Effects of pre-slaughter sheep handling and animal-related factors on Creatine Kinase levels and physico-chemical attributes of mutton

By

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Declaration

I, **Chulayo Amanda Yucca**, vow that this dissertation has not been submitted to any University and that it is my original work conducted under the supervision of Prof. V. Muchenje. All assistance towards the production of this work and all the references contained herein have been fully acknowledged.

12 January 2011

Amanda Yucca Chulayo **Date**

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Abstract

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The objective of the study was to determine the effects of pre-slaughter sheep handling and animal-related factors on Creatine kinase (CK) levels and physico-chemical characteristics of mutton from South African sheep breeds. One hundred and seventy-three castrated male sheep from four breeds (15 Dormer, 46 South African Mutton Merino, 77 Dorper and 35 Blackhead Persian) were used. The animals were grouped according to age categories. The grouping was as follows: Group 1 ($6 \leq 8$ months), Group 2 ($9 \leq 12$ months) and Group 3 ($13 \leq 16$ months). Blood samples for CK determination were collected at exanguination using disposable vacutainer tubes. Representative samples of the *Muscularis longissimuss thoracis et. lumborum* (LTL) muscle were taken for the determination of ultimate pH (pH_u), colour (L*-lightness, b*- redness and a*-yellowness), thawing and cooking losses and meat tenderness. Significant ($P<0.01$) breed effects were observed on CK levels with Dormer having the highest CK values. Significant linear relationships were observed between pre-slaughter condition and CK levels. There were positive correlations between colour coordinates L* and b* (r =+0.22) and between a* and b* (r =+0.63). Cooking loss and Warner Braztler Shear Force (WBSF) values were positively correlated (r =+0.29). The Principal Component Analysis (PCA) showed a significant variance for CK and between physico-chemical characteristics of mutton. There were no relationships between CK levels and physico-chemical characteristics of mutton. Significant breed and age effects on pH_u, L, a*, b*, thaw %, CL and WBSF of mutton were observed.

It was concluded that, pre-slaughter conditions affect CK levels and physico-chemical characteristics of mutton. However, there were no relationships between CK levels and physico-chemical characteristics of mutton.

Keywords: temperature, distance, enzymes, season, Principal Component Analysis, physico-chemical characteristics, bruising

List of abbreviations

a* - Redness

ANMA - Adelaide Nxuba Municipal Abattoir

b*- Yellowness

BP- Blackhead Persian

CK - Creatine kinase

CL% - Cooking loss percentage

DP- Dorper

DM -Dormer

L*- Lightness

LTL - *Muscularis longissimuss thoracis et. lumborum*

PCA - Principal Component Analysis

pH_u - Ultimate pH

U/l - Units per litre

SAMM- South African Mutton Merino

SAS- Statistical Analysis System

Thaw % - thawing loss percentage

WBSF - Warner Bratzler Shear Force

Dedication

I dedicate this thesis to the Lord God Almighty who is the pillar that holds my life and to my family for everything they have supported me with to finish my studies.

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To my family (Spokazi 'Mpora', Zonwabele 'Dad' and Kokoko 'H.N.'), who through thick and thin supported me with all they could to see me doing it to the best of my knowledge. In Xhosa we say "Ukwanda kwaliwa ngumthakathi" meaning "something good is prohibited by the enemy". Lord I give you all the glory.

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Chapter 1: Introduction

Pre-slaughter welfare of sheep refers to the influence of internal challenges or *ante-mortem* conditions on the psychological or biochemical state of sheep at the time of observation (Broom, 2000). It is a combination of both subjective and objective aspects of life for animals such, as the health of an animal (Fitzpatrick, Scott & Nolan, 2006). Prior to slaughter, sheep are exposed to physical and psychological stimuli stressors through human-animal or animal-animal interactions. These include handling, loading, transportation, waiting in the lairage and feed deprivation, solidarity, gastro-intestinal infection, crowding, noise, stocking density, poor handling facilities, agitation, bullying by others and temperature extremes (Kadim, Mahgoub, Al-Kindi, Al-Marzooqi, Khalaf, Al-Sinawi *et al.,* 2009; Liste, Villarroel, Chacon, Sańudo, Olleta, Garcia-Belenguer *et al.,* 2009; Miranda-de la Lama, Villaroel, Liste, Escos & Mariá, 2010). Although sheep may be adapted to the harsh environment, these procedures have a major impact on their welfare and the quality of mutton (Schaefer, Dubeski, Aalhus & Tong, 2001; Kadim, Mahgoub, Al-Kindi, Al-Marzooqi & Al-Saqri, 2006).

Many researchers have assessed stress through physiological measures related to changes in hormonal levels, blood chemistry as well as behavioural reactions (Kadim *et al*., 2006; Campo, Prieto & Davila, 2008; Muchenje, Dzama, Chimonyo, Strydom & Raats, 2009). Although pre-slaughter stress responsiveness is influenced by previous experience and specific features at the time of observation, the welfare status of the animals also depends on breed, age of the animal, sex and health status (Muchenje *et al*., 2009). According to Fitzpatrick *et al.* (2006), it is difficult to recognise the effects of improper welfare in sheep. Improper handling of sheep during pre-

slaughter results in stress and depletion of muscle glycogen, which negatively affects meat quality attributes such as juiciness, colour, flavour and tenderness (Gregory, 2008).

Stress due to transportation has been found to reduce meat quality and quantity. In addition, transportation and additional handling prior to slaughter increases the risk of injury in muscle cells, leading to bruising (Hoffman, Spire, Schwenke & Unruh, 1998). Bruising is the rapture of the muscle as a result of physical exertion in the animal. It is not easy to detect bruising in sheep due to the presence of fleece and the thickness of the skin. It can be detected by measuring the amount of Creatine Kinase (CK) present in blood plasma (Braun, Medaille & Trumel, 2008). Creatine kinase (CK) is as an enzyme found in tissues and cell types of the animal (Lewis, 2000). The enzyme (Figure 1) breaks down phosphocreatine to creatine and phosphoric acid (Vojtic, 2000). In buffaloes, Creatine phosphokinase (CPK) is used as a prognostic indicator for the occurrence of uterine torsion. With higher serum concentrations increases with uterine torsion such that CK levels are above 500 U/l (Amin, Amer, Hussein & Hazzaa, 2011). It acts as a catalyst in the conversion of the creatine which consumes Adenosine Tri-Phosphate (ATP) to make Phosphocreatinine and Adenosine Di-Phospahte (ADP) (Radostits, Arundel, Gay, Hinchcli & Blood, 2000).

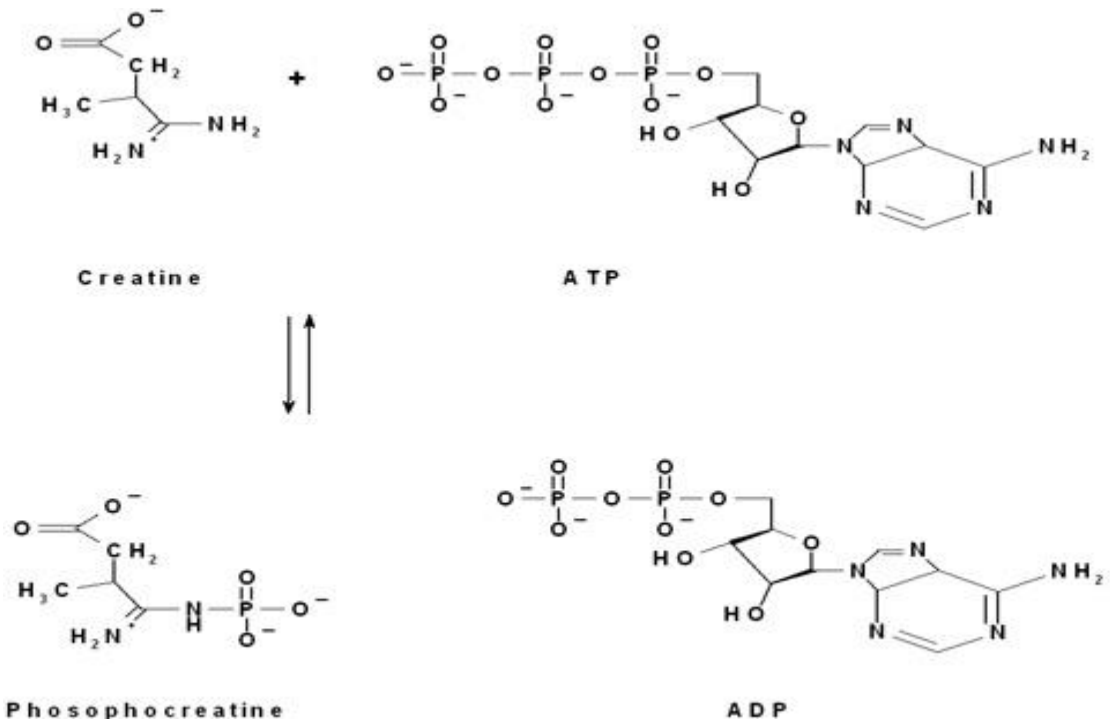

Figure 1 The creatine kinase reaction which occurs in skeletal muscle to resynthesize ATP to be used for energy production, (Adapted from Kaddurah-Daouk and Wyss, 2000).

1.2 Problem statement

Research has been conducted to determine the effects of transportation on plasma cortisol (Hall, Broom & Kiddy, 1998) and also on the effect of pre-slaughter animal handling on meat quality traits in large and better-equipped abattoirs (Miranda-de la Lama *et al.*, 2009). In addition relationships between reactivity profiles were measured in blood during rearing including plasma cortisol levels (Deiss, Temple, Ligout, Racine, Boux, Terlouw *et al.*, 2009). Although this is the case, there is little or no information about community-based abattoirs. A community-based abattoir is an abattoir that is situated in the communities or villages. Most of the animals slaughtered in this abattoir come from the local communities and sourrounding towns. As speculated by Smith *et al.* (2005), traceability of livestock products by farmers is gradually increasing and traditional rearing methods with high levels of animal welfare and product specificity may soon assume economic relevance (Napolitano, Cifuni, Pacelli, Riviezzi & Girolami, 2002; Miranda-de la Lama *et al.*, 2011). However, there is a need for more information such as the processes that induce stress, causes bruises and ultimately affect the quality of mutton to be addressed in order to improve animal production and animal handling methods.

1.3 Justification

The processes that occur before slaughter may cause aversive handling which may raise ethical concerns. They will also cause carcass down grading and concequently lead to economic losses. Producers are faced with difficulties in determining the actual causes of stress, that occur either at the farm before transportation or at the slaughter house.

Furthermore, small-holder farmers are faced with challenges in grouping animals awaiting slaughter to reduce fights and physical activity that compromise biochemical reactions and meat quality.

As animals are moved to the abattoir, they might get injured. It probably because of the long hours of transportation, distance travelled, stocking density and lairage period. Pre-slaughter conditions such as loading and bullying of animal by others have an impact on meat quality. In addition, these conditions causes bruising (cells become damaged) leading to deposition of CK into the blood stream (Sa´ncheza, Canaliasa, Palenciab & Gella, 1999). Moreover, meat quality attributes such as pH, colour, thawing loss, cooking loss and tenderness are affected (Muchenje *et al.*, 2009; Miranda-de la Lama *et al.*, 2011). Research on the pre-slaughter conditions to which sheep are subjected will enhance the understanding of proper handling and welfare issues associated with sheep pre-slaughter. Such a study will also unravel the factors relating to pre-slaughter animal handling and mutton quality attributes in communal small-scale abattoirs.

1.4 Objective

The objective of the current study was to determine the effects of pre-slaughter sheep handling and animal-related factors on CK levels and physico-chemical characteristics of mutton.

Specific objectives

Specific objectives were to:

a. determine the effect of pre-slaughter variables (distance travelled, loading density lairage period, ambient temperatures and time of slaughter) and animal-related factor on CK levels and mutton quality.

b. determine the relationship between CK levels and mutton quality.

c. determine the effect of pre-slaughter conditions and animal-related factors on the physico-chemical characteristics of mutton.

1.5 Hypothesis

The null hypothesis tested was that there are no effects of pre-slaughter sheep handling and animal-related factors on physico-chemical characteristics of mutton.

Specific Hypothesis:

a. There are no effects of pre-slaughter variables (distance travelled, loading density lairage period, ambient temperatures and time of slaughter) and animal-related factors on CK levels and mutton quality.

b. Creatine kinase and mutton quality are not related.

c. Pre-slaughter conditions and animal-related factors do not affect the physico-chemical characteristics of mutton.

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Chapter 2: Literature Review

2.1 Introduction

In many countries, sheep are believed to produce more meat than cattle and chickens (Morris, 2009). There are almost no cultural barriers to keeping sheep or eating mutton. Therefore, the rearing of sheep is of wider benefit across the cultures as compared to cattle (Gatenby, 1991). Sheep provide meat, wool, fat and horns (Zygoyiannis, 2006). In the Eastern Cape (EC) Province, both communal and commercial farmers keep sheep. However, the cumulative effects of various continued environmental stressors affect sheep. The environmental stressors include handling methods, transportation and the activities that occur from the farm to the abattoir (Miranda-de la Lama *et al.*, 2009). Handling methods have negative effects on animal welfare as they cause physical lesions, physical discomfort, muscle compression, increased production of enzymes such as creatine kinase, hormonal release as well as subsequent meat quality (Bourguet, Deiss, Tannugi & Terlouw, 2011). It is possible that these effects also slow down ante- and *post-mortem* muscle metabolism.

