Glycolytic potential and meat quality from Dorper and Merino sheep

By

Stempa Thuthuzelwa

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Department of Livestock and Pasture Science

Faculty of Science and Agriculture

University of Fort Hare

P/Bag X1314

Alice

South Africa

University of Fort Hare

Together in Excellence

Supervisor: Professor V. Muchenje

Declaration

I, Thuthuzelwa Stempa , vow that this dissertation has not been submitted to any University, and that it is my original work conducted under the supervision of Professor V. Muchenje. All assistance towards the production of this work and all the references contained herein have been fully accredited.

Stempa T.  **Date: 09 October 2015**

Approved to style and content by:

Prof. Muchenje V  **Date: 09 October 2015**

Abstract

Glycolytic potential and meat quality from Dorper and Merino sheep slaughtered at a commercial abattoir

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Thuthuzelwa Stempa

The objective of the study was to determine glycolytic potential and meat quality from Dorper and Merino sheep of both sexes slaughtered at a commercial abattoir. Dorper (*n*=52) and Merino (*n*= 48) breeds aged eight years, consisting of 50 intact rams and 50 non-pregnant ewes were used in the study. The sheep used in the study were reared, transported and lairised under identical conditions. Blood samples were collected at exsanguination for the measurement of glucose, lactate and cortisol levels. Samples were also collected from the *Muscularis longmissius thoracis et lumborum* (LTL) for the measurement glycogen, lactate levels, pH decline and colour. Correlations amongst blood stress indicators, muscle metabolites and meat quality attributes were also determined. Sex and breed had no effect on muscle glycolytic potential, glycogen and lactate levels from Dorper and Merino sheep of both sexes at the abattoir. Although sex and breed had an effect on pre-slaughter stress indicators (lactate and cortisol) collected at exsanguination. Ewes had higher levels of blood lactate (7.4 3 ± 0.49 mmol/L) and cortisol (293.92 ± 14.32 nmol/L) than the rams which had (5.19 ± 0.49 mmol/L) and (179.50 ± 14.32 nmol/L) lactate and cortisol levels, respectively. Furthermore, higher levels of lactate were observed in Dorper (7.54 ± 0.42 mmol/L) compared to the Merino sheep (4.97 ± 0.49 mmol/L). Meat pH decline and colour were also significantly affected by sex and breed. Ewes had higher levels of at pH_{45} minutes post slaughter

(7.05 ± 0.04), pH_3 hours (6.45 ± 0.04) , pH_{24} hours (6.00 ± 0.03), a* (14.31 ± 0.33) , b* (8.84 ± 0.29), H* (31.47 ± 0.73) and C* (16.75 ± 0.24) compared to the rams which had pH_{45} minutes (6.44 ± 0.04), pH_3 hours (6.12 ± 0.04), pH_{24} hours (5.88 ± 0.03), a* (12.25 ± 0.33), b* (7.00 ± 0.29), H* (29.36 ± 0.73) and C* (14.15 ± 0.42) values. Moreover, Merino sheep had higher levels of L* (38.17 ± 0.48) and H* (31.59 ± 0.74) compared to the Dorper which had (36.39 ± 0.46) and (29.33 ± 0.71) L* and H* values, respectively. Blood cortisol was also positively correlated ($P < 0.05$) to glucose ($r = 0.27$), lactate ($r = 0.37$) but was negatively correlated ($P < 0.001$) to meat lightness ($r = -0.44$). Furthermore, blood cortisol was positively correlated ($P < 0.001$) to pH_{45} ($r = 0.34$), pH_{24} ($r = 0.22$), meat yellowness ($r = 0.24$) and chroma ($r = 0.37$), but was negatively correlated to meat lightness ($r = -0.47$). Glycolytic potential was positively correlated ($P < 0.001$) to muscle glycogen levels ($r = 0.66$) and muscle lactate ($r = 0.71$).

Key words: cortisol, ewes, glycogen, glycolysis, glucose, lactate, post-mortem, pH decline, rams

Dedication

I would like to dedicate this dissertation to my family, my mother, father, sister Nosisa, brothers Lonwabo and Siyamthanda and lastly my niece Asemahle and nephew Ntando. Without their prayers and support this journey would not have been possible.

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Abbreviations

a^* = Redness

b^*= Yellowness

B.Glu = Blood glucose

C*= Chroma

DFD = Dark firm dry

ELISA= enzyme linked immunoassay

GP= glycolytic potential

H*= Hue angle

HPA - Hypothalamic-pituitary-adrenal axis

L^* = Lightness

LSD = Least Significant Differences

LTL = *Muscularis longmissius thoracis et lumborum*

mmol/L = Millimoles per litter

nmol/L = Nanomole per litter

M.Gly = Muscle glycogen

M.Lac = Muscle Lactate

n= sample size

pH_{24} = pH at 24 hours

pH_3 = pH at 3 hours

pH_{45} = pH at 45 minutes

PROC GLM = Generalised linear model procedure

r = Pearson's correlation coefficient

rpm = runs per minute

SAM = sympatho-adrenal system

SAS = Statistical Analysing System

SSTTM II = serum separating tubes

µl = Micro litre

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Chapter 1: General introduction

1.1 Background

During the pre-slaughter phase it is inevitable that sheep will experience some level of stress. Response to stress may be influenced by intensity, duration or the individual susceptibility to the stressor (Ferguson and Warner, 2008). Furthermore animal species respond differently to exposure to stress for example, the manner in which pigs and cattle respond to stress differs from that of sheep. Sheep are known to be naturally stoic creatures as they do not exhibit obvious signs of distress (González *et al.,* 2013). Sheep usually tolerate severe injury without evident signs of distress (Hemsworth *et al.,* 2011).Hence there is a need to measure blood stress indicators, muscle glycogen levels and meat quality attributes in order to quantify sheep response to pre-slaughter stress.

Exposure to pre-slaughter stress leads to deviations in sheep's normal physiological functioning. In addition, these deviations are mechanisms by which sheep cope with the external (physical stress) and internal environment (homeostasis) (Ferguson and Warner, 2008). These modifications lead to an increase of stress indicators in the blood. Therefore, elevated levels of cortisol, lactate and glucose at exsanguination are indicators of exposure to a stressful encounter (Gruber *et al*., 2010). Moreover an escalation in blood glucose, lactate and cortisol is associated with high pH values, lower lightness (L*) and redness (a*) values, thus indicating that blood stress indicators have an impact on sheep meat quality attributes (Škrlep *et al.,* 2009; Dokmanovic *et al.,* 2015).

The extent and rate of post-mortem drop in pH of the muscle is directly influenced by the "glycolytic potential" (Hudson, 2012). Glycolytic potential refers to the measure amount of glycogen and glucose substrates available to produce lactic acid for consequent decline in pH (Hamilton *et al.,* 2003). Monin and Sellier (1985) also described glycolytic potential as an index for muscle's capacity to carry out glycolysis at post-mortem and regulate the extent of pH decline. The glycolytic potential model indicates that high muscle glycogen concentrations pre-slaughter regulate the rate of pH decline in sheep (Daly *et al.,* 2006; Hudson, 2012). Consequently low glycogen reserves are detrimental to meat quality since it directly contributes to reduced acidification in the muscle thus a high pHu. Furthermore, high pHu in muscles is often observed as a quality defect known as dark cutting (Tarrant, 1989). Consequently, dark cutting meat results in large economic losses due to reduced shelf life because of high microbiological activity. Moreover, dark cutting meat is deemed aesthetically unacceptable to consumers due to the dark colour and reduced tenderness (Warner *et al.,* 2005). Exposure to stress triggers muscle glycogen depletion in the live animal, thus reducing glycogen reserves at post-mortem (Pethick *et al.,* 1995).

