the establishment of both somatic and neural conditions that ensures maximal efficiency in coping with stressful situations. Some researchers refer to catecholamines as coping hormones as catecholamines make energy available to the brain. Animal temperament was not quantified in the present study, but Brahman cattle showed less urinary catecholamines compared to Simmental and Nguni. As Brahman is known to be more frightful or temperamental towards humans it seems as if a catecholamine deficiency may be responsible for this breed's inability to cope with fearful situations. Dopamine-β-hydroxylase is involved in biochemical pathways where dopamine is converted to norepinephrine and epinephrine. Both the expression of dopamine-β-hydroxylase and animal temperament (h^2 = 0.45) are genetically heritable. More research in this regard could give more conclusive evidence for the expression of this gene and its contribution towards animal behaviour and animal temperament. Slaughter animals with poor temperaments are difficult to handle and carcasses are often downgraded as a result of bruising. By quantifying the relationship between catecholamines and animal temperament in cattle, selection of animals with a more favourable catecholamine profile, could enhance the ease of handling slaughter animals and reduce carcass bruising. Slaughter animals that are less fearful will be safer to handle by the animal handler. Furthermore, there will be a reduction in carcass bruising and condemned meat from animals that were less fearful in the *ante mortem* period which will benefit the meat producer economically.

Catecholamine turnover rate from dopamine to norepinephrine and epinephrine, differed between breed types and feed withdrawal periods. Results indicated a relationship between energy metabolism, feed withdrawal period and catecholamine turnover. Twenty four hours feed withdrawal increased the rate of catecholamine turnover for Brahman and lower early *post mortem* glycogen together with higher glucose-6-phosphate indicated that glycogenolysis increased for this treatment group just before slaughter. It is concluded that hunger triggers glycogenolysis via increased catecholamine turnover from dopamine to norepinephrine and epinephrine after 24 hours feed withdrawal for Brahman. In contrast, feed restriction had no effect on catecholamine turnover and slightly higher early *post mortem* glycogen and lower glucose-6-phosphate indicated lower energy metabolism for the Simmental breed type just before slaughter. It is concluded that 24 hours feed withdrawal for Simmental does not increase glycogenolysis just before slaughter or may even lower energy metabolism. Catecholamine turnover for the Nguni breed type were intermediate between Brahman and Simmental breed types.

Warner-Bratzler shear force results for treatment groups where feed was not restricted for longer than 3 hours did not differ between breed types which emphasises the importance of providing feed to animals kept in lairages or transported over long distances. When feed is available up until 3 hours before slaughter, irrespective of breed type, meat tenderness will not be affected negatively. However, variation in meat tenderness within each treatment group was explained by its pH profile which indicates that *post mortem* glycolysis and energy metabolism in *post mortem* muscle, still causes variation in tenderness. Feed restriction of 24 hours *post mortem* causes significant variation in *post mortem* glycolysis and subsequently meat tenderness.

When feed was not restricted for more than 3 hours and within each breed type variation in Warner-Bratzler shear force can be explained by factors such as enzymatic profile, sarcomere length. For Brahman, although Warner-Bratzler shear force did not differ from Nguni or Simmental, variation in Warner-Bratzler shear force could be explained by its calpastatin activity. It is concluded that for Brahman, variation in tenderness could be reduced through its proteolytic enzyme profile. In contradiction with past studies, meat from Brahman had lower Warner-Bratzler shear force values, irrespective of feed withdrawal period.

Variation in Warner-Bratzler shear force for Nguni breed type cattle was explained mostly by its enzymatic profile, positive myofibrillar fragmentation lengths and sarcomere length. Tenderness for this breed type is ensured by proteolytic enzyme degradation. Further improvement in meat tenderness can be ensured through the reduction in cold shrinkage for the Nguni breed type by implementing methods to reduce carcass temperature decline.

When feed was restricted, additional sarcomere shortening and creatine phosphate depletion while the carcass was still warm, occurred to such a degree that the meat from Simmental was most tough. It is clear that feed withdrawal of 24 hours has a significantly negative impact on Warner-Bratzler sheer force of Simmental. For results in the present study, and non-electrically stimulated carcasses it is clear that feed must be provided to Simmental up until 3 hours before slaughter to prevent excessive sarcomere shortening and meat toughness. In cases where feed withdrawal for longer periods of time (24 hours) is inevitable, animals transported over long distances or kept in holding pens for extended periods of time without food, carcasses must be electrically stimulated in order to drive complete glycolysis at a faster rate early *post mortem*.

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