2.2 Pre-slaughter handling of sheep

Pre-slaughter handling refers to activities that occur before animals are slaughtered. Its phases include farm, haulier and abattoir activities. These activities may affect animal welfare and quality of meat (Gade, 2004). Sheep welfare is a combination of both physical and mental aspects of the quality of life (Goddard, Waterhouse, Dawyer & Stott, 2006; Dawyer, 2009).

According to Fitzpatrick, Scott and Nolan (2006), welfare of sheep encompasses five freedoms; freedom from hunger and thirst; freedom from discomfort, pain, injury or disease; freedom to express normal behaviour and the freedom from fear and distress. Due to the effects of stress and injury, bruises develop which then affect the quality of meat (Grandin, 2009; Miranda-de la Lama *et al.,* 2009). In addition, meat quality can be affected either negatively or positively depending on the loading procedures, weather conditions, stocking density during transportation, lairage time and time of slaughter (Kannan, Terrill, Kouakou & Galipalli, 2007; Kadim, Mahgoub, Al-Kindi, Al-Marzooqi, Khalaf, Sinawi *et al.,* 2009). All these factors constitute the supply chain of animal welfare. According to Yu *et al.* (2009), duration of transportation is an important factor of meat quality. According to Strappini, Frankena, Metz, Gallo and Kemp (2010), these conditions are associated with rough handling, violent impact of the animals against sharp-edged surfaces, aggression between animals and also mechanical damage to animal tissue. For all these reasons, pre-slaughter conditions must be monitored in order to reduce stress in animals and limit the occurrence of bruises (De Perre, Permentier, De Bie, Verbeke & Geers, 2010; Strappini *et al.,* 2010).

2.2.1 Phases of animal handling

The activities and processes that occur from the farm until the animal is slaughtered make up the logistic chain. A logistic chain encompasses successive steps that comprise processes in a particular environment or industry (Miranda-de la Lama *et al.,* 2009; Gregory, 2009; Strappini *et al.,* 2010). According to Miranda-de la Lama, Rivero, Chacón, Garcia-Belenguer and Villarroel, María (2010), small ruminant animals are reared widely under traditional extensive production

where resources are limited. Animal handling by humans has important welfare implications on animals and meat production (Figure 2.1). This affects both the animal and the stockperson at the farm. Handling of animals involves farm personnel, haulier and abattoir staff.

2.2.1.1 Farm personnel

Preparation of animals for collection, feeding, classification of animals and counting the stock constitute farm activities (Gade, 2004). As animals are raised extensively having lesser contact with humans, they should be brought together in one place. During this period, the animals tend to stay without food. In addition, some are identified for fattening, weighing and body condition scoring depending on the motives behind. In a food competition test (Bonney, 2006), pigs were found to fight more, were reactive to humans and were more reactive at slaughter. This implies that too much activity before slaughter causes hyper activity. In steers, higher reactivity due to humans resulted in higher serum cortisol scores at exsanguinations (Deiss, Temple, Ligout, Racine, Boux, Terlouw *et al.*, 2009). In contrast, animals that fear humans usually have lower heart rates during loading and unloading. They also have fewer incidents of slipping and falling at the abattoir (Terlouw, Arnould, Auperin, Berri, Le Bihan-Duval, Deiss *et al.*, 2008). This implies that is an important aspect in reducing fear in animals (Jago, Krohn, & Mathews, 1999).

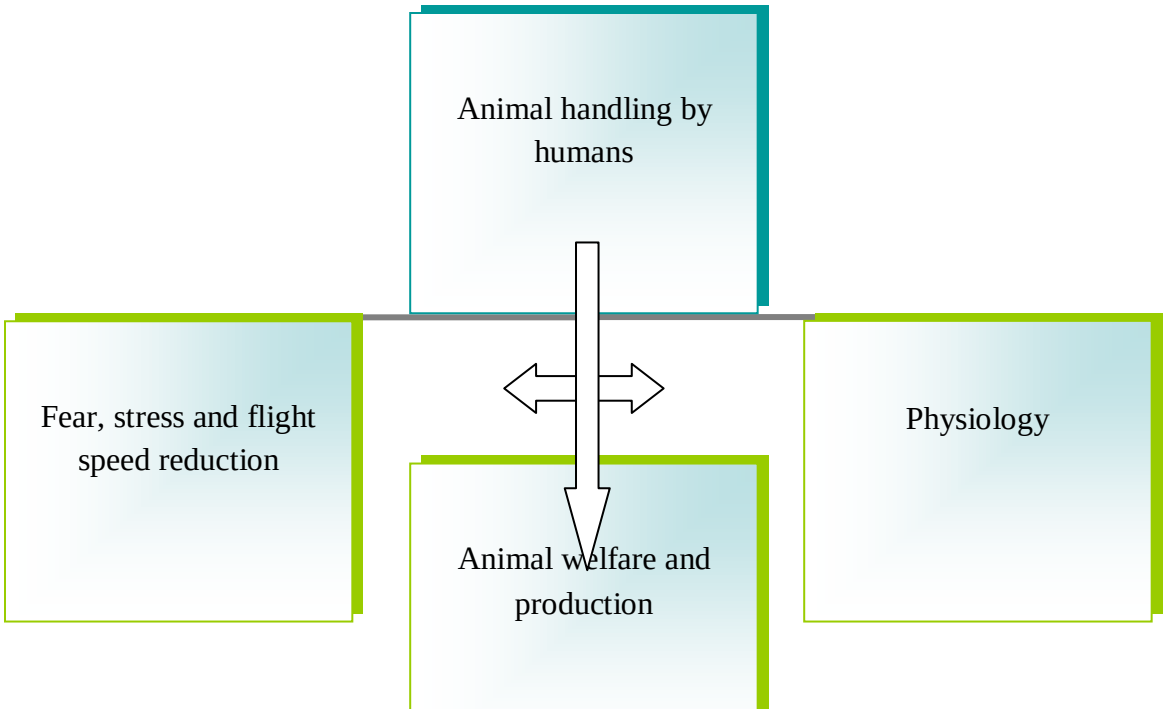

Figure 2.1 Effect of rough handling imposed to animals (Hemsworth & Coleman, 1998; Grandin, 2006).

2.2.1.2 Haulier and abattoir staff

Haulier includes activities such as loading, transport and off-loading. Loading is a complex that needs critical attention. Loading and transportation factors that occur at the farm up to the abattoir may differ depending to how the animals are sold. According to Strappini *et al.* (2010), other animals such as cattle are sold and shipped from the farm to the slaughter plant. Stress is also caused by loading. Loading can be stressful to the animals and is oftencan be likened with hot-iron branding in cattle (Grandin, 2006). During transportation, animals can be affected physiologically and physically. This depletes muscle glycogen leading to high ultimate pH (pH_u) and dark firm dry (DFD) meat. Due to its dark $ph_{u,}$ DFD meat is susceptible to bacteria (Vergara & Gallego, 2000; Genaro, Miranda de-la Lama, Monge, Villarroel, Olleta, García-Belenguer *et al.*, 2011).

Abattoir staff is responsible for moving animals to the lairage, resting them and also movingto stunning (Gade, 2004). According to Vergara and Gallego (2000), stunning induces the secretion of stress hormones such as dopamine, noradrenaline and adrenaline in sheep. This affects the quality of meat. Other physiological effects of stress caused by advesre weather conditions, hunger and thirst have been detected to increase cathecholamines in pigs and steers (Muchenje, Dzama, Chimonyo, Strydom and Raats, 2009b). During movement of animals to the slaughter area, rough handlingand violent impact of animals against sharp-edged surfaces may occur. This causes damage to animal tissues that can even develop into bruises (Strappini *et al.*, 2010).

2.2.2 Logistic chain on animal physiology

Few developed countries use semi to intensive animal production systems. In extensive production, animals seem to have dominance over the others with respectto priority to resources. This phenomenon is mostly high when there are limited resources such as the feeding system, water and spacing prior transportation. This process has been found to reduce the effects of loading procedures. Animals that grow in the veld, harsh and arid environments usually have better quality meat than those growing indoors. This is due to the fact that they consume a lot of green grass and that their muscles are always working (Martinezo-Cerezo, Sańudo, Panea, Medel, Delfa, Sierra *et al.*, 2005). However, according to Kadim, Mahgoub and Marzooqi (2008) temperatures higher than 32^0C reduce the reserves of glycogen and also lead to darker meat. In addition, the physiology of the animal is also affected, including reduction in economic returns. Utilisation of energy is reduced due to heat stress, resulting in high water holding capacity and ultimately, dark meat (Gregory, 2009). Therefore, pre-slaughter logistics chain effects should be evaluated in order to reduce the usage of energy trying to avoid the occurence of injuries.

2.2.3 Impact of bruises on meat quality

A bruise is an injury that does not break the skin but results in some discolouration in the muscle. Bruises reduce the quality and quantity of meat (Von Borrel, 2001). According to Jarvis, Selkirk and Cockram (1995), bruises also lead to economic losses in the meat industry. They usually occur during loading, transportation, in the lairages, during handling and stunning (Gregory, 2008; Vimiso 2010). The occurrence of injuries in extensive systems seems to be greater than in

intensive systems mainly due to the fact that there is limitedhuman-animal contact in the farmer (Goddard *et al.,* 2006). This implies that improper welfare of sheep is greater in extensive systems than in extensive systems. This could be due to the fact that in intensive systems, animals are housed and are taken care. Sheep have been found to even survive in harsh environments even though the impact on meat quality is not yet indicated. Muchenje, Dzama, Chimonyo, Raats and Strydom (2008) indicated that Nguni animals are adapted to harsh environments, suspecting that even sheep can withstand the condtions reffered to above. In South African region, there is a wet and a dry season whereby animals are supposed to adapt to feeding and temperature variations (Bakare & Chimonyo, 2010). In both seasons, there is a huge change from scarce to abundant resources including shelter when transportation takes place. During hot seasons, animals are transported in open trucks, suspecting that economic and welfare implications are not taken into account (Kadim *et al.,* 2009). Animals are also more familiar with handling in intensive systems compared toextensive farming (Goddard *et al.,* 2006). Rough handling facilities increase the risk of animal bruising. The levels of the, CK, in the blood can be used to determine the extent of animal cellular injury.

2.3 The potential use of Creatine kinase levels as an indicator of bruising and stress in animals

Creatine Kinase is used to show the response of animals to stress caused by transportation, handling, loading and off-loading. The enzyme is a member of phosphoryl transfer enzymes usually called guanidine or phosphagen kinases (Radostits, Arundel, Gay, Hinchcli & Blood, 2000; Braun, Medaille, & Trumel, 2008).

It participates in the homeostasis of Adenosine Tri-Phosphate (ATP) (Bertin *et al.,* 2007). Adenosine Tri-Phosphate is the primary source of energy in living organisms and it is also found in tissues especially in cardiac and skeletal muscles (Figure 2.2), brain, retina and primitive-type spermatozoa (Grzyb & Skorkowski, 2005).

Poor handling and long hours of transportation result in the breaking of muscle fibres (Grzyb & Skorkowski, 2005). Creatine kinase is therefore used to indicate cellular injury, heart and liver damage (Zhang, Xin, Bao, Hartung & Yue, 2010). Pre-slaughter, muscular activity and damage is severe due to transportation and handling. This increases the CK levels in the blood (Kannan, Kouakou, Terrill & Gelaye, 2003). In addition, when animals are transported for more than 6 hours, CK activity increases (Kannan *et al.,* 2007). In goats, higher levels of CK in the plasma may also be caused by breed temperament, excitability and fighting against each other (Grzyb & Skorkowski, 2005).

Enzymes [Creatine kinase (CK), alanine aminotransferase (ALT) and aspartate transminase (AST)] are deposited to the bloodstream because of stress caused by bruises (Radostits *et al.,* 2000). Kannan *et al.* (2003) found that if goats were transported for more than 2 hours, deposition of CK in the blood stream is high. Therefore, CK activity and its circulation in the blood become elevated because of long hours of transportation. In addition, vigorous handling, loading, herding and unloading procedures can also damage muscles (Yu *et al.,* 2009). Adenkola and Ayo (2010) reported that in sheep, loading, herding and rough handling resulted in increased CK`s.