Response to pre-slaughter stress differs depending on animal related factors like sex (Johnson *et al.,* 2005; Hopkins *et al.,* 2007) and breed (Mortimer *et al.,* 2010; 2014), thus affecting meat quality (Hopkins *et al*., 2011; Guerrero *et al.,* 2013; Hopkins and Mortimer, 2014). Hence it is important to take into account sex and breed differences when investigating glycolytic potential, meat quality and their correlations from Dorper and Merino sheep slaughtered at a commercial abattoir.

1.2 Problem statement

During the pre-slaughter period sheep are exposed to a variety of stressors starting from the farm to the abattoir. They are stressed by novelty of the environment, disruptions in social groups, unfamiliar noises, smells and handling (Gradin, 1997; Terlouw *et al.,* 2008). However, sheep

usually tolerate severe discomfort without exhibiting evident signs of distress (González *et al.*, 2013). Hence most studies on measuring stress responsiveness using blood stress indicators (glucose, lactate and cortisol), glycolytic potential, meat quality and their correlations were carried out on pigs (Hambrecht *et al.*, 2005; Edwards *et al.*, 2010; Choe and Kim, 2014; Dokmanovic *et al.*, 2015) and cattle (Immonen *et al.*, 2000; Hopkins *et al.*, 2014; Boles *et al.*, 2015). However, similar work on sheep has mostly been conducted in Australia (Daly *et al.*, 2006; Ferguson *et al.*, 2008; Pighin *et al.*, 2014) but such evidence is also important in South Africa. Most studies have not investigated sex and breed differences on blood stress indicators, glycolytic potential, mutton quality and their correlations.

Furthermore, exposure to stress leads to activation of the hypothalamus –pituitary- axis (HPA) thus releasing cortisol. Cortisol is a well-known stress indicator which activates the release of catecholamines into the blood stream and also increases glycogenolysis. This whole process increases glucose levels in the blood as the body tries to maintain homeostasis (Bolander, 2004). During exposure to stress prior to slaughter glycogen reserves are significantly depleted due to the release of stress indicators and catecholamines into the blood stream. When glycogen reserves are depleted prior to slaughter the muscle is unable to produce the required amounts of lactic acid in the muscle to allow the muscle acidification to occur. Hence a high ultimate pH is evident which leads to various quality defects such as dark colour and susceptibility to microbial spoilage a condition known as dark firm and dry (DFD). Such meat is condemned and deemed unacceptable by consumers, which results in large economic losses (Hemsworth *et al.*, 2011).

1.3 Justification

South African sheep meat consumption in 2012, was reported to be approximately 149 000 tons, from this number merely 140 000 tons were from within South Africa, which is an indication

that there is a need to improve mutton production efficiency (DAFF, 2012). Hence, it is important to raise awareness on how sheep pre-slaughter stress response affects physiological reactions, muscle metabolites and mutton in order to optimise sheep meat production. It is also important to understand the relationships between blood stress indicators, glycolytic potential and meat quality from non-pregnant ewes and intact rams of Dorper and Merino breeds. This study will have a significant impact in providing recommendations to abattoirs on how they can improve the slaughter process and to produce meat of high quality to prevent economic losses due to exposure to stress prior to slaughter.

1.4 Objectives

The main objective of the study was to determine the levels of blood metabolites (cortisol, glucose and lactate), glycolytic potential, mutton quality and their correlations from intact rams and non-pregnant ewes in Dorper and Merino sheep

The specific objectives were:

- To determine sex and breed effects on blood glucose, lactate, cortisol during exsanguination and their correlations
- To determine sex and breed effects on muscle glycogen, muscle lactate levels, glycolytic potential, mutton quality and correlations amongst blood, muscle metabolites and some meat quality attributes

1.5 Hypothesis

- Null hypothesis were that:

- Sex and breed do not have an effect on blood glucose, lactate, cortisol at exsanguination and their correlations
- Sex and breed do not have an effect on muscle glycogen, muscle lactate levels, glycolytic potential, mutton quality and correlations amongst blood, muscle metabolites and some meat quality attributes

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Chapter 2: Literature review

2.1 Introduction

When sheep are presented for slaughter at the abattoir, it is inevitable that they will experience exposure to stress (Bourguet *et al.,* 2010; Ferguson *et al.,* 2014). Sheep are stressed due to transportation, feed deprivation, temperature extremes social disturbance, pain and discomfort (Terlouw *et al.,* 2008). Furthermore, pre-slaughter stress response affects physiological reactions through the release of stress indicators (glucose, lactate and cortisol) into the blood stream thus affecting metabolism post slaughter (Coombes *et al*., 2014). Cortisol induces rapid glycogen metabolism through glycogeonesis (Grandin, 1993), thus increasing the levels of glucose in blood. Moreover, lactate is the finale product of glycogen metabolism during anaerobic environments. It is expected that when cortisol and glucose levels in the blood increases, lactate levels also increase due to the positive correlation which exists among these stress indicators (Choe and Kim, 2014). These reactions occur when sheep try to maintain homeostasis within the body and may have an influence on the quality of meat produced (Coombes *et al*., 2014). Consequently, stress indicators mobilise glycogen which also has an influence on the reserves to be used to lower the muscle pH post-mortem (Ferguson and Gerrard, 2014).

Post mortem glycogen reserves are the primary source for ATP in the muscle and are also responsible for the production of lactate which lowers the pH of meat (Ferguson *et al.*, 2008). If muscle glycogen reserves decrease lower than of 45 to 55 mmol/kg, which is critical threshold the standard ultimate pH of meat (5.5-5.6) will not be reached (Tarrant, 1989) thus leading to reduced meat quality. Moreover, meat that has a pH higher than 6.00 is referred to as a dark cutting (Thompson, 2002). The aim of this review is to understand the impact of pre-slaughter stress

response on glycolytic potential and mutton quality of Dorper and Merino sheep slaughtered at a commercial abattoir.

2.2 Abattoir characteristics

The main role of an abattoir is to convert of animals to meat that is safe and suitable for human consumption. Abattoirs differ based on species slaughtered, structure and management practices (RMAA, 2011). Although there are such differences all abattoirs are obliged to operate abiding by Meat Safety Act No 40 of 2000 (SAMIC, 2006) to ensure public health and safety. This act is one of the laws governing abattoir operations in South Africa. Moreover there are mainly three classes of red meat abattoirs in South Africa namely the rural, low throughput and high throughput (Roberts *et al.,* 2009; Tshabalala, 2011).

The manner in which abattoirs are designed is usually based on conventional architectural criteria, such as optimal usage of space to facilitate movement of animals. However, meat quality is also affected by abattoir operations which are influenced by structure, machinery and handling practices (Chulayo *et al.*, 2012). Meat produced from less intensive production systems is often of lower quality compared to that from more intensive (Ahnstrom *et al.,* 2012). Furthermore, commercial abattoirs invest more in equipment and advanced machines (Gregory, 2005), whilst majority of municipal abattoirs in rural areas usually have less sophisticated equipment and handling facilities (Ndou *et al*., 2011; Njisane and Muchenje, 2013).