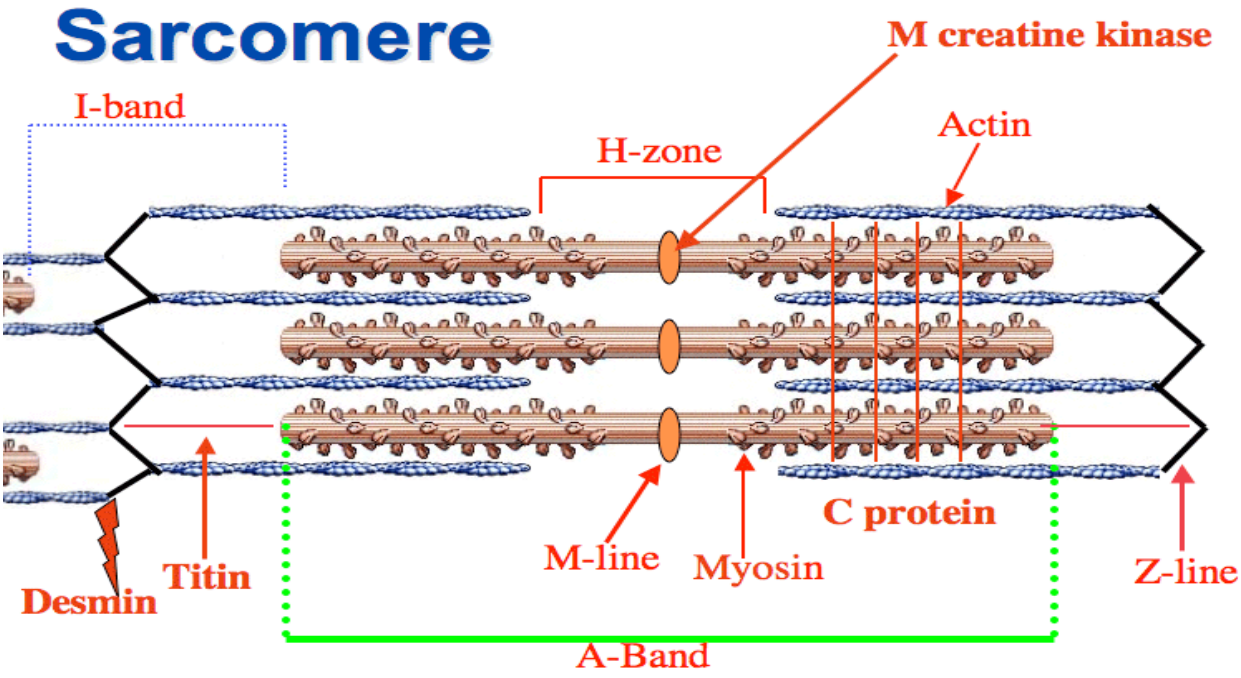

Figure 2.2 Sarcomere lengths showing the muscle creatine kinase in the M-line of the H-zone (www.google.com/structure_of_the_sarcomere_length)

2.3.1 Characterisation of Creatine Kinase

Creatine kinase is found mostly in proto-chordates and craniates with other enzymes including phosphorylated-arginine kinase. The enzyme can be measured from the blood of the animal before and after transportation (Bertin *et al.*, 2007). Kannan *et al.* (2003) and Ndou, Muchenje & Chimonyo (2011) indicated that blood samples could be collected pre- and post-slaughter by trained personnel using vacutainer needles. The collected blood must be stored either in 15% Ethylenediaminetetraacetic acid (EDTA) or ice, while enables separation of plasma after 2-3 hours of collection. This is done in order to determine response to physiological stress (Kannan *et al.*, 2003). Determination of CK in vertebrates showed three cytosolic isoenzymes that only form diametric structures (Votjic, 2000). These included muscle type (MM-CK), Brain-type (BB-CK) and cardiac muscle-type (MB-CK). Cytosolic isoenzyme MM-CK is basically the same as skeletal type (S-CK) (Galarraga *et al.*, 2003; Grzyb & Skorkowski, 2005). There are additional two tissue specific isoenzymes that are ubiquitous (uMtCK) and sarcomeric (sMtCK).

The existence of the tissue specific isoenzymes (MtCK) is determined by the concentration in the tissues, pH and temperature (Hornikova, Herman, Mejsnar, Vecer & Zurmanova, 2009). Most of the MtCK are found in between the membrane space where they bind as octamer. The reaction generates the high energy compound, phosphocreatine (PCr) which is then channelled as a substrate and later generates CK isoenzymes and ultimately, ATP pool (Grzyb & Skorkowski, 2005).

2.3.2 Administration and measuring of creatine kinase

Creatine kinase can either be drenched or drawn from the blood to diagnose usage of enzymes. The diagnostic use of enzymes in veterinary and human clinical pathology is mostly aimed at detecting, evaluating and monitoring organ damage based on the increase organ-specific enzymes (Braun *et al.*, 2008). However, enzymes are also used to evaluate the synthetic capacity of an organ, to diagnose the adverse effects of toxic compounds which inhibit enzymes and to monitor the inductive activity of exogenous compounds or enzyme activation by minerals or vitamins. In all these cases, interpretation is usually based on physiopathological data regarding input of the enzyme into a body fluid, most often blood plasma, and sometimes urine, digestive contents and cerebrospinal fluid. There is little consideration of the distribution of enzymes within the body and the clearance of enzymes from the body fluids. When interpreting the decrease in plasma activity of an enzyme used as a marker of organ damage, the plasma half-life of enzymes is sometimes used to address their clearance (Radostits *et al.*, 2000).

Enzymes such as alanine aminotransferase (ALT) and aspartate transminase (AST) are indicators of heart and liver diseases, damage or hepatic injury during stressful transport (Zhang *et al.*, 2010). According to Kannan, Terrill, Kouakou, Gazal, Gelaye, Amoah (2000), pre-slaughter conditions can lead to increased production of catecholamines in an animal. However concentrations of plasma cortisol and creatine kinase are useful indicators of stress and muscle damage. In addition, Creatine kinase activity is easy to determine when animals (sheep, pigs and cattle) are handled vigorously loaded and unloaded (Kannan *et al.*, 2003; Adenkola and Ayo, 2010). Therefore, physiological stress induced to an animal can be determined by using CK as an

indicator in order to improve meat quality. It is then separated into fractions that contain cells and serum. In the serum, tests are done to determine the amount of CK present. Any presence is measured and reported in units (U) of enzyme activity per litre (L) of serum.

2.4 Mutton quality attributes

Meat quality is a function of tenderness, pH, colour, juiciness, flavour and nutritive value (Webb & O`Neill, 2008; Muchenje *et al.*, 2009a). Pre-slaughter factors such as loading, transportation, distance travelled, unloading and time spent in the lairages, the procedure and method of slaughtering used affect these attributes (Roth, Imsland, Gunnarsson, Foss & Schelvis-Smit, 2007).

2.4.1 Ultimate pH of meat

As soon as the animal is slaughtered, muscles are converted to meat due to a number of metabolic and structural processes that occur immediately *post-mortem*. These changes that occur in the muscle *post-mortem* can be measured by the level of pH and temperature (Deiss *et al.*, 2009). Muscle glycogen will then be metabolised through anaerobic glycolysis (Maltin, Balcerzak, Tilley, & Delday, 2003), while generates lactate that accumulates in the muscles and in turn lowers intracellular pH. This occurs until it reaches ultimate pH (pHu) of about 5.4-5.7. In sheep, pHu is expected to be 5.75-6.00 (Gregory, 2008). According to Maltin *et al.* (2003), the rate of fall of pH has an impact on the eating quality of meat although it is not well understood.

Meat quality attributes such as ultimate pH (pH_u) and colour affect consumption (Simela, 2005). Conditions that occur prior and post-slaughter from the farm to the abattoir affect these attributes. They increase or decrease pH_u of meat depending on levels of glycogen and lactic acid in the muscle. The meat will either be Dark Firm Dry (DFD) or Pale Soft Exudative (PSE) (Zhang *et al.*, 2010). Ultimate pH below 5.0 has leads to tougher muscles (Sen, Santra & Karim, 2004; Muchenje, Dzama, Chimonyo, Raats & Strydom, 2008; Yu *et al.*, 2009). This is due to the formation of bonds between the myosin head and actin resulting in the occurrence of rigor mortis as already alluded to stressful handling facilities at the abattoir and the rate at which meat is cooled mainly affect pH_u (Toohey & Hopkins, 2006). Showering animals before slaughter reduces acidosis which can lead to higher pH although in chickens this seems to be different (Dyubele, Muchenje, Nkukwana & Chimonyo, 2010).

2.4.2 Colour of meat

The colour of meat is an important physical property that is usually used as an indicator of the quality of the meat, by consumers (Chen, 2007). Differences in intramuscular fat, ultimate pH, cooling rate, muscle fiber and myoglobin and moisture content in the muscle determine meat colour (Kadim *et al.*, 2008; Muchenje *et al.*, 2008). Colour of meat is defined in terms of Hunter colorimetric co-ordinates with L*, a* and b* values (Kannan *et al.*, 2003). Lightness (L*) of meat, indicates the lightness of the meat and it ranges from 0 (all light absorbed) to 100 (all light reflected). Co-ordinate a* is the redness of meat and it ranges from -60 (green) to + 60 (red); and b* ranges from -60 (blue) to +60 (yellow) (Simela, 2005). Colour of meat depends on the concentration and the chemical pigment myoglobin which varies in beef, mutton and pork.

According to Kannan *et al.* (2003), dark colour shows that animals were exposed to situations that exhausted glycogen levels. Furthermore, Warriss, Kestin, Young, Bevins and Bevins (1990) indicated that darker meat in sheep is caused by depletion of muscle glycogen before slaughter.

2.4.3 Tenderness of meat

Tenderness refers to the physical and chemical interaction of meat in the mouth, any feel of non-tactile sensation, the ease of mastication that is determined by the amount of residue that remains in the teeth (Maltin *et al.*, 2003; Tshabalala, Strydom, Webb & De Kock, 2003). In addition, it is the function of the collagen content, heat stability and the myofibrillar structure of muscle (Muchenje *et al.*, 2008). Consumers prefer meat that is tender (Toohey & Hopkins, 2006; Muchenje *et al.*, 2008) with less connective tissue and has desirable flavour (Muchenje *et al.*, 2010). Age of the animal, muscle type and size of the muscle fibre, post mortem, sarcomere length, proteolysis, distribution of intramuscular fat, pre-slaughter stress, cold shortening, cooking time and method and storage period including aging determine tenderness (Fanatico *et al.*, 2007; Kadim, Mahgoub, Al-Kindi, Al-Marzooqi, Khalaf, Al-Sinawi *et al.*, 2009; Muchenje *et al.*, 2008, Muchenje, Dzama, Chimonyo, Strydom & Raats, 2009b). According to Gregory (1996) and Muchenje *et al.* (2009b), animals exposed to pre-slaughter stress tend to have tougher and darker meat due to the depletion of glycogen in the muscle.

2.4.4 Thawing and cooking loss of meat

When meat is thawed several processes that are at play such as frost formation, frost consolidation, transition, condensation, evaporation and drying stages. Under conditions normally used in the thawing of foods, the drying stage is rarely reached. During thawing, the initial and final sample weights are recorded. In all the experiments, the final sample weight becomes greater than the initial sample weight. Thawing loss is the amount of water lost before cooking (Delgado & Sun, 2007). In general, thawing occurs more slowly than freezing, potentially causing further damage to frozen tissue (Tironi, Lamballerie & Le-Bail, 2010). The methodology and technique used during freezing and thawing processes play an important role in the preservation of the quality of frozen foods (Fernández, Otero, Martino, Molina-García & Sanz, 2008). Retention of fresh-like quality is the primary focus of freezing as the method of preservation and expectation of consumers, and numerous studies (Delgado & Sun, 2007) related to quality have been reported over the past several decades. Due to microbial and enzyme problems, a minimal ambient temperature must be ensured for a preferred thawing process to take place (Delgado & Sun, 2007).

Thawing at low temperatures can help to prevent the loss of food quality. This is obviously a challenge for traditional thawing processes because use of lower temperatures reduces the difference between the frozen and the ambient sample (Zhu, Ramaswamy & Simpson, 2004). According to (Aaslyng, Bejereholm, Ertbejerg, Bertram & Andersen, 2003), the eating quality in pork is the combination of appearance, flavour, tenderness and juiciness of which pH has been used to explain the variation in juiciness.

Cooking forms part of the catering industry and thus, it is of significant economic importance (Aaslyng *et al.,* 2003). It determines the appearance of meat as it is expected to explain the variation in juiciness which is the sensory attribute that influences acceptability of meat (Osman & Aldosari, 2006).

The time taken to cook the meat, temperatures and also heating method define the cooking procedure (Jama *et al.,* 2008). Cooking is the heating of meat to a sufficiently high temperature in order to denature proteins (Combes, Lepetit, Darche & Lebas, 2003). Variations in temperatures seem to affect the juiciness of meat in the cooking procedure. Higher temperatures results in less juicier meat. This decreases its flavour. It has been deduced that high cooking loss results in less juicier meat hence there is a negative correlation between the two factors (Muchenje *et al*., 2009a). Cooking loss is a breed dependant attribute, although in sheep there is little or no information available about cooking loss (Aaslyng *et al.,* 2003). According to Muchenje *et al.* (2008), pH_u does not necessarily affect meat tenderness as in the case with other parameters. It can result in DFD meat.

2.5 Factors affecting mutton quality

2.5.1 Animal management factors

Age, breed, species and muscles in an animal have different effects on meat quality. Older animals usually have tougher meat as compared to younger one (Maltin *et al.,* 2003). This is also determined by the feeding patterns and nutrition. Nutrition plays an important role in optimising the functioning of the body. This is due to its regulatory effect on biological processes in the

muscles that are reflected in the quality of meat (Descalzo, Rossetti, Grigioni, Irurueta, Sancho, Carrete *et al.*, 2007). Muscles in various species differ in classification because of the glycolytic and oxidative activities that occurs at different ages of the animal (Sańudo, Sanchez & Alfonso, 1998). In addition, sex of the animal affects the classification of meat (Maltin *et al.*, 2003). Jeremiah, Tong and Gibson (1998) indicated that meat palatability might be different among breeds due to their ages and slaughter weights.