2.3 Transportation

Transportation is a novel experience to sheep as they are used to being at the farm throughout their lives (Knowles, 1998; Zhong *et al.,* 2011; Chulayo *et al.,* 2012). When sheep are being transported they are often grouped by farm workers (Mpakama *et al.,* 2014). This is a complex process that

has many risks that induce stress in sheep such as live weight loss, dehydration and even death may occur (Kannan *et al*., 2000; Warriss, 2004; Fazio *et al*., 2005) thus resulting in large economic losses.

The level of stress during transport varies depending on factors such as duration, intensity and breed (De la Fuente *et al*., 2010). Moreover, transportation stress results in physiological modifications due to newness of environment, noise and smell (Galipalli *et al.*, 2004; Erkiz *et al.*, 2012). Whilst physical stress may result from activities such as such as loading, maintenance of posture and balance during transportation long distances, poor driving skills and high stocking densities also contribute to physical exhaustion (Crockram *et al.*, 2004; Mareko, 2005). Both physical and physiological stress results in an escalation in blood metabolites such as glucose, lactate and cortisol. These stress indicators may also affect the quality of the meat produced, hence the manner in which sheep are transported has a major influence on the meat quality (Gruber *et al*., 2010).

2.4 Lairages

Lairages are where animals are kept to allow them to recover from transportation stress at the abattoir (Kannan *et al.*, 2000; Gallo *et al.*, 2003; Weeks, 2008). Lairages allow sheep to get a chance to rehydrate and recover from transportation stress before slaughter (Erkiz *et al*., 2012; Díaz *et al*., 2014). Particularly, in commercial abattoirs it is a common practice for sheep to be kept to overnight prior to slaughter (Kannan *et al*., 2000). This is done to enable restoration of muscle glycogen levels to ensure that the final meat quality is not compromised (Mounier *et al*., 2006). However, the rate of recovery at lairage may be affected by factors such as duration (Knowles, 1998), environmental conditions (Weeks, 2008), social interactions, access to food and water (Manteca, 2008).

Moreover, if lairage is not conducive for rest and recovery it can be a stressful experience to sheep (Díaz *et al*., 2014). During the lairage period it is necessary to allow the sheep to lie down and rest. Furthermore, it is highly recommended for sheep to remain in their original social grouping and not be crowded in order to facilitate rest and recovery (Jarvis and Cockram, 1995). Consequently, high levels of blood cortisol were reported after transportation however, they later decreased after six hours of lairage (Knowles *et al.,* 1993). This is an indication that lairage significantly reduces stress after transportation. However, even though lairage is meant to replenish glycogen reserves after transportation some experiments have found a decrease in muscle glycogen and a high meat pH after prolonged lairage duration (Jones *et al.,* 1990). Purchas (1992) reported that meat pH increased from 5.64 to 5.92 after lairage time was increased from 4 hours to 24 hours.

2.5 Pre-slaughter fasting

Pre-slaughter fasting of sheep facilitates the evisceration process and reduces risk of carcass contamination with gastrointestinal contents (Gregory, 1998). However, feed deprivation has been reported to reduce live and carcass weights in sheep. Sheep fasted for 24 hours pre-slaughter could lose up to seven percent of their body weight due to the reduction of gut contents. Whilst in short term feed deprivation live weight change is mainly be due to gut fill variation (Chillard *et al.*, 1995). During the early hours of fasting small ruminants utilise glucose as an initial source of energy. Furthermore, the body manufactures more blood glucose thus concentrations elevate during the breakdown of glycogen from the liver (Murray *et al.*, 1990). Consequently, after 24 hours of fasting glycogen levels are severely depleted thus negatively affecting the meat quality (Gregory, 1998). Particularly a decrease in muscle glycogen results in meat with a high ultimate pH due to reduced lactate production during glycolysis (Partanen *et al.,* 2007). Prolonged sheep

fasting increases the circulating levels of corticosterone (Muranyama *et al.,* 1986), which is an indication that feed deprivation increases stress levels.

2.6 Classification of stress indicators in relation to pre-slaughter stress

2.6.1 Cortisol

Cortisol is an adrenocortical hormone which is regarded as a one of the most important stress hormones in animals (Gregory, 1998; Choi *et al.*, 2012; Russell *et al.*, 2012).When an animal is exposed to a stressful situation the hypothalamic-pituitary-adrenal axis (HPA) is stimulated to produce cortisol from the adrenal gland into the blood stream (Minton, 1994; Terlouw, 2005). Exposure to stress disrupts normal homeostasis, hence cortisol is secreted in higher levels as a means to try and cope with the internal homeostatic imbalance (Schulze *et al.,* 2009). Cortisol has many important functions in the animal's body such as, the maintenance of blood glucose levels and enhancement of the activity of catecholamines at target tissues (Djuric *et al.,* 2008). Furthermore there is supporting evidence that cortisol and catecholamines (stress hormones) cause metabolic and /or structural post-mortem modifications in muscles (Kraemer and Ratamess, 2005; Ferguson and Warner, 2008). Hence high levels of cortisol reduce the amount of glycogen present for lactate production and meat pH decline (Terlouw, 2005).

2.6.2 Glucose

Glucose is known as one of the most important compounds in life processes. It is referred to as the primary source of energy for important life processes such as respiration and glycolysis (Galant *et al.,* 2015). Despite its many functions, high glucose levels in the blood are regarded as an acute indication of stress. The relationship between stress and glucose levels is intermediated via catecholamines and glucocorticoids (Shaw and Tume, 1992) through a HPA response.

Furthermore, the release of catecholamines and cortisol elevates the concentration of glucose in the blood stream (Gruber *et al.,* 2010). This occurs through a process known as tachycardia, where catecholamines are released and they cause an increased metabolic rate resulting in increased body temperature (Gruber *et al.,* 2010). These catecholamines then fuel a process known as glucogenolysis, which results in the utilisation of hepatic and muscle glycogen reserves thus an increase in the concentration of glucose in blood. Consequently, elevated levels of glucose are noticeable during the pre-slaughter period in stressed sheep (Knowles and Warriss, 2007). Furthermore, glucocorticoids promote the breakdown of muscle protein and fatty acid reserves which release glucose into the blood stream (Mareko, 2005), thus increasing blood glucose levels due to stress response. Deviations in blood glucose levels may be associated with stress (Mota-Rojas *et al*., 2012) since elevated glucose concentrations in the blood are an indication of physiological stress (Kannan *et al*., 2002).

2.6.3 Lactate

Elevated levels of lactate in exsanguination blood are often associated with pre-slaughter stress. However, there are many factors which influence blood lactate accumulation these factors include, transportation, rough handling, breed and sex (Bórnez *et al.,* 2010). Increased blood lactate is known as a possible indicator of both physical and psychological stress (Dokmanović *et al.,* 2014) such as physical exercise and fatigue (Fernandez *et al.,* 1994). Moreover, the circulating levels of lactate are also elevated by the animal's behavioural responses to stress (Edwards *et al*., 2010), since lactate is finale product of glycogen of mobilised glycogen in anaerobic conditions. Therefore high levels of blood lactate indicate exposure to stress and may result in reduced meat quality (Hambrecht *et al*., 2005; Edwards *et al*., 2010), since the glycogen levels will be depleted at post mortem (Coombes e*t al.,* 2014). Elevated lactate levels are also associated with lower initial

pH, a higher drip loss leading to decreased meat quality due to rapid muscle metabolism pre and post slaughter (Edwards *et al*., 2010).