Animals find it difficult to move towards a certain direction due to aggressiveness, age and body weight, breed, sex, experience and also the possession of horns. In addition, changes to the availability of these resources can lead to changes to social environment of the animals, including their development (Miranda-de la Lama & Mattiello, 2010). Although sheep are not more aggressive than goats, they need to be monitored at the farm, in the truck and lairages so as to improve social behaviour and adaptation of sheep to environmental factors.

2.5.2 Mechanical factors

Knowles (1998) postulated that most animals undergo a process of being castrated and tail docking. There are issues whereby species such as sheep are endangered such that meat quality becomes affected. This is because the processes are performed using several procedures which may have a negative impact on mutton quality (Miranda-de la Lama *et al.*, 2009). These processes include ear tagging, castration using the Burddizzo, shearing and vaccination. Due to vaccination, slaughtered animals may even have puss in the hind and fore-legs as well as in the neck due to vaccination. This affects meat colour and the quantity of meat (Genaro *et al.*, 2011).

2.6 Summary

Pre-slaughter handling of sheep involves several activities that occur prior to animal slaughter. The slaughter period starts at the farm with the preparation of the animals for transport and ends when they are killed. During this period, animals react in differentways as they try to cope with the environment and the handling procedures while their physical and biochemical well being are affected. Animals are also subjected to stress. In order to evaluate stress-inducing factors, behavioural and physiological measurements are used together with indicators of post-mortem muscle metabolism. Muscle metabolism results in CK being released to the blood, which also affects meat quality. In order to improve sheep handling and mutton quality, there is a need to determine the effects of pre-slaughter sheep handling on CK levels, mutton quality and their relationship.

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Chapter 3: The effects of pre-slaughter conditions and intrinsic animal factors on Creatine Kinase levels and their relationship with physico-chemical characteristics of mutton

A.Y. Chulayo

Abstract

The objective of the current study was to determine the effect of pre-slaughter conditions and animal-related factors on creatine kinase (CK) levels and physico-chemical attributes of mutton and how CK levels relate to physico-chemical characteristics of mutton. One hundred and seventy-three (173) castrated male sheep from four breeds (15 Dormer, 46 South African Mutton Merino, 77 Dorper and 35 Blackhead Persian) were used in the study. Blood samples for CK determination were collected at exsanguination using disposable vacutainer tubes. Representative samples of the *Muscularis longissimuss thoracis et. lumborum* (LTL) of mutton were analysed for ultimate pH (pH_u), colour, thawing and cooking loss and WBSF. There was a significant effect ($P<0.001$) of the season on CK levels and physico-chemical characteristics of mutton. Significant breed effects were also observed on CK levels ($P<0.01$) with the Dormer having the highest CK values. Significant linear relationships were observed between pre-slaughter conditions and CK levels ($P<0.05$) and physico-chemical characteristics of mutton ($P<0.001$). The Principal Component Analysis (PCA) showed a significant variance for CK levels than physico-chemical characteristics of mutton. There were no relationships between CK and physico-chemical characteristics.

It was concluded that except temprerature, pre-slaughter conditions did not affect CK levels. There there were no relationships between CK levels and physico-chemical characteristics of mutton.

Keywords: Temperature, ultimate pH, creatine kinase, season, Warner-Braztler Shear Force

3.1 Introduction

Intrinsic and extrinsic factors affect the quality of mutton. Breed, age, gender and health status of the animal are some of the intrinsic factors while extrinsic factors include pre-slaughter conditions (Vergara & Gallego, 2000; Muchenje *et al.,* 2009a; Muchenje, Dzama, Chimonyo, Strydom & Raats, 2009b). Pre-slaughter conditions have been found to have deleterious effects on the physiology (lactate, glucose, CK and cortisol) of the animal and the quality of meat. Blood metabolites are commonly used in monitoring the health and nutritional status of the herd, diagnosing metabolic problems and pre-slaughter effects (Kannan, Kouakou,Terrill, & Gelaye, 2003; Partida, Olleta, Campo, Sañudo, & María, 2007; Muchenje *et al.,* 2009a, 2009b; Ndlovu, Chimonyo, Okoh, Muchenje, Dzama, Dube *et al.,* 2009; Veiseth-Kent, Grove, Færgestad & Fjæra, 2010). Pre-slaughter stress also results in animals releasing cortisol and catecholamines (Muchenje *et al.,* 2009b; Mapiye, Chimonyo, Marufu & Muchenje, 2011) into the blood stream leading to a series of secondary effects that involve energy metabolism, respiratory function, immune function, and osmotic regulation. In addition, catecholamines deplete glycogen reserves in muscles reducing lactic acid production which increases ultimate pH (pH_u) (Veiseth-Kent *et al.,* 2010).

Increased pH_u is another factor that causes pre-slaughter stress which then results in darker meat (Muchenje *et al.,* 2009b; Veiseth-Kent *et al.,* 2010; Early *et al.,* 2011; Rodriquez *et al.,* 2011). The animal's previous experiences and specific features at the time of observation influence pre-slaughter stress (Muchenje *et al.,* 2009b; Dodzi & Muchenje, 2011; Ndou, Muchenje & Chimonyo, 2011). Some of the pre-slaughter conditions that affect meat quality are temperature,

lairage duration and distance. In addition, transportation has also been found to cause rapture in muscle cells depositing some enzymes into the bloodstream (Hoffman, Spire, Schwenke & Unruh, 1998; Ali, Al-Qarawi & Mousa, 2006).

According to Warriss *et al.* (1998), fatigue caused by transportation increases the activities of these enzymes. The diagnostic use of enzymes in veterinary and human clinical pathology is mostly aimed at detecting, evaluating and monitoring organ damage based on the increased organ-specific enzymes (Braun, Medaille & Trumel, 2008). However, enzymes are also used to evaluate the synthetic capacity of an organ, to diagnose the adverse effects of toxic compounds which are enzyme inhibitors and to monitor the inductive activity of exogenous compounds or enzyme activation by minerals or vitamins (Galarraga *et al.*, 2003; Grzyb & Skorkowski, 2005). These enzymes include aspartate aminotransferase (AST/ASAT) and Creatine Kinase (CK).

Creatine kinase is found in the skeletal muscles of animals responsible for maintaining energy homeostasis at sites of high Adenosine Tri-Phosphate (ATP) (Dieni & Storey, 2009). When CK is found in the blood it indicates muscle damage (Sa´ncheza, Canaliasa, Palenciab & Gella, 1999). Rapture of muscles releases CK from the muscles and it is deposited into the blood (Vojtic, 2000). As total activity of CK can also be used to measure the sum of enzyme activities from different organs, it was detected that CK can either be an M or a B monomer subunit. In muscle, M-CK which can also be denoted by S-CK is found in the M-line of the H-zone in the sarcomere length (Hornikova, Herman, Mejsnar, Vecer & Zurmanova, 2009). Therefore, when CK is found in the blood, it could indicate severe muscle damage.

Creatine kinase lasts for about two to five hours after the obstruction of blood supply (Braun *et al.,* 2008). This indicates that CK measurements can be taken within the specified period before it disappears in the blood stream (Sa´ncheza *et al.,* 1999; Kannan, Terrill, Kouakou, Gazal, Gelaye, Amoah *et al.,* 2000). Presence of CK in the blood indicates muscles exertion by adverse conditions before slaughter that indicate bruising. Bruises are indicative of violence and pain that animals underwent, indicating poor welfare conditions during the pre-slaughter period (Tadicha, Gallob, Bustamantea, Schwertera, & van Schaik, 2005). Pre-slaughter conditions such as loading and bullying of animal by others have a negative impact on meat physico-chemical quality characteristics (Vimiso, 2010) such as pH, colour, thawing loss, cooking loss and tenderness.

The physico-chemical characteristics are affected by conditions that occur prior to slaughter. Some researchers (Muchenje *et al.,* 2009a; Zimerman, Grigioni, Taddeo & Domingo, 2011) revealed that pre-slaughter stressors have an effect on the physiology of the animal and meat quality. In sheep, CK is a good indicator of a stressful environment before slaughter as sheep do not exhibit physical signs of stress such as bruising. This is because of the cushion wool they possess (Kannan *et al.,* 2000). However, there is limited information regarding the relationship between CK and physico-chemical characteristics of meat as affected by pre-slaughter conditions and intrinsic animal factors. The objective of the current study was therefore to determine the effects of pre-slaughter conditions and animal-related factors on CK levels and how CK levels relate to physico-chemical characteristics of mutton.

3.2 Materials and Methods

3.2.1 Description of the study site

The study was carried out at the Adelaide Nxuba local municipal abattoir located 32`.8$^0$ S and 26`.9^0 E in the Amathole District of the Eastern Cape Province, RSA. Adelaide is situated 586m above sea level. It has vegetation that ranges from grasslands and thicket to forests and bushveld with *Acacia karroo, Themeda triandra and Digitaria eriantha* being the most dominant plant species. The place receives approximately 480 mm of rainfall per year of which mostly it is during the summer months. It is situated in the semi-arid False Thornveld of the Eastern Cape. It has a generally flat topography with few steep slopes. The temperatures in Adelaide during the period of study were ranging from 15^{0}C to 36^{0}C with mean temperatures of 21.5^{0}C.

3.2.2 Study animals and management

One hundred and seventy-three (173) sheep from four breeds of different farms presented for slaughter at the abattoir were used in the study. The numbers of animals per breed were 15 Dormer, 46 South African Mutton Merino, 77 Dorper and 35 Blackhead Persian. The animals used were male castrates and were grouped according to age categories of different months. The grouping was as follows: Group 1 (6 $\leq$ 8 months), Group 2 (9 $\leq$ 12) and Group 3 (13 $\leq$ 16 months). Animals were transported by the same motor vehicle from the farms to the abattoir. The size of the vehicle was 3.6m x 4.4m with the stocking density of 0.75m^2, approximately 4 units of sheep (1 unit = 5 sheep).

The experiment was conducted during the cold-dry (April – August) and the hot-wet (September – March) seasons. Information on pre-slaughter conditions was gathered by recording departure time from the farm, distance travelled and the size of the truck. On arrival at the abattoir, off-loading activities were monitored. These included; transferring animals to the lairages and the period of stay in the lairage. Sheep were given water a*d-libitum* prior to slaughter.

3.2.3 Biochemical determination of blood

3.2.3.1 Blood collection and plasma separation

The sheep were slaughtered using an electric stunner of 650Volts for five seconds. A sharp knife was used to cut the throat and they were allowed to bleed for 6 minutes. Exsanguination blood was collected from each sheep while they were hanging, after cutting of the throat. Disposable vacutainer tubes with anticoagulant to assist during blood centrifuge were used to collect blood. Each blood sample was kept in ice until plasma was separated within 2 hours after collection. Blood tubes were centrifuged at 21°C for 10 min at 3550 rpm placed in 1.5 ml Eppendorf tubes and stored at -20°C (Model 5403 Centrifuge, Gatenbay Eppendorf GmbH, Engelsdorp, Germany). The samples were then arranged for easy identification during transportation for determination of CK.

3.2.3.2 Creatine Kinase level determination

The samples of stored plasma were analysed for CK using a Model DXC 600 machine (Beckman Coulter, Ireland) at the National Health Laboratory Services, Port Elizabeth. Creatine Kinase Reagent for SYCHRON Systems (CK 2 x 200) had reactive ingredients. The contents included 2 x 61ML CK Reagent, 1 Preparation insert and the reactive ingredients included Creatine Phosphate, Disodium Salt 461mmol/L, Nadide 30:0mmol/L, Adenosine-5-Diphosphate, Monopotassium Salt, Dihydrate 36.0 mmol/L, Glucose 24.0mmol/L, Glucose-6-Phosphate Dehydrogenase 46.1kU/L and Hexokinase 136kU/L. All the ingredients were added for quantitative determination of CK activity of units per litre (U/L) in plasma.

3.2.4 Meat quality measurements

Representative samples of mutton were collected 24 hours after slaughter for measurements of pH_u, colour (L*, a*, b*), thawing and cooking losses and tenderness of meat. The measurements were taken from the *Muscularis longissimuss thoracis et. lumborum* (LTL) muscle. The muscle was removed by cutting a sample between the 4th and 6th ribs (14 x 9 x 3 cm) of the loin region while the carcasses were still hanging. The weight of the meat samples ranged from 191 to 210g. Each sample was vacuum-packed, kept in a cooler box an hour after collection and further stored in the refrigerator at -4^0C.

3.2.4.1 pH measurements

A portable pH meter, with a fibre-optic probe (CRISON pH 25 Instruments S.A., Alella, Spain) was used to measure ultimate pH (pH_u) of the carcasses 24 hours after slaughter. It was measured from the cut *Muscularis longissimuss thoracis et. lumborum* muscle. The pH_u was first calibrated using pH 4, pH 7 and pH 9 standard solutions (CRISON Instruments, SA, Spain). The measurement was then performed with a sharpened metal sheath to prevent probe breakage from raw meat contamination.