2.7 Role of muscle glycogen at post-mortem

At post mortem the muscle comprises a trivial amount of a specific carbohydrate referred to as glycogen. The glycogen concentration in live sheep and cattle muscles ranges from 75 to 120 mmol/kg (Immonen *et al.,* 2000). Particularly, in the living animal glycogen is an energy reserve for muscles and it is used as aid in muscle contraction. It also has important biochemical functions in sheep homeostatic control of glucose and energy balance. Specifically in sheep and cattle (ruminants) glycogen levels can be affected by exercise, nutrition and pre-slaughter management. In the first 12 hours after slaughter muscle glycogen is broken down to lactic acid, a process referred to as the glycolytic cycle (Pethick *et al.,* 1995).

The extent of muscle pH reduction after slaughter has an important influence on meat quality. Therefore, adequate glycogen levels at slaughter are essential to ensure sufficient lactic acid production post-mortem to enable acidification of the muscle thus an ultimate pH of 5.5 in meat will be reached (Pethick *et al.,* 1995). An amount of 0,81g / 100g of glycogen is necessary to reduce the pH of 1 kg of muscle from 7.2 in live animal to 5.5 at post-mortem. This means a negative exponential association between glycogen concentration and pHu exists (Warriss, 1990). Low ultimate pH (5.5) is essential for high quality meat since it is associated with a bright red colour, improved tenderness, flavour, shelf-life and cooking qualities (Devine *et al.,* 1993, Pethick *et al.,* 1995).

When sheep experience stress prior to slaughter glycogen levels plunge lower than the normal range of 45-55 mili-mole per kilogram hence the acceptable ultimate pH (5.5) of meat will not be reached (Tarrant, 1989). When sheep are stressed prior to slaughter, the physiological response

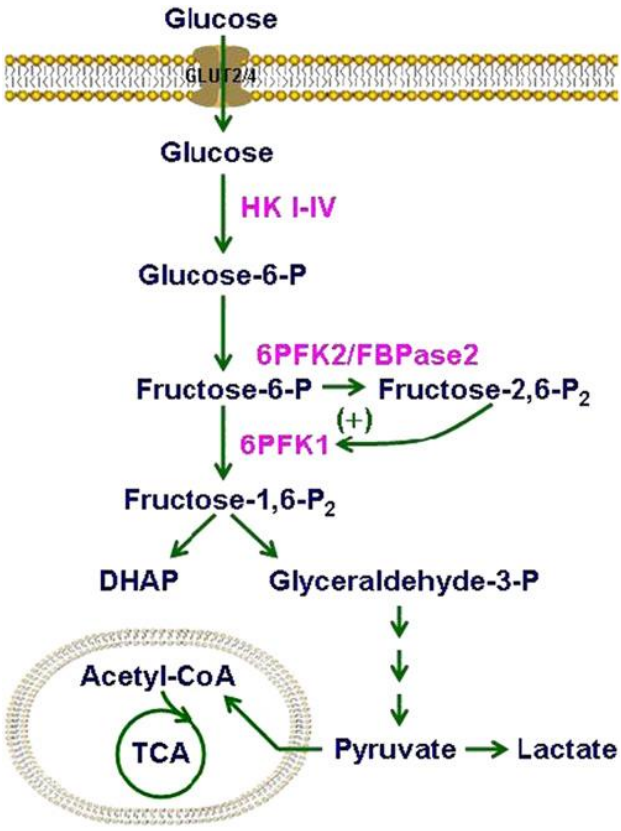
leads to the release of catecholamines thus reduction of glycogen (Lacourt and Tarrant, 1985). This results in high pHu and darker colour (Muchenje *et al.,* 2009a). Such meat is highly susceptible to bacterial deterioration and thus making it unacceptable to consumers (Ferguson *et al.,* 2001). Furthermore dark-firm-dry (DFD) mutton is a direct resultant of small amounts of glycogen during slaughter and this quality defect causes significant financial losses to the meat industry (Warriss, 1990). Glycogen concentration also has an effect on the post mortem glycolytic rate in sheep muscles (Daly *et al.,* 2006). Consequently, when glycogen reserves are depleted in the muscle during the pre-slaughter period mutton quality characteristics for example, colour, tenderness, ageing, pHu, tenderness and water-holding capacity are negatively affected (Gregory, 2003).

2.8 Post-mortem glycolysis

In a live animal's body there are two main energy production routes, namely the oxidative and the glycolytic pathway (Pösö and Puolanne, 2005). These pathways differ depending on the presence of oxygen. The oxidative pathway is most efficient in energy production when oxygen is present whilst when there is limited or no oxygen; glycolysis is suitable to provide energy (Scheffler and Gerrard, 2007). After the animal has been slaughtered the muscles lack oxygen thus glycolysis is a very important pathway post-mortem. Furthermore, glycolysis is responsible for the conversion of glycogen to lactate, which is responsible for acidification of the muscle from pH 7.00 in a live animal to 5.5 thus improving the quality of the meat to be produced (Bowker *et al.*, 2000; Ryu *et al*., 2005). Consequently, glycogen and lactate content which are products of post-mortem metabolism have an influence in the quality of meat, thus glycolysis is an important pathway in the meat industry (Choe *et al*., 2008). Glycolysis is defined as the intracellular biochemical conversion on a glucose molecule into two pyruvate/lactate molecules with the simultaneous production two adenosine triphosphate (ATP) molecules (TeSlaa and Teitell, 2014). Moreover, it

provides substrates for production of energy through the formation of ATP, it also provides substrates for glycogenesis and lipogenesis as illustrated in Figure 2.1 (Guo *et al.,* 2012).

Glycolysis induces the accumulation of lactate in the muscles and this is an important metabolic process during the post-mortem as it determines the pHu of mutton (Bowker *et al.,* 2000; Pösö and Puolanne, 2005). The increase of lactate in the muscle fibres facilitates the rapid decline in pH during the early stage of post mortem (Ryu *et al.,* 2005). The speed and magnitude of post mortem pH decline has a major influence on mutton quality (Scheffler and Gerrard, 2007). Furthermore, differences in ultimate pH, temperature and pH fall can greatly influence meat characteristics (Pösö and Paolanne, 2005).The rate of glycolysis is affected by a number of intrinsic and extrinsic factors. Intrinsic factors such as age, species, temperament, type of muscle, sex and breed cannot be manipulated at the abattoir (Lawrie, 1992). In contrast, extrinsic factors such as stress levels, environmental temperature, pre-slaughter drug administration and electrical stimulation can be manipulated at the farm or abattoir (Lawrie, 1992; Varnam and Sutherland., 1995).