3.2.4.2 Determination of meat colour

The colour of meat (L* = lightness, a* = redness and b* = yellowness) (Commission International De I` Eclairage, 1976) was determined at the butchery 24 hours after slaughter from carcases transported from the abattoir. A portable refrigerator vehicle was used to move carcasess from the abattoir to the butchery an hour after slaughter. A Minolta colour-guide 45/0 BYK-Gardener GmbH machine with a 20mm diameter measurement and illuminant D65-day light, 10^0 standard observer was used for colour measurement. The machine was calibrated each day before taking measurements using the green, black and white standard colour samples provided for this purpose. The readings were taken by rotating the Colour Guide 90^0 between measurements so as to obtain the average value for the colour.

3.2.4.3 Determination of meat tenderness, thawing and cooking losses

The *Muscularis longissimuss thoracis et. lumborum* muscle samples were weighed after refrigeration, thawed and weighed again. The recorded weight differences were expressed as the thawing loss using the following formulae:

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

The meat samples were placed in a plastic bag and cooked using a water bath at 85^0C for 45 minutes (Ding, Kou, Cao & Wei, 2010). Cooking loss was then calculated using the following formulae:

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

The tenderness of mutton was determined using the Instron- Warner-Bratzler Shear Force (WBSF). Following cooking, sub samples of specified core diameter were cored parallel to the grain of the meat. Three sub samples measuring 10 mm core diameter were cored parallel to the grain of the meat. The samples were sheared perpendicular to the fibre direction using a Warner Bratzler (WB) shear device mounted on an Instron (Model 3344) Universal Testing apparatus (cross head speed at 400mm/min, one shear in the centre of each core). The mean maximum load (N) was recorded for the batch.

3.2.5 Statistical analyses

Generalised Linear Models procedure of SAS (2003) was used to determine the effect of breed, age and season on CK levels and mutton quality attributes (pH_u, L*, a*, b*, thaw%, CL% and WBSF values).

$$У_{ijk} = \mu + \alpha_i + \beta_j + \lambda_k + \varepsilon_{ijk}$$

Where

$У_{ijk}$ - Response variable (CK, pH_u, L, a*, b*, thaw %, CL% and WBSF)

μ - Overall mean

α_i - Breed effect (Blackhead Persain, Dorper, Dormer and SA Mutton Merino breeds)

β_j - Age group effects (Groups 1, 2 and 3)

λ_k - Seasonal effects (Hot-wet and cold-wet season)

ε_{ijk} - Random error term

Significant differences between least square group means for CK levels and mutton quality attributes were compared using PDIFF procedure. The data was analysed using PROC Regression model of SAS (2003) to determine the relationship between continuous variables (distance duration, lairage duration and temperature), CK and physico-chemical charateristicss of mutton. The Principal Component Analysis (PCA) was used to determine the relationship between CK and physico-chemical characteristics of mutton.

3.3 Results and Discussion

Creatine kinase was higher during the hot-wet season than in the cold-wet season. This is in agreement with the results by Miranda-de la Lama, Villarroel and María (2011b) who reported higher CK levels as a result of physical stress during the summer season. Muscle enzyme activity increases during transport because of increased muscle cell permeability or muscle cell damage, making CK a very sensitive indicator of muscular damage and fatigue (Miranda-de la Lama *et al.*, 2010b). The current study does not agree with Zimerman *et al.* (2011), who reported lower CK levels in goats as pre-slaughter conditions are concerned. Pre-slaughter conditions are known to induce stress and negatively affect meat quality attributes such as colour, tenderness and ultimate pH (Mazzone, Vignola, Giammarco, Manetta & Lambertini, 2010).

The effect of season on CK levels are shown in Table 3.1. There were significant seasonal effects on CK levels. Higher values for CK (1026.3 ± 105.06) were observed during the hot-wet season than the cold-dry season. Results in this study are in agreement with the results that were obtained by Pollards, Littlejohna, Ashera, Pearsea, Stevenson-Barrya, McGregora *et al.* (2002) in the deer.

Table 3.2 shows breed effects on CK levels. Creatine kinase levels were highest (1358.6 ± 191.08) in Dormer. Dormer is a breed from Dorset Horn and Germany Merino bred to adapt to conditions in winter rainfall areas of South Africa. Dorper is a South African meat breed that can be raised under harsh conditions hence had the lowest CK levels (Fourie, Neser, Olivier & van der Westhuizen, 2002).

Table 3.1 Mean values (±SE) for creatine kinase, ultimate pH, colour and cooking loss % of mutton as affected by season.

Parameter	Season		Significance level
	Cold wet	Hot wet	
CK (U/L)	723.3 ± 77.75	1026.3 ± 105.06	***
pH_u	5.7 ± 0.04	5.8 ± 0.06	NS
L*	29.4 ± 0.64	33.7 ± 0.94	***
a*	16.3 ± 0.60	16.3 ± 1.09	NS
b*	10.2 ± 0.33	11.5 ± 0.48	*
CL%	32.8 ± 1.63	31.3 ± 2.37	NS

[a, b]Means with the same superscript in the same row are significantly different.

Significance difference = *P < 0.05, ***P < 0.001, NS = No significance difference

CK = creatine kinase

pH_u = ultimate pH

L* = lightness

a* = redness

b* = yellowness

CL% = cooking loss %

Table 3.2 Means (±SE) for the effect of sheep breed on creatine kinase levels.

Breed	CK U/L	Significance level
Blackhead Persian (*n*=36)	726.2 ± 117.08[a]	***
Dormer (*n*=78)	1358.6 ± 191.08[b]	***
Dorper (*n*=14)	656.3 ± 81.79[a]	***
SA Mutton Merino (*n*=45)	758.0 ± 107.47[a]	***

[a, b]Means with the same superscript in the same column are significantly different.

Significance difference = ***P < 0.001.

CK = creatine kinase

U/l = units per litre

The current study is in agreement with Kannan *et al*. (2000) and Miranda-de la Lama *et al*. (2000), who reported that CK activity indicate the extent of muscular activity and damage during hauling and holding. Warries *et al*. (1998) also reported that farm of origin had an effect on CPK in pigs. Furthermore, other animals are susceptible to transport stress leading to highest levels of CK in the blood (Yu *et al*., 2009).

Table 3.3 shows the relationship between pre-slaughter conditions and creatine kinase. A positive linear relationship was observed between temperature and CK levels. The study also agrees with findings by Melesse, Maak, Schmidt and von Lengerken (2011) who reported increased CK levels due to high temperatures. Partida *et al*. (2007) also reported that increasing environmental temperatures and pre-slaugter conditions such as loading, transporation and lairage duration also increased CK levels. According to Earley *et al*. (2011), long distance transportation is also a source of stress and increases CK levels in blood. There was no relationship observed between pre-slaughe conditions such as distance, lairage duration, stocking density and an enzyme CK levels.

During transportation, the truck stocking density of $0.75m^2$ with 20 sheep was acceptable. This is due to the fact that there was no muscle injury associated with stocking density. This is agreement with Miranda-de la Lama *et al.* (2011a) who reported fewer or no injuries associated with stocking density. However, in a moving truck and high stocking density, animals require more energy in order to keep themselves balanced.

Table 3.3 Relationships between pre-slaughter conditions and creatine kinase

Parameter	Variable	Relationship	Equation	Significance
CK	Temperature	Linear	$Y = 16.27 + 8.330_X$	*
	Distance duration	NS	$Y = -3.22 + 1.838_X$	NS
	Lairage duration	NS	$Y = -0.43 + 0.472_X$	NS
	Stocking density	NS	$Y = 466.22 + 290.117_X$	NS

Significance difference = *P < 0.05, **P < 0.01, ***P < 0.001, NS = Not significant.

X= Temperature, Distance duration, Lairage duration, Stocking density.

CK = creatine kinase

Figure 3.1 shows the PCA for correlations between CK and physico-chemical characteristics of mutton. Creatine kinase was not related to any of the meat quality parameters, however, Warries, Kestin, Young, Bevins & Bevins (1990); Mach, Bach, Velarde and Devant (2008) reported that CK can be linked to muscle exhaustion that occurs prior to slaughter. Even though muscle glycogen was not measured in the current study, physiological stress factors have an impact on the physiology of an animal thereby causing muscle damage, glycogen depletion, CK production and negatively affects the quality of meat (Yu *et al.,* 2009). Positive correlations were observed between physico-chemical characteristics; pH_u, L*, a*, b*, CL% and WBSF. Higher pH_u found in the muscles was associated with darker meat (Muchenje *et al*., 2009b; Miranda-de la Lama *et al.,* 2009). Meat pH_u affects other important meat quality attributes such as colour, tenderness and water holding capacity (WHC) (Muchenje *et al*., 2008).

According to Cloete, Hoffman and Cloete (2008), high pH_u has an effect on the colour and tenderness of meat. It was also indicated that higher pH_u (>5.8) leads to undesirable beef colour (Ferguson *et al.,* 2001; Hoffman, Muller, Cloete & Schmidt, 2003; Muchenje *et al.,* 2009b) which is unattractive to the consumer (Vegara & Gallego, 2000; Esenbuga *et al.,* 2009). Furthermore, the relationship between physico-chemical characteristics is also dependent on the differences in intramuscular fat, ultimate pH, cooling rate, muscle fiber and myoglobin.

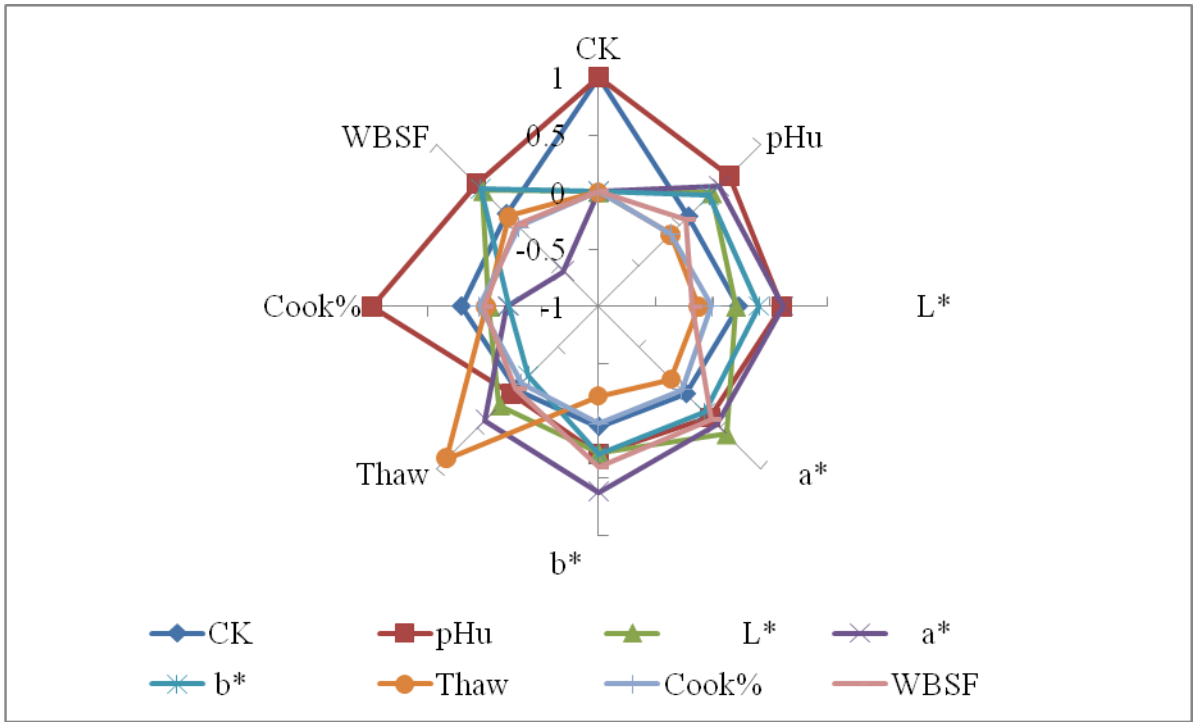

Figure 3.1 Principal Component Analysis for Creatine Kinase and physico-chemical characteristics of mutton

Moisture content in the muscle, cooking temperatures and the amount of water lost during cooking have been found to be contributing to physico-chemical characteristic (Combes, Lepetit, Darche & Lebas 2006; Kadim, Mahgoub & Al-Marzooqi, 2008; Muchenje *et al.*, 2008; Miranda-de la Lama *et al.*, 2009). Martinez-Cerezo, Sańudo, Panea, Medel, Delfa, Sierra *et al.* (2005) indicated that the differences in the physico-chemical characteristics and the activities that occur in the muscle likewise determine meat tenderness to the extent of muscle glycogen, pH_u, water holding capacity and cooking loss percentage. However, these characteristics of mutton differ in their importance as shown by PCA analyses (Kadim *et al.*, 2009).

The results of the PC analysis for CK and physico-chemical characteristics are presented in Table 3.4. The four first PC values explained about 100% of the total variation for blood and carcass quality measurements. The components are derived in decreasing order of their importance (Brand, 2006). Creatine Kinase, $pH_{u,}$ L* and a* were found to contribute about 99%, 0.02%, 0.01% and 0.01% respectively. In other experiments, Principal Component Analysis method has been used to characterise processed meat (Laville, Martin & Bastien, 1996; Destefanis, Barge, Brugiapaglia & Tassone, 2000), meat quality (Hernández, Pla, Oliver, & Blasco, 2000) and meat products. Santos, Silva, Silvestre, Silva & Azevedo (2008) reported that PCA explained about 67.3% of the variation for meat quality measurements in goats. Kumar and Singh (2010) used PCA to analyse the main parameters that were responsible for the main variability in water. They reported about 94.5% of the factors explaining the total variability.

Table 3.4 Results from the principal component analyses for the first four components of creatine kinase and physico-chemical characteristics of mutton.

Component	Eigenvalue	Proportion of variance (%)	Cumulative variance (%)
1	573556.891	99	99
2	137.357	0.02	99
3	48.883	0.01	99
4	33.611	0.01	100

1 = creatine kinase

2 = $pH_{u,}$

3 = lightness (L*),

4 = redness (a*).

In lamb, about 49.67% was explained by PCA for the first four meat quality characteristics (Cańeque, Pérez, Velasco, Díaz, Lauzurica, Álvarez *et al.*, 2004). In the current study, CK contributed a greater proportion of variance as compared to the meat quality characteristics. This could also show that there is no relationship between CK and physico-chemical characteristics of mutton. As explained earlier, the second, third and fourth components ($pH_{u,}$ L* and a*) were influenced by chemical compositions that include glycogen content in the muscle. However, it could not be identified yet as to which components did contribute to the indicated variances. Therefore, further studies are needed to identify the sources of variance for CK levels and each physico-chemical characteristics of mutton.

3.4 Conclusions

With the exception of temperature, pre-slaughter conditions did not affect CK levels. Season negatively affected CK levels, L*, b* and WBSF of mutton. There were animal-related factor (breed) effects on bruising with Dormer having higher levels of CK. It was concluded that, pre-slaughter conditions do not affect CK levels and but physico-chemical characteristics of mutton were negatively affected. Creatine kinase levels did not relate to physico-chemical characteristics of mutton.

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Chapter 4: Effects of pre-slaughter conditions and animal-related factors on physico-chemical characteristics of mutton

By

A.Y. Chulayo

Abstract

The objective of the current study was to determine the effects of pre-slaughter conditions and animal-related factors on physico-chemical characteristics of mutton. Furthermore, correlations among the mutton quality attributes were also determined. Data from 84 sheep of different breeds (28 South African Mutton Merino, 28 Dorper and 28 Blackhead Persian) brought for slaughter were used in the current study. The following pre-slaughter conditions: season, ambient temperature, distance travelled and distance duration, stocking density and lairage duration, were recorded at Adelaide Nxuba Municipal Abattoir (ANMA). Representative samples of the *Muscularis longissimuss thoracis et. lumborum* (LTL) muscle were used to determine ultimate pH (pH_u), colour (L*-lightness, b*- redness and a*- yellowness), thawing and cooking losses, and meat tenderness. The South African Mutton Merino had the highest values for pH_u (5.9±0.64), L* (34.2±0.97) and b* (12.2±0.50). Age group effects were observed on pH_u, cooking loss% and Warner Braztler Shear Force (WBSF) with Age Group 3 (13≤ 16 months) having the highest values. There were positive correlations between colour coordinates L* and b* (r =+0.22) and between a* and b* (r =+0.63). Cooking loss and WBSF values were positively correlated (r =+0.29). Linear relationships were observed between pre-slaughter variables (temperature, lairage duration, stocking density, distance travelled and distance duration) and

pH_u, colour, thawing loss, cooking loss and WBSF of mutton. It was therefore concluded that the age at which animals are slaughtered, breed, season and pre-slaughter conditions affect physic-chemical characteristics of mutton. Furtheromre, the Principal Component Analysis (PCA) showed a significant relationship among physico-chemical characteristics of mutton.

Keywords: temperature, distance, ultimate pH, colour, WBSF, age at slaughter, Principal Component Analysis

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4.1 Introduction

In Chapter 3, it was observed that most pre-slaughter conditions did not affect CK levels. It was also observed that CK levels are not related to mutton quality attributes. However, the quality of meat is likely to be affected by animal-related factors and pre-slaughter conditions (Vimiso, 2010). Animal-related factors are the underlying, inborn characteristics. These include age, breed and gender. The pre-slaughter period is the period before animal slaughter that includes loading of the animals, transportation, unloading times and the period of stay in the lairages (Geesink, Mareko, Morton & Bickerstaffe, 2001; Ljungberg, Gebresentbet & Aradom, 2007; Arain, Khaskheli, Rajput, Rao, Faraz, Fazlani *et al*., 2010; Rodriquez *et al*., 2011). During the pre-slaughter period, an animal becomes stressed because it experiences changes in the nearby environment which can either cause it to respond to exogenous or endogenous stimuli (Ndou, Muchenje & Chimonyo, 2011; Odore, Badino, Re, Barbero, Cuniberti, D`Angelo *et al*., 2011). Ferguson and Warner (2008) and Ndou *et al*. (2011) reported that prior to slaughter, animals become conscious and begin to react due to previous experience.

Some studies indicate that stress lowers *post mortem* pH_u values that produce tougher meats due to the fact that repletion of glycogen in ruminants is slow (Devine, Lowe, Wells, Edwards & Edwards, 2006; Gregory, 2010) while others reported that pre-slaughter stress resulted in higher pHu and darker cuts (Martinezo-Cerezo, Sańudo, Panea, Medel, Delfa, Sierra *et al*., 2005; Tejeda, Pena & Andres, 2008; Muchenje, Dzama, Chimonyo, Strydom & Raats, 2009a). Moreover, pre-slaughter handling has been reported to affect other meat quality attributes (Okeudo & Moss, 2005; Miranda-de la Lama *et al*., 2009; Muchenje *et al.*, 2009ab).

Meat quality is a generic term used to describe properties and perceptions of meat (Maltin, Balcerzak, Tilley & Delday, 2003). Meat quality encompasses attributes such as carcass composition and conformation, eating quality and health-related issues. Eating quality includes tenderness, flavour, juiciness and cooking loss (Martinezo-Cerezo *et al*., 2005; Muchenje, Dzama, Chimonyo, Raats & Strydom, 2008a; Tejeda *et al*., 2008). Schönfeldt and Strydom (2011) indicated that tenderness and juiciness are some of the determinants of overall meat eating quality while pH and colour affect the keeping quality and visual appeal of meat. Ultimate pH is regarded as more important than the others followed by colour (Hopkins & Fogarty, 1998; Muchenje *et al*., 2009b; Rodriguez *et al*., 2011). Consumers desire products depending on their personal, situational, product characteristics and production system.

Consumers demand tender, juicier, flavoured meat and healthy meat (Dalle Zotte, 2002; Font i Furnols, Realini, Montossi, Sańudo, Campo, Oliver *et al*., 2011). Physico-chemical characteristics are the determinants of meat quality and its acceptability by the consumers (Tejeda *et al*., 2008; Muchenje *et al*., 2008b). There is also a degree of correlation within and between these factors (Muchenje *et al*., 2008a, b). Correlations assist in measuring and providing more information on the strength of the relationship between meat quality traits and pre-slaughter variables. In addition, knowledge of the relationships among physico-chemical characteristics can be used to predict meat characteristics that are expressed *post mortem* (Okeudo & Moss, 2005; Muchenje *et al*., 2009a).

Available information shows that gender, breed, slaughter weight and ageing affect meat quality (Martinez-Cerezo *et al*., 2005). Cloete, Hoffman and Cloete (2008) compared carcass composition and meat quality from the progeny of Dormer and Suffolk sires and reported that breed had an effect on Ph and shear force values. Information on the effects of pre-slaughter variables such as distance, lairage duration and stocking density on physico-chemical characteristics of mutton in South Africa is limited. Therefore, the objective of the current study was to determine the effects of pre-slaughter conditions and animal-related factors on physico-chemical characteristics of mutton. Correlations among physico-chemical characteristics were also determined. The null hypothesis being tested was that pre-slaughter conditions and animal-related factors do not affect mutton physico-chemical characteristics and that there is no correlation among physico-chemical characteristics.

4.2 Materials and Methods

4.2.1 Study site

The study site is the same as described in Section 3.2.1.

4.2.2 Animal Management

Eighty-four sheep of different breeds that were brought for slaughter were studied. The breeds were South African Mutton Merino (SAMM), Dorper (DP) and Blackhead Persian (BP). There were 28 animals for each breed. The animals used were male castrates and were grouped according to age categories. The grouping was as follows: Group 1 (6-8 months), Group 2 (9-12) and Group 3 (13-16 months). Information gathered included; season, ambient temperatures, departure time from the farm and arrival, distance travelled and size of the truck. On arrival at the abattoir, animals were given water in the lairages at *ad-libitum*. They were not given feed. The lairage time was 1 hour. The sheep were slaughtered using an electric stunner of 650Volts for 5 seconds. They were hanged upside down and the throat was cut using a sharp knife. The animals were allowed to bleed for 6 minutes. After slaughter, carcasses were stored in a cold room at -2^{0}C for 24 hours.

4.2.3 Meat quality determination

Determination of pH_u, colour (L*, a* and b*), cooking and thawing loss percentage and WBSF are the same as detailed in Section 3.3.2.

4.2.4 Statistical analysis

The effects of breed, age group and season on pH_u, L*, a*, b*, thawing loss (Thaw %), cooking loss (CL) and WBSF of mutton were analysed using the PROG GLM of SAS (2003). The statistical model used was as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \lambda_k + \varepsilon_{ijk}$$

Where

Y_{ijk} - Response variable (pH_u, L, a*, b*, Thaw %, CL% and WBSF)

μ - Overall mean

α_i - Breed effect (Blackhead Persain, Dorper and SA Mutton Merino breeds)

β_j - Age group effects (Groups 1, 2 and 3)

λ_k - Seasonal effects (Hotwet and coldwet season)

ε_{ijk} - Random error term

Significant differences between least square means of pH_u, L, a*, b*, Thaw %, CL% and WBSF values were compared using the PDIFF procedure (SAS, 2003).

The strength of the relationships between physico-chemical characteristics were determined using Pearson`s correlation coefficient (SAS, 2003). Prediction equations between distance, lairage duration, stocking density and temperature in relation to physico-chemical characteristics of mutton were determined using regression analysis (PROG REG, SAS, 2003). The data was analysed using the following model:

$У = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_4 X_4,$ where

У = Response variable (pH_u, L, a*, b*, Thaw %, CL% and WBSF)

a = Intercept

b = Co-efficient variable

X_1 = Distance duration,

X_2 = Distance travelled,

X_3 = Lairage duration,

X_4 = Stocking density,

X_5= Temperature

The Principal Component Analysis (PCA) was used to interpret the relationship between physico-chemical characteristics (SAS, 2003).

4.3 Results and Discussion

Significant breed effects on pH_u, L, a*, b*, Thaw %, CL and WBSF of mutton were found in the current study (Table 4.1). Mutton from South African Mutton Merino had higher (P <0.001) values than Dorper and Blackhead Persian sheep for $pH_{u,}$ L* and b* of mutton. The differences observed between Blackhead Persian (BP), Dorper (DP) and SA Merino (SAMM) breeds for pH_u were expected. The results were not comparable to those of Teixeira, Batista, Delfa and Cadavez (2005) where he found no differences 24 hours after slaughter. However, Cloete *et al*. (2008) found difference in pH_u when comparing Merino breed with other breeds of sheep. The pH_u from DP and SAMM were above the normal range of pH 5.4 and 5.7 (Hoffman, Muller, Cloete & Schmidt, 2003) making the meat undesirable. Higher levels of pHu affect the conversion of muscles to meat because of reduced glycogen reserves and low lactic acid in the muscles after slaughter (Ferguson & Warner, 2008; Rodriguez *et al*., 2011).

Ultimate pH (pH_u) found in muscles is one of the contributing factors to the quality of meat. Mach, Bach, Velarde and Devant (2008) indicated that beef with pH_u above 6.0 is undesirable for human consumption because it leads to DFD (Dark Firm dry) meat. Dark Firm Dry meat is measured by L* coordinate which also depends on differences in pH_u (Rodriguez *et al*., 2011). The darker meat (low L* values) from Blackhead Persian may be attributed by reduced muscle glycogen and increased myogobin because of the light scattering protperties of meat. Moreover, degradation of proteins is increased at *post mortem* due to the dark colour of meat.

Table 4.1 Mean (±SE) for pH_u, L*, a*, b*, CL% and WBSF of mutton as affected by breeds

Meat quality characteristics	Breed			Significance level
	Blackhead Persian	Dorper	Merino	
No.	28	28	28	
pH_u	5.6 ± 0.06^a	5.8 ± 0.05^b	5.9 ± 0.06^b	***
L*	28.8 ± 0.86^a	31.7 ± 0.82^b	34.2 ± 0.97^c	***
a*	16.7 ± 0.49	17.2 ± 0.47	17.6 ± 0.56	NS
b*	9.1 ± 0.44^a	11.2 ± 0.42^b	12.2 ± 0.50^b	**
CL %	35.5 ± 2.17^b	33.0 ± 2.08^a	29.3 ± 2.46^a	***
WBSF	22.9 ± 1.33^a	26.4 ± 1.28^b	26.8 ± 1.51^b	**

^{a,b,c}Means with the different superscript in the same row differ significantly.