Source: Guo *et al.*, 2012

Figure 2.1: Diagram showing the major steps of glycolysis

2.9 Animal characteristics and meat quality

The quality of mutton is a function of factors such as palatability, tenderness, colour, pH and nutritional value (Pleasant *et al.,* 2005; Webb and O'Neill, 2008; Hopkins and Geesink, 2009; Muchenje *et al.,* 2009a). During the pre-slaughter period animals are exposed to handling, restraint, hunger, thirst and fatigue (Apple *et al.,* 2005). Therefore, these factors can lead to stress thus affecting the meat quality (Muchenje *et al.,* 2009b). Moreover, the quality of meat can also be affected by animal characteristics such as age, physiological status, sex and breed (Hopkins and Mortimer, 2014).

Age of the animal has an important impact on meat quality characteristics and it is of higher importance in grading and marketing decisions (Purchas, 2007). The age of the animal may also affect the eating quality of mutton. It has also been reported that sheep aged two years and above produce tougher meat in comparison to yearlings (Kirton *et al.,* 1983). Consequently, differences in the toughness of mutton produced from sheep with different ages may be caused to the increase in the muscle level of collagen as the animal gets older (Hopkins and Mortimer, 2014). Furthermore, there is a positive correlation between age of the animal and intramuscular fat, as the animal gets older intramuscular fat is expected to increase (Martínez-Cerezo *et al.,* 2005; Pethick *et al.,* 2005), thus leading to differences in mutton quality produced from sheep of different ages.

Breed is also an important factor that influences mutton quality (Hofman *et al.,* 2003; Hopkins and Mortimer, 2014). Merino dams were reported to have higher loin pH values compared to Dohne Merino and South African Mutton Merino breeds (Hofman *et al.,* 2003). Another study reported that the Dohne Merino and Merino had higher pH values compared to the South African Mutton Merino (Cloete *et al.,* 2012), this is evidence of differences in meat quality from different breeds. Moreover, breed types have different physiological responsiveness to pre-slaughter stress thus

affecting muscle glycogen concentration, ultimate pH, cooking loss, colour and tenderness of meat (Muchenje *et al.,* 2009b).

Sex also has an influence on the meat quality due to hormonal and developmental differences between the sexes (Dransfield *et al.,* 1990; Hopkins and Mortimer, 2014). However, the impact of gender on tenderness of meat is not quite clear, as some researchers did not find significant differences (Kemp *et al.,* 1981; Lee, 1986) whilst others have reported lamb from males and castrates to be tougher than that of ewes (Johnson *et al.,* 2005; Hopkins *et al.,* 2007; Cloete *et al.,* 2012).

2.10 Role of muscle glycogen on meat quality

At post-mortem anaerobic glycolysis commences, through this process glycogen reserves break down to produce lactate thus lowering the pH of meat (Gardner and Thompson, 2003; King *et al.,* 2006). Glycogen reduction dependent upon physical exercise and psychological stress prior to slaughter (Immonen *et al*., 2000). Furthermore, glycogen is depleted through the production of lactic acid using glycogen as a substrate in the reaction thus reducing the pH of meat by increasing its acidity. This process ceases when post-mortem glycolysis comes to a halt meaning that the ultimate pH of meat has been reached.

Thus pre-slaughter stress activities such transportation, high temperatures, rough handling have an important influence on ultimate pH (O'Neill *et al.,* 2006). These activities reduce glycogen reserves prior to slaughter thus negatively affecting the development of ultimate pH (Muchenje *et al.,* 2009a) in red meat. Glycogen concentration in the muscle has a very important influence on meat ultimate pH as it accounts for about 40 to 50 percent of its (pH) variation (Laack *et al.,* 2001). The standard pH range for mutton and lamb is 5.75 to 6 (Gregory, 2005). However, when sheep meat has a pH higher than 6 undesirable characteristics result such as aesthetically unacceptable

characteristics such as dark colour (Mounier, 2006), differences in tenderness (Silva *et al.*,1999) and high WHC (Apple, 2005) thus low palatability. Furthermore a high ultimate pH promotes the growth of microorganisms thus reducing the shelf-life of meat, through development of off odours (Gallo *et al.,* 2003). Such meat is unacceptable to consumers thus leading to financial losses. Hence, it is quite important to avoid exposing sheep to stress prior to slaughter, since it results in glycogen depletion thus producing meat with high pHu and dark colour (Lacourt and Tarrant, 1985; Ferguson and Warner, 2008).

2.11 Meat quality

Meat quality depends on the composition and palatability of meat. It is also a function of flavour, pH, nutritive value, juiciness, tenderness and colour (Lawrie and Ledward, 2006; Hopkins and Geesink, 2009). Furthermore, meat quality can also be determined using traits perceived desirable by consumers these include both visual and sensory traits (Warner *et al.,* 2010). However, starting from development to slaughter stress, age, nutrition and breed to name a few have an effect on meat quality. During the transformation of a live animal to meat, the pre-slaughter period consist of a chain of events such as handling, transportation, loading, lairage, stunning and slaughter technique affects meat quality attributes (Roth *et al*., 2007). During the pre-slaughter period substandard operational procedures, equipment and facilities may lead to distress, pain, injury thus reduced meat quality (Muchenje *et al*., 2009b).

2.12 Colour of meat

Meat colour has an important influence on consumers purchasing decisions since it represents perceived freshness (Mancini and Hunt, 2005; Jacob *et al.,* 2014). It can be defined in terms of Hunter colimetric co-ordinates (Kannan *et al.,* 2003). Where L* indicates the lightness of meat ranging from 0 (all light absorbed) to 100 (all light reflected), a* represents redness of meat with

a range from - 60 (green) to + 60 (red) and lastly coordinate b* which is the yellowness of meat ranges from - 60 (blue) 60 + yellow (Simela, 2005).

Meat colour is affected by intrinsic and extrinsic factors. Intrinsic factors include genetics, age, gender, muscle type, muscle fiber composition, pH, myoglobin concentration. However, extrinsic factors include nutrition, post-mortem condition, temperature and pre-slaughter stress (AMSA, 2012). Consequently, when sheep are exposed to stress prior to slaughter, glycogen levels are significantly depleted thus resulting in dark cutting meat (Kannan *et al.,* 2003). Thus stress needs to be reduced at pre-slaughter in-order to produce meat products with the desired colour to avoid economic loss (Mancini and Hunt, 2005).

2.13 Relationship between glycogen and ultimate pH

The level of pH decline is an important element in determining the quality of fresh meat. The rate and extent of pH decline is influenced by glycogen reserves at post-mortem (England *et al.,* 2014). There is a strong link between the amount of muscle glycogen prior to slaughter and the ultimate pH of meat (Tarrant, 1989). At post-mortem lactate is formed through the anaerobic environments in the muscle where glycogen is metabolised at glycolysis (Coombes *et al.,* 2014). Lactate formation leads to the formation of hydrogen ions which reduce the intracellular pH from 7.00 in a live animal down to an ultimate pH of 5.4 to 5.7 in the first 24 - 48 hours after slaughter (Maltin *et al.,* 2003). If the animal's glycogen reserves are depleted prior to slaughter glycolysis will occur to a lesser extent thus meat will have a high ultimate pH resulting in DFD meat (Bendal, 1973). The study of post-mortem muscle metabolism is essential in making advances in meat quality improvements. Since, it brings an understating of how to improve feeding strategies, animal welfare and slaughter procedure in order to preserve meat quality (England *et al*., 2013).