Level of Significance = **P < 0.01***P < 0.001, NS = Not significant

pH_u = ultimate pH

L* = lightness

a* = redness

b* = yellowness

CL% = Cooking loss percentage

WBSF =Warner Braztler Shear Force

The interaction of individual factors determines the colour of meat and it can also be affected by chemical stability of myoglobin (Muchenje *et al*., 2009b). Myoglobin is a pigment responsible for the colouration of meat and it is also determined by the rate of oxidation and is species dependant (Kannan, Kouakou & Gelaye, 2001; Kadim *et al*., 2008; Troy & Kerry, 2010). The dark colour of meat is undesirable to the consumers (Muchenje *et al*., 2009b).

The *M. longissimus thoracis et. lumborum* muscle from the SA Merino breed was lighter as compared to Dorper and Blackhead Persian. The differences found these breeds shows that animals have different levels of susceptibility to stress (Mota-Rojas, Becerril, Lemus, Sanchez, Gonzalez, Olmos *et al*., 2006). The higher L* value for SAMM is associated with the production of lactic acid prior slaughter. Higher glycogen reserves credited other activities that occur at *post moterm* that require energy such as breaking down the bond between muscles to improve on tenderness of meat (Martinez-Cerezo *et al*., 2005). Increased toughness for SAMM was not expected as increase in pH_u results in more tender meat. The WBSF values between BP, DP and SAMM could be attributed to the fact that they have different types of muscles (Muchenje *et al*., 2008a).

There were no breed effects (P >0.05) on a*. However, significant breed effects were observed on CL and WBSF values. The highest (P <0.001) CL and (P <0.01) WBSF values were observed in meat from BP and SAMM respectively. Increased tenderness could be due to the effects of pH_u activity of the proteolytic enzymes that degrade myofibrillar muscle structure (Wanatabe, Daly & Devine, 1996). However, Muchenje *et al*. (2008a) indicated that higher pH_u may not always be involved in toughness of meat as other meat parameters do.

The results contradict with the findings by Miranda-de la Lama *et al*. (2009) and Genaro, Miranda-de la Lama, Monge, Villarroel, Olleta, García-Belenguer *et al*. (2011) where tenderness increased with higher pH_u. Meat for BP was tender than DP and SAMM with decrease in pH_u. In the current study, Age Group 1 had tender meat as compared to age group two and three. Age-related tenderness depends on the amount of connective tissue, heat stability and myofibrillar structure found in muscles. An older animal has a connective tissue that is difficult to break down (MTU, 2000). Furthermore, Okeudo and Moss (2008) indicated that tenderness (shear force) is influenced by sarcomere length, connective tissue and proteolysis. However, in the current study, sarcomere length and proteolyses were not determined. Tender meat found in younger animals indicates that the diameter of the fibres and connective tissue has not fully developed (Brown, 2007).

Table 4.2 shows the effects of different age groups on mutton quality characteristics. Ultimate pH differed among the different age groups. Age Group 3 had the highest (P <0.01) pH_u values. The study agrees with findings by Tejeda *et al*. (2008) who repoted that greater pH_u is undesirable. Age had significant effects on cooking loss with the lowest (P <0.05) values observed for the youngest age group. Age affected WBSF values with meat from older sheep having higher (P <0.001) values than mutton from younger animals. Tougher meat for older animals was expected because the older the animal, the tougher the meat (Jeremiah, Tong & Gibson, 1998; Okeudo & Moss, 2008). Cooking loss % for group three was higher (42.2); this may lead to tougher meat (Jama *et al.*, 2008).

Table 4.2 Age (months) effects on pH_u, cooking loss % and WBSF values of the *M. longissimus thoracis et. lumborum* muscle

	Age (months)			
Meat quality characteristics	6≤ 8	9≤ 12	13≤ 16	Significance level
No.	28	28	28	
pH_u	5.7 ± 0.03^b	5.6 ± 0.05^a	5.9 ± 0.09^c	*
Cooking loss %	26.9 ± 1.30^a	28.4 ± 1.99^b	42.4 ± 3.72^c	*
WBSF	17.7 ± 0.80^a	26.1 ± 1.22^b	33.33 ± 2.29^c	***

$^{a, b,c,}$ Means with the same superscript in the same row are significantly different.

Significance difference = *P < 0.05, ***P < 0.001, NS = No significance difference

pH_u = ultimate pH

WBSF =Warner Braztler Shear Force

Cooking loss consists of non-aqueous fluid due to the fat that melts and structures that contain it are destroyed (Beattie, Burrows, Moss & Weatherup, 2002). During cooking, meat shrinks due to the effect of heat transfer reducing the weight of the meat (Jama *et al*., 2008; Geesink, Sujang & Koohmaraie, 2011). This is because at higher temperatures, the water content of meat becomes reduced while that of fat increases (Aaslyng, Bejereholm, Ertbjerg, Bertram & Andersen, 2003). As indicated by Chiavaro, Rinaldi, Vittadini and Barbanti (2009), cooking loss is a combination of soluble substances lost from the meat during cooking.

Table 4.3 shows the effect of season on pH_u, L*, cooking loss % and WBSF values of mutton. Higher (P <0.001) L*, b* and WBSF were observed during the hot wet season than in the cold wet season. The darker meat during cold wet season could be due to the shortage of feed however, hot wet season increased WBSF values. This indicates that availability of feed do affect meat quality. Cold wet season affects the colour of meat such that the shelf life is affected due to the fall of pH_u and normal muscle metabolism leading to dark cutting (Kim, Yoon, Song & Lee, 2003; María, Buil, Liste, Villarroel, Sańudo & Olleta, 2006).This also confirms that in dry season, livestock animals in poor condition (Gregory 2010; Dodzi & Muchenje, 2011) because forage is limited (Bakare & Chimonyo, 2011). The study agrees with the findings by Kadim, Mahgoub, Al-Kindi, Al-Marzooqi and Al-Saqri (2006); María *et al*. (2006) and Miranda-de la Lama *et al*. (2009) who reported that shortage of feed during winter season causes dark cutting. Stressful environment results in the reduction of glycogen levels in the muscles resulting to lower lactic acid production and high pH_u.

Table 4.3 Means (±SE) for pHu, Lightness (L*), cooking loss % and WBSF of mutton as affected by season

	Season		
Meat quality characteristics	Cold wet	Hot wet	Significance level
pH_u	5.7 ± 0.04	5.8 ± 0.06	NS
L*	29.4 ± 0.64[a]	33.7 ± 0.94[b]	***
b*	10.2 ± 0.33[a]	11.5 ± 0.48[b]	*
Cooking %	32.8 ± 1.63	31.3 ± 2.37	NS
WBSF	21.2 ± 0.99[a]	29.5 ± 1.46[b]	***

[a, b,] Means with the same superscript in the same row are significantly different.

Significance difference = *P < 0.05, ***P < 0.001, NS = No significance difference

pH_u = ultimate pH

L* = lightness

b* = yellowness

WBSF = Warner Braztler Shear Force

The b* values were lower during the cold-wet season than in hot-wet season. The results are in agreement with Kim *et al.* (2003) who indicated colour coordinates as affected by temperature, winter season and *ante-mortem* short-term stressors as they change the normal muscle metabolism. However, these values were in the acceptable range as indicated by Muchenje *et al.* (2009a). The yellow fat colour is due to higher levels of beta-carotene found in the grass during the hot-wet season. Studies indicate that the availability of grass is season-dependent (Bakare & Chimonyo, 2011; Dodzi & Muchenje, 2011) and in winter, there is scarcity of feed. Scarcity of feed also affects the grazing behaviour of the animal, muscle metabolism and meat quality attributes such as tenderness, $pH_{u,}$ and the colour (Kim *et al.,* 2003; Muchenje, Dzama, Chimonyo, Raats & Strydom, 2008, Muchenje *et al.,* 2009a, 2009b). Most attributes of meat are related to pH_u (Muchenje *et al.,* 2009b). Muscle glycolysis is governed by the activity of glycogen phosphorylase.

Depletion of glycogen reserves affects glycolysis which in turn results in increased pH_u. At high pH levels the oxymyoglobin is rapidly turned into dark red coloured reduced myoglobin and the muscle does not reflect well as it is less compact (Lambertini, Vignola, Badiani, Zaghini & Formigoni, 2006). This then affects the tenderness of meat. Tenderness could be affected by the extent of glycolysis through ultimate pH_u in the muscles (Ferguson *et al.,* 2001; Purchas, Sobrinho, Garrick & Lowe, 2002; Esenbuga, Macit, Karaoglu, Aksakal, Aksu, Yoruk *et al.,* 2009).

In the current study, increased toughness of meat was found during cold-wet season. The results agree with the findings by Miranda-de la Lama *et al.* (2010b) in lambs slaughtered in winter. According to Kadim *et al.* (2009), there is a relationship between myofibrillar disruption and improved tenderness although myofibril determination was not determined in the current study. Both myofibrillar and connective tissue are also used to determine the tenderness of meat although they are affected by cold weather (Kim *et al.,* 2009). This shows that the cold season was more stressful, probably due to feed scarcity to the animals than the hot season.

Correlation coefficients between physico-chemical characteristics of mutton are presented in Table 4.4 There was a negative correlation between L* and a* (r =-42), a* and cooking loss % (r =-0.28) of mutton. A positive correltation (r =+0.22) between L* and b* was also observed. Redness and yellowness of mutton were positively correlated (r =0.63) and cooking loss % and WBSF were positively correlated (r =+0.29). A positive relationship between L* and b* was expected because they are determined by glycogen levels in the muscles (Muchenje *et al.*, 2008a). Higher myoglobin and decreased glycogen levels results in darker and yellow meat (Priolo, Micol, & Agabriel, 2001). Forage fed animals tends to have yellow fat with darker colour (Muchenje *et al*., 2009b; Ekiz, Ozcan, Yilmaz, Tolu & Savas, 2010; Rodriguez *et al*., 2011). Meat colour is an attribute that is used to judge the freshness and quality of meat by consumers. Consumers associate yellow meat with deceased animals. The negative relationship between L* and a* in the study contradicts with the findings of Esenbuga *et al*. (2009).

Table 4.4 Pearson's correlation coefficients between physico-chemical characteristics of mutton

Physico-chemical characteristics	pH_u	L*	a*	b*	TL %	CL %	WBSF
pH_u	–	0.12	-0.04	0.13	-0.11	0.13	-0.11
L*		–	-0.42***	0.22*	-0.11	-0.09	0.10
a*			-	0.63***	0.08	-0.21	-0.15
b*				–	-0.03	-0.20	-0.04
TL %					–	0.04	-0.18
CL %						–	0.29*
WBSF							–

Significantly correlated at *P<0.05, **P<0.01, ***P<0.001, NS= Not significant

pH_u = ultimate pH

L* = lightness

a* = redness

b* = yellowness

TL% = thawing loss percentage

CL% = cooking loss percentage

WBSF = Warner Braztler Shear Force

Table 4.5 represents relationship between pre-slaughter variables (temperature, lairage duration, stocking density, distance and distance duration) and the physico-chemical characteristics (pH, colour, thawing loss, cooking loss and WBSF) of mutton. A positive relationship (P <0.001) between pH_u, cooking loss with pre-slaughter variables was observed. There is evidence that pre-slaughter conditions have deleterious effects on mutton quality (Miranda-de la Lama, Villarroel, Liste, Escos & María, 2010) as pH_u and colour coordinates were affected temperature, stocking density, distance and distance duration. Long distances increase pH_u leading to high risk of DFD meats and WHC. As indicated by Dalle Zotte (2002) and Adenkoya and Ayo (2010), ambient temperature and transportation are the major causes of stress.

Pre-slaughter conditions have been found to accelerate the depletion of ATP which leads to lower glycogen reserves of the *post mortem* muscle immediately after exsanguinations (Yu *et al.*, 2009). Reduced pH_u results in darker meat due to the exhaustion of glycogen reserves in the muscles. The results obtained for the colour of mutton were expected since an increase in muscle pH_u indicates increased muscle metabolism during pre-slaughter. An increase in transportation distance results in decreases in lightness and yellowness of meat while redness and ultimate pH increases. Animals do suffer during long journeys because of fasting and exhaustion (Van de Water, Verjans & Geers, 2003). In agreement with the current study, Lambertini *et al.* (2006) reported that the meat from rabbits was affected by pre-slaughter lairage duration and transportation. In addition, L* and b* were reduced while a* values increases. It therefore becomes important to measure the sources of stress in animals so as to improve meat quality and quantity (Mapiye *et al.*, 2011).