2.14 Role of muscle glycogen on meat quality

After the animal has been slaughtered anaerobic glycolysis commences a process where glycogen reserves at post-mortem are used to acidify meat. The amount of glycogen in the muscle is a gathering of glycogen produced at the farm minus glycogen synthesised for muscle energy in the before slaughter as the animal tries to cope with exposure to stress (McGilchrist *et al.*, 2012). This is done through the production of lactic acid using glycogen is a substrate in the reaction. Lactic acid reduces the pH of meat by increasing its acidity, this process ceases when post-mortem glycolysis comes to a halt meaning that the ultimate pH of meat has been reached. Pre-slaughter stress activities such transportation, high temperatures, rough handling have an important influence on ultimate pH (pHu) (O'Neill *et al.,* 2006). Since these activities reduce glycogen reserves prior to slaughter thus negatively affecting development of ultimate pH (Muchenje *et al.,* 2009b) in red meat.

Glycogen concentration in the muscle is a very factor as it accounts for about 40 to 50 percent of variation in meat ultimate pH hence it is a very important determinant of ultimate pH (Laack *et al.,* 2001). The relationship that exists between glycogen concentration and meat pHu can be represented by a negative exponential graph (Warriss, 1990). Glycogen depletion in muscle results in meat with high ultimate pH (Kannan *et al.,* 2002). Gregory (2005) reported that, the accepted range for sheep meat is between 5.75 to 6.00 any value higher than 6.00 is undesirable. Since, it results in meat with aesthetically unacceptable characteristics such as dark colour (Mounier *et al.,* 2006). Such meat will have differences in tenderness (Silva *et al.,* 1999) and high water holding capacity (WHC) (Apple, 2005) thus low palatability. Furthermore a high ultimate pH promotes the growth of microorganisms thus reducing the shelf-life of meat, through development of off odours (Gallo *et al.,* 2003). Such meat is unacceptable to consumers and may lead to economic

losses. It is quite important to avoid unnecessary exposure to stress prior slaughter, as stressed animals release catecholamine which depletes glycogen thus producing meat with high pHu and dark colour (Lacourt and Tarrant, 1985; Ferguson and Warner, 2008).

2.15 Summary

From the preceding review of literature it can be deduced that the pre-slaughter environment affects the levels of blood metabolites, glycolytic potential and meat quality. The manner in which sheep react to stress prior slaughter varies depending on differences in animal class. Hence it is important to understand the role of sex and breed in response to stress and post-mortem muscle metabolism affects the quality of meat. The broad objective of the study was therefore to determine glycolytic potential and mutton quality from Dorper and Merino sheep of both sexes slaughtered at a commercial abattoir.

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Chapter 3: Effect of sex and breed on blood glucose, lactate, cortisol collected at exsanguination from Dorper and Merino sheep

Abstract

The study was conducted to determine sex and breed effects on blood glucose, lactate and cortisol levels from sheep slaughtered at a high throughput abattoir. Two different types of breeds (Dorper $n = 52$; Merino $n = 48$) eight years of age, consisting of 50 intact rams and 50 non-pregnant ewes were used in the study. Blood samples were collected at exsanguination for the measurement of glucose, lactate and cortisol levels. Ewes had higher levels of blood lactate (7.4 3 ± 0.49 mmol/L) and cortisol (293.92 ± 14.32 nmol/L) than the rams which had (5.19 ± 0.49 mmol/L) and (179.50 ± 14.32 nmol/L) lactate and cortisol levels ($P < 0.001$) respectively. Significant differences ($P < 0.01$) were also observed between the breeds on blood lactate. Higher levels of lactate were observed in Dorper (7.54 ± 0.42 mmol/L) than on Merino sheep (4.97 ± 0.49 mmol/L). Blood glucose levels were positively correlated with both lactate ($P < 0.01$) and cortisol levels ($P < 0.05$). Furthermore stronger positive correlations ($P < 0.001$) were also found between blood lactate and cortisol levels. It was therefore concluded that sex and breed have an effect on blood glucose, lactate and cortisol levels on sheep exposed to pre-slaughter stress.

Key words: ewes, fearful, pre-slaughter, rams, reactive and stress

3.1 Introduction

The manner in which sheep respond to stress varies depending on intrinsic animal factors such as physiological state, past exposure, age, breed and sex (Hemsworth and Barnett, 2001; Moberg, 2001). During the pre-slaughter period, sheep are exposed to a range of endogenous and exogenous stressors such as loading, noise, vibration and unfamiliar environment (Hall and Bradshaw, 1998;

Chulayo and Muchenje, 2013). Furthermore, stress can be assessed through changes in the animal's behaviour or physiology as it tries to cope with environmental challenges (Broom, 1987; Fraser *et al.,* 1975). Particularly, these physiological changes in response to stress result in an increase on the levels of certain blood metabolites hence they are known as stress indicators (Chulayo and Muchenje, 2015; Franco *et al*., 2015). These changes occur when sheep try to maintain homeostasis and adapt to exposure to stress (Zimmerman *et al.,* 2013).

Pre-slaughter stress encourages the two main stress-responsive neuroendocrine systems namely, the hypothalamic-pituitary-adrenocortical axis (HPA) and the sympatho-adrenal medullary system (SAM). In response to stress the HPA releases cortisol (Foury *et al.,* 2011). Cortisol is an important stress indicator as it reflects the handling conditions during the pre-slaughter procedure (Minton, 1994; Warriss, 2010; Russel *et al.,* 2012). On the other hand, when the sympatho-adrenal medullary system (SAM) is stimulated, the adrenal medulla releases catecholamines (Matteri *et al.,* 2000). These catecholamines rapidly breakdown glycogen from the liver resulting in increased levels of blood glucose (Warriss, 2010).

Blood lactate is also seen as an important pre-slaughter stress indicator. This is mainly because increased blood lactate levels are linked with strenuous muscular action that metabolises muscle glycogen and discharges huge amounts of lactic acid into the blood (Shaw and Tume, 1992). Thus increased levels of biochemical parameters such as cortisol, glucose and lactate give an insight of the nature of sheep welfare during pre-slaughter handling (Gregory, 1998; Ferguson and Warner, 2008).

The associations between pre-slaughter stress, blood glucose, lactate and cortisol levels in pigs have been well researched (Warriss *et al.,* 1994; Hambrecht *et al.,* 2004; Hambrecht *et al.,* 2005;

Terlouw *et al.*, 2008; Edwards *et al.*, 2010; Choe and Kim, 2014) nonetheless, such work has not been published in sheep. Therefore, the aim of this study was to determine the effects of sex and breed on blood glucose, lactate and cortisol levels collected at exsanguination and their correlations.

3.2 Materials and Methods

3.2.1 Ethical considerations

The study was conducted following normal routine farm to abattoir practices. Ethical clearance was applied for and obtained from the ethical clearance committee of the University of Fort Hare (Certificate reference number: MUC091SSTE01).

3.2.2 Description of the study site

Data was collected from sheep slaughtered at a commercial high throughput abattoir in East London. The abattoir is situated 542 m above sea level at a latitude and longitude of 32.2 ^{o}S and 27.5 ^{o}E, respectively in the Amathole district of the Eastern Cape Province of South Africa. The normal weather conditions of the area are mild with an average rainfall of 850 mm. The day temperatures range between 18 to 26 ^{o}C with a mean average temperature of 22 ^{o}C.

3.2.3 Study animals and management

A total of 100 eight-year old sheep consisting of Dorper (n = 52) and Merino (n = 48) breeds, were used in the study. There were 30 intact-rams and 22 non-pregnant ewes from the Dorper breed whilst, 20 intact-rams and 28 non-pregnant ewes from the Merino breed. All sheep were reared under identical free range conditions with access to *ad-libitum* water at a commercial farm in Steynsburg, Eastern Cape province of South Africa. The sheep were transported from the farm to East London abattoir which is approximately 331 km away under the same handling conditions.

Upon arrival in the evening at 21:00 pm they were kept together in their original social grouping over-night at the lairages. Feed was withdrawn, but fresh clean water was made available *ad-libitum*. The sheep were kept at the lairages for nine hours and were humanely slaughtered in the morning at 6.45 am. The slaughter procedure was in accordance with the rules and regulations stipulated in the Meat Safety Act no. 40 of 2000. Sheep were electrically stunned at 110 volts with concave tongs with a current of 0.6 amperes for 60 seconds to induce unconsciousness. After stunning, the throat was slit open with a sharp knife to initiate exsanguination and sheep were hung by the hind legs on the rails to facilitate the bleeding process.

3.2.4 Biochemical determination of blood

3.2.4.1 Blood collection and plasma separation

Exsanguination blood was collected immediately after the throat was slit whilst the sheep were hanging on the rails. Three disposable Becton Dickinson vacutainer 10.0 ml tubes treated with fluoride oxalate (grey top) were used to collect blood for the analysis of glucose and lactate levels. Whilst serum separating vacutainer tubes 10.0 ml (SSTTMII gold top) were used to collect blood for cortisol levels determination. After collection, the blood samples were kept on ice until plasma was separated within two hours. The blood tubes were centrifuged (Model 5403 Centrifuge, Gatenbay Eppendorf GmbH, Engelsdorp, Germany) at 21^{o} C for 10 minutes at 3550 runs per minute (rpm). Then they were placed in 1.5 ml Eppendorf tubes and stored at -20^{o} C refrigerator until analysis.

3.2.4.2 Determination of blood glucose and lactate levels

Laboratory analysis of blood glucose and lactate levels was carried out using an enzymatic method (Trinder, 1969; Barham and Trinder, 1972). The analyses were automated using an Olympus

AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Melville, New York) and the Olympus reagent kit for glucose and lactate (Olympus Cat.No.OSR6193). The results were expressed in mmol/L.

3.2.4.3 Cortisol level determination

The levels of blood cortisol were determined using a commercial cortisol enzyme linked immunoassay (ELISA) kit for the in-vitro diagnostic quantitative determination of cortisol in plasma (Palme and Möstl, 1997; IBL International, 2013) according to manufacturer instructions. Results for blood cortisol levels were represented in nmol/L.

3.3.4 Statistical analysis

The effect of sex and breed on blood glucose, lactate and cortisol levels from Dorper and Merino sheep was analysed using the PROC GLM procedure of SAS 2009, the following statistical model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} = response variable (glucose, cortisol, lactate)

μ = overall mean

α_i = i^{th} effect of sex (Rams and Ewes)

β_j = j^{th} effect of breed (Dorper and Merino)

$(\alpha\beta)_{ij}$ = interaction between sex and breed

ε_{ijk} = Random error

The strength of relationships between blood stress indicators were determined using Pearson`s correlation coefficient (SAS, 2009). Significant differences among group means were tested using Least Significant Differences (LSD) and differences at $P < 0.05$ were considered to be statistically significant. Interactions between sex and breed were also assessed.

3.3 Results and Discussion

Effect of sex on the levels of blood glucose, lactate and cortisol

The results for the effects of sex on blood glucose, lactate and cortisol levels are represented in Table 3.1. There were no interactions between sex and breed. Furthermore, no significant ($P > 0.05$) sex differences on blood glucose levels between the rams and ewes (4.5 ± 0.19 mmol/L and 4.8 ± 0.19 mmol/L, respectively). These results concur with the findings by AL-Hadity and Bawadi (2015) who also found no significant differences in the normal glucose levels between rams and ewes (2.6 ± 0.09 mmol/L and 2.2 ± 0.09 mmol/L, respectively). Conversely these results are in contrast with those reported by (Carlos *et al.*, 2015),reported significant sex differences in blood glucose.

Table 3.1: Levels of glucose (mmol/L), lactate (mmol/L) and cortisol (nmol/L) in blood (LSMeans ± standard error of the mean) between rams and ewes

	Sex		
Stress indicators	**Rams**	**Ewes**	**P value**
	n = 50	*n* = 50	
Glucose	$4.5^{a} \pm 0.19$	$4.8^{a} \pm 0.19$	0.4033
Lactate	$5.2^{b} \pm 0.49$	$7.4^{a} \pm 0.49$	0.0016
Cortisol	$179.5^{b} \pm 14.32$	$293.0^{a} \pm 14.32$	0.0001

Means in the same row with different superscripts are significantly different at $P < 0.05$

However, significant differences ($P < 0.001$) between ewes and rams (5.2 ± 0.49 mmol/L and 7.4 ± 0.49 mmol/L, respectively) on the levels of blood lactate. These results indicated that the ewes were more stressed than the rams. These results contradict reported by Bertol *et al.* (2011), where males to have higher blood lactate than the females in pigs. However these sex differences in the levels of blood lactate are in accordance to results by Gruber *et al.* (2010) on cattle, where heifers expressed higher blood lactate levels than the steers. Ewes are known to be more fearful than rams (Hernandez *et al.,* 2010).

Furthermore behaviourally reactive sheep tend to have elevated amounts of blood lactate than calm sheep (Coombes *et al.,* 2014). Higher levels of blood lactate are an indication that the ewes were stressed (Tadich *et al.,* 2009).

A difference ($P < 0.001$) blood cortisol levels was observed between the ewes and rams (179.5 ± 14.32 nmol/L and 293.0 ± 14.32 nmol/L, respectively). The ewes had higher levels of cortisol compared to the rams. This may be attributed to differences in sexual glucocorticoid regulation which is responsible for cortisol secretion causing the ewes to be more easily disturbed than the rams (Canny *et al.,* 1999; Dodd *et al.,* 2012). Where male sex hormones (androgens) become suppressive whilst female sex hormones (oestrogens) stimulate the HPA axis (Handa *et al.,* 1994; McCormick *et al.,* 1998), hence females have higher cortisol levels. It has been well established that elevated levels of cortisol are an indication of exposure to stressful conditions in sheep as it serves as a worthy guide for the response of an animal to any environmental adversity such as the pre-slaughter environment (Gregory, 1998; Minka and Ayo, 2010). When animals are presented for slaughter they are exposed to a variety of stressors such as handling, transportation, novelty of the environment and feed withdrawal (Hall and Bradshaw, 1998).

When blood cortisol levels are greater than, 175.00 nmol/L, it is an indication of severe stress (Grandin, 1997). The results from this study have shown that the response of sheep to stress varies depending on sex, and ewes were more stressed than the rams. These results concur with the results reported by Gruber *et al.* (2010); Hernandez *et al.* (2010); Bourguet *et al.* (2011) and Probst *et al.* (2012). This is not surprising as females have been reported to perceive the slaughter processes more stressful than males (Boissy and Boissou, 1994; Taylor *et al.*, 2001).