Table 4.5 Linear relationships between meat quality parameters and pre-slaughter variables (distance, temperature, distance, stocking density and lairage duration)

Parameter (Y)	Equation	Significance
pH_u	$3.46(0.41) + 0.008(0.001)\ X_1 + 0.01(0.002)\ X_2 + 0.001(0.0003)X_3 + 0.61(0.17)X_4 + 0.02(0.006)X_5$	***
L*	$52.2(7.16) – 0.06(0.02)X_1 - 0.02(0.006)X_3 - 0.34(0.10)X_5$	***
a*	$13.2(5.5) + 0.01(0.01)X_1$	**
b*	$18.2(3.3) – 0.01(0.003)X_3 - 3.07(1.41)X_4 - 0.11(0.05)X_5$	***

Significance difference = *P < 0.05, **P < 0.01, ***P < 0.001, NS = Not significant,

X_1 = Distance duration, X_2 = Distance, X_3 = Lairage duration, X_4 = Stocking density, X_5= Temperature

pH_u = ultimate pH

L* = lightness

a* = redness

b* = yellowness

Lightness had a negative (P <0.001) relationship with distance duration, lairage duration and temperature. Distance duration and a* were negatively related (P <0.001). There was a negative relationship observed between b* and lairage duration, stocking density and temperature. In the current study, both temperature and lairage duration had a negative impact on meat quality. This also implies that transportation affects glycogen levels (Devine *et al*., 2006; Ferguson & Warner, 2008; Muchenje *et al*., 2009b). Miranda-de la Lama *et al.* (2009) indicated that rough road causes stress but Kadim *et al*. (2009) reported stress on smooth road which then increases muscle pH and this is in agreement with the current study.

Figure 4.1 shows the relationship between $pH_{u,}$ L*, a*, b*, Thawing and cooking loss, WBSF, carcass weight, slaughter weight and dressing percentage (DP). Significant relationships were observed between $pH_{u,}$ L*, cooking loss, WBSF and DP. The relationship observed between pH_u and L*, a*, CL and WBSF could mean that the rate at which lactic acid is produced determines the colour of meat, amount of water lost during cooking hence tenderness of meat (Aasylyng *et al*., 2003). Aasylyng *et al*. (2003) and Chiavaro *et al*. (2009) reported that cooking loss depends on the cooking method, rate of cooking and temperatures. Darker meats tend to lose more water leading to tougher and undesirable meat (Font i Furnols *et al*., 2011). The increase in toughness of meat with increased pH_u was reported by Miranda de-la Lama *et al*. (2009). A relationship between L* and a*, SLW, DP and WBSF could be attributed to the amount of intramuscular fat, age of the animal prior to slaughter and changes in the myoglobin content in the muscle (Muchenje *et al*., 2009b). The bright-red meat was expected because of the amount of myoglobin content of the muscle.

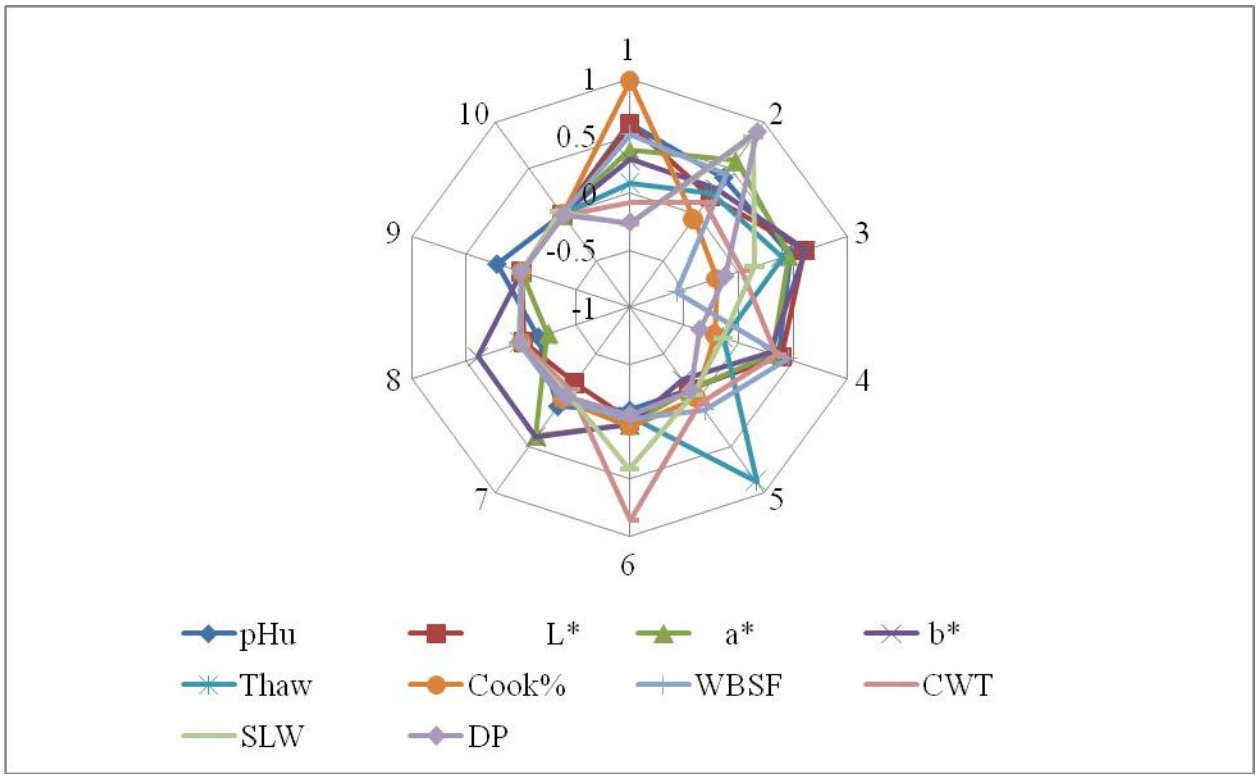

Figure 4.1 Plot of the relationship between physico-chemical characteristics of mutton

4.4 Conclusions

Results from this study show that pre-slaughter conditions, such as temperature, distance and lairage duration have a negative effect on physico-chemical characteristics of mutton hence we therefore reject the null hypothesis. In addition, the age at which animals were slaughtered, breed and season affected physico-chemical characteristics of mutton. Correlations were observed between physico-chemical characteristics of mutton.

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Chapter 5: General Discussion, Conclusions and Recommendations

5.1 General Discussion

Pre-slaughter conditions include activities that occur from the farm to the abattoir. When these conditions prevail, animals strive to cope with the novelty of the setup and environment. The conditions impose stress and cause bruises resulting in poor meat quality. Stress is a condition that negatively affects glycogen levels in the muscles as the animal tries to withstand and survive the conditions (Bourguet *et al.*, 2010). Some animals are bruised without exhibiting the signs of stress and the meat quality becomes affected (Gregory, 2008). Since sheep do not exhibit signs of bruising, Creatine Kinase (CK) levels maybe used to determine the extent of muscle damage. Bruises may affect both CK levels and the quality of meat (Martínez-Cerezo, Sańudo, Panea, Medel, Delfa, Sierra *et al.*, 2005). Animal welfare is affected; more cuts are made after slaughter which may assume economic relevance. Therefore, the focus of the current study was to determine the effects of pre-slaughter sheep handling and animal-related factors on physico-chemical characteristics on mutton from South African sheep breeds (Dormer, Dorper, South African Mutton Merino and Blackhead Persian).

In Chapter 3, effects of pre-slaughter conditions on CK levels and their relationship with physico-chemical characteristics of mutton were determined. With the exception of temperature, pre-slaughter conditions did not affect CK levels. High ambient temperatures cause exhaustion in animals thereby affecting the ability of the animals to cope with adverse conditions (Mach, Bach, Velarde & Devant, 2008). Temperature is also linked with season such that during the hot-wet

season, animals had higher CK levels than in the cold-wet season. This proves that, during hot conditions, animals require more energy to withstand the adverse situations, simultaneously maintaining their body weight (Kadim, Mahgoub & Al-Marzooqi, 2009). Creatine kinase production was found to be breed dependent, with Dormer (DM) recorded the highest CK levels. Animals differ in their tolerance of stressful situations and consequently in their stress status (Bourguet *et al.*, 2010). The Principal Component Analysis (PCA) results showed a significant variance for CK and physico-chemical characteristics of mutton. This highlighted the important physico-chemical characteristics of mutton including pH_u and colour of mutton. There were no relationships among CK and physico-chemical characteristics.

The results of the study presented in Chapter 4 revealed that animal-related factors such as breed and age affected mutton quality. Among the three breeds, South African Mutton Merino had higher values for ultimate pH (pH_u), lightness (L*) and yellowness (b*) than Blackhead Persian and Dorper breeds. However, mutton with higher pH_u, had, unexpectedly, higher L* values. This unexpected relationship could be due to the complex interaction of factors affecting meat quality. Examples of such factors are feeding patterns (Mapiye, Chimonyo, Dzama, Muchenje & Strydom, 2010), pre-slaughter treatment (Santos, Silva, Silvestre, Silva & Azevedo, 2008), age (Schönfeldt & Strydom, 2011), breed (Martínez-Cerezo *et al.*, 2005; Dyubele, Muchenje, Nkukwana & Chimonyo, 2010), ticks and diseases (Muchenje, Dzama, Chimonyo, Raats & Strydom, 2008a; Marufu, Chimonyo, Dzama & Mapiye, 2010) and pre-slaughter conditions (Muchenje, Dzama, Chimonyo, Strydom & Raats, 2009; Becerril-Herrera, Alonso-Spilsbury, Ortega, Guerrero-Legarreta, Ramírez-Necoechea, Roldan-Santiago *et al.*, 2010; Miranda-de la Lama, Villarroel, Liste, Escósa & María, 2010). Differences in meat colour could be associated

with variations in intramuscular fat and moisture content, age (Muchenje, Dzama, Chimonyo, Raats & Strydom, 2008b) and species-dependent changes in muscle myoglobin content (Kannan, Kouakou, & Gelaye, 2001). This is due to the fact that animals have different growth rates that affect intramuscular fat content (Ndlovu, Chimonyo & Chimonyo, 2009). The colour of meat is also linked to cooking loss and meat tenderness (Warner Braztler Shear Force -WBSF).

Consumers use colour and tenderness to access the quality of meat and decide on its acceptability (Strydom, Naude, Smith, Scholtz & Van Wyk, 2000; Muchenje, Dzama, Chimonyo, Strydom, Hugo & Raats, 2008c; Dyubele *et al.*, 2010; Chulayo, Muchenje, Mwale & Masika, 2011). Bright colour is associated with fresh and tender meat and usually has a smaller proportion of cooking loss although cooking loss is mostly dependent on muscle pH and cooking conditions (Kannan *et al.*, 2001). Moreover, this indicatesthat thedegree of correlations exist among meat quality attributes. As shown in Chapter 4, WBSF was significantly correlated with cooking loss but negatively correlated with the meat colour (a*).

5.2 Conclusion

It was concluded that pre-slaughter conditions, such as distance and lairage duration do not affect CK levels although they negatively affected physico-chemical characteristics of mutton. However, high temperatures and animal related factors affected CK levels. In addition, the age at which sheep were slaughtered, breed and seasonal variations affected the physico-chemical characteristics of mutton. Correlations were observed among physico-chemical characteristics of mutton. Therefore, pre-slaughter, sheep should be monitored in order to reduce stress-related factors. This improves meat quality.

5.3 Recommendations

Based on the findings reported in the current study, it is recommended that a checklist of animal welfare for farmers that are keeping animals, transporting and handling animals should be drawn. This would help improve pre-slaughter sheep handling from the farm to the abattoir, which ultimately improves meat quality. There is also a need for farmers to work hand-in-hand with abattoir employees to familiarise themselves with the conditions that affect animal welfare and meat quality. In addition to the issues raised above, more research should be conducted focusing on the following:

1. Physico-chemical characteristics of mutton such as $pH_{u,}$ colour coordinates and WBSF could be affected by the amount of intramuscular fat, myofibrillar index, collagen content and sarcomere length. Further instrumental measurements on these factors are desirable such as the sarcomere length, MFI, intramuscular fat and collagen content.
2. Physiological and biochemical parameters should be considered in relation to meat quality. This is due to the fact that pre-slaughter handling affects the animal's physiologic, biochemical changes and meat quality. Although CK was measured in the current study, other physiological parameters such as levels of catecholamines, glycogen and other enzymes were not determined. This is necessary as it ultimately indicate the release of these hormeones due to pre-slaughter stress. Meat quality maybe affected due to higher levels of catecholamines and glycogen in the muscles.
3. Responsiveness to stress and depletion of glycogenof sheep to external stimuli such as thermal stress, heart and respiration rates should be determined. All of them are indicators of stress that affect mutton quality. Classification of carcass bruising also is likely to be beneficial because it provides information relating to harmful events that occur prior to slaughter.
4. Seasonal variations in nutrition also affect meat quality. Therefore, the nutritional composition needs to be evaluated.
5. Consumers are concerned about the conditions under which sheep are reared, slaughtered and processed. This necessitates undertaking a survey on consumer patterns (sensory evaluation) of mutton and specific preferences.

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List of Appendices

Appendix 1: Sheep transport Recording Sheet

Delivery Date	Loading/ departure time	Time of arrival	Slaughter date	Time of slaughter	Vehicle dimensions Length x Width	No. of sheep in vehicle	Day temp ^{0}C	Farm to Abattoir distance (km)	Type of breed

Appendix 2: Carcass record sheet for pH an Colour

									Colour 24hr		
Date	Sheep No.	Cas. No.	Breed	Age	Gen (M/F)	Wgt (1)	Wgt (2)	24hr pH	L*	a*	b*

Appendix 3: Thawing and cooking loss record sheet

Date	**Sample No.**	Wt Frozen (g)	Plastic Wt (g)	Initial wt (g)	Final wt (g)	**Thawing loss %**	Wt aft cooking (g)	**Cooking loss%**