Effect of breed on the levels of blood glucose, lactate and cortisol

The results for the effects of breed on blood glucose, lactate and cortisol levels are represented in Table 3.2. There were no significant ($P > 0.05$) breed differences on blood glucose levels between the Dorper and Merino (4.8 ± 0.18 mmol/L and 4.5 ± 0.19 mmol/L, respectively). These results contradict those with by (Catunda *et al.*, 2013) who found significant breed differences in glucose levels. However, there were significant differences ($P < 0.001$) between Dorper and Merino (7.5 ± 0.42 mmol/L and 4.1 ± 0.49 mmol/L, respectively) on the levels of blood lactate. The Dorper had higher levels of blood lactate than the Merino, indicating that the Dorper was more stressed than the Merino. Differences in the levels of blood glucose were also influenced by variations in physiological status between sheep breeds (Carlos *et al.*, 2015). These results are in agreement with results by (Kaneko *et al.*, 2008) who reported breed differences in blood lactate. Conversely, these results are in contrast with the results by Cloete *et al.* (2005) which indicated that the Merino is more susceptible to stress. Furthermore, no significant ($P > 0.05$) differences on blood cortisol levels were observed between the Dorper and Merino (245.4 ± 16.1 nmol/L and 227.3 ± 16.78 nmol/L, respectively).

Table 3.2: Levels of glucose (mmol/L), lactate (mmol/L) and cortisol (nmol/L) in blood (LSMeans ± standard error of the mean) between the Dorper and Merino

	Breed		
Stress indicators	**Dorper**	**Merino**	**P value**
	n = 52	*n* = 48	
Glucose	$4.8^{a} \pm 0.18$	$4.5^{a} \pm 0.19$	0.2494
Lactate	$7.5^{a} \pm 0.42$	$4.1^{b} \pm 0.49$	0.0003
Cortisol	$245.4^{a} \pm 16.12$	$227.3^{a} \pm 16.78$	0.4405

Means in the same row with different superscripts are significantly different at $P < 0.05$

Pearson's correlation between blood glucose, lactate and cortisol levels from Dorper and Merino of both sexes

The relationships amongst blood glucose, lactate and cortisol levels are represented in Table 3.3. Blood glucose levels were positively correlated ($P < 0.01$) with blood lactate ($r = 0.32$). Blood glucose was also positively correlated ($P < 0.05$) with blood cortisol ($r = 0.27$). Blood lactate levels were also positively ($P < 0.001$) correlated to blood cortisol ($r = 0.37$). A stronger correlation between blood lactate and cortisol was observed than the relationship between blood glucose and cortisol these results are in accordance with those reported by Choe and Kim (2014). The positive correlation between glucose and lactate has also been reported by (Tadich *et al.,* 2009; Miranda-de la Lama *et al.,* 2011). Furthermore, the positive correlation between blood glucose and cortisol is similar to results reported by Miranda-de la Lama *et al.* (2011). These positive relationships in agreement with findings by Saeb *et al.* (2010), who reported that after sheep are exposed to stress an increase in blood cortisol concentrations are seen which is later followed by an increase in glucose in the blood. This relationship is referred to as the gluconeogenic effect of cortisol (Hellhammer *et al.,* 2009). When sheep are stressed the HPA is stimulated to release cortisol (Foury *et al.,* 2011). Elevated levels of cortisol result in rapid breakdown of glycogen in the liver through gluconeogenesis, where fat is converted to intermediate metabolites that are later converted to glucose, thus an increase in blood glucose concentration (Saeb *et al.,* 2010).

Table 3.3: Pearson's correlations coefficients (*r*) for blood glucose, lactate and cortisol from Dorper and Merino sheep of both sexes

Stress indicators	Glucose	Lactate	Cortisol
Glucose	-	0.32**	0.27*
Lactate		-	0.37***
Cortisol			-

Significantly correlated at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, NS= Not significant

The pre-slaughter period leads to two main stress responses, namely the HPA, and the SAM. When an animal is stressed the HPA is stimulated to release cortisol (Foury *et al.,* 2011) whilst the SAM releases catecholamine leading to rapid breakdown of glycogen in the liver. This is done through a process called gluconeogenesis, where fat is converted to transitional metabolites which are later changed to glucose (Saeb *et al.,* 2010). Hence the blood glucose levels are increased (Warriss, 2010). However blood glucose levels are not only affected by pre-slaughter stress conditions they are also affected by hormonal changes and feed withdrawal (Terlouw *et al.,* 2008; Moja-Rojas *et al.,* 2012). This results in lower correlation coefficient within blood glucose and cortisol compared to the lactate and cortisol levels in this study. Furthermore these results may lead to the assumption that blood glucose levels at exsanguination may be an unintended stress indicator.

3.4 Conclusion

It can therefore be concluded that sex and breed have an effect on lactate and cortisol levels of Dorper and Merino sheep. Blood lactate and cortisol levels collected at exsanguination reveal that the ewes were more reactive to the pre-slaughter period compared than the rams. Sex had an effect on blood lactate and cortisol, but not on glucose levels. The results also indicate that the Dorper breed had higher blood lactate levels than the Merino. However there were no breed effects on blood glucose and cortisol. There were significant correlations positive amongst blood stress indicators (glucose, lactate and cortisol), meaning when sheep are exposed to stress glucose, lactate and cortisol levels will increase in the blood.

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Chapter 4: Effect of sex and breed on muscle glycolytic potential, glycogen, lactate levels, mutton pH decline and colour

Abstract

The study was conducted to determine the effect of sex and breed on muscle glycolytic potential, lactate, glycogen, pH decline and colour from sheep slaughtered at a high throughput abattoir. Two different types of breeds (Dorper *n*= 52; Merino *n*= 48) eight years of age, consisting of 50 rams and 50 ewes were used in the study. Samples were collected at slaughter from the *Muscularis longmissius thoracis et lumborum* (LTL) for the measurement of glycogen, lactate levels, pH and colour. No significant differences ($P > 0.05$) were observed for sex and breed on muscle glycolytic potential, lactate and glycogen levels. However, significant differences ($P < 0.001$) were observed between ewes and rams on pH_{45} minutes, pH_3 hours and pH_{24} hours and meat colour (L*, a*, b*, H* and C*). Ewes had higher levels of pH_{45} minutes post slaughter (7.05 ± 0.04), pH_3 (6.45 ± 0.04) , pH_{24} hours (6.00 ± 0.03), a*(14.31 ± 0.33) , b*(8.84 ± 0.29), H* (31.47 ± 0.73) and C*(16.75 ± 0.24) compared to the rams which had pH_{45} (6.44 ± 0.04), pH_3 (6.12 ± 0.04), pH_{24} hours (5.88 ± 0.03), a* (12.25 ± 0.33), b* (7.00 ± 0.29), H* (29.36 ± 0.73) and C* (14.15 ± 0.42) values. Significant differences ($P < 0.001$) were also observed between breeds on L* and H*. Merino sheep had higher levels of L* (38.17 ± 0.48) and H* (31.59 ± 0.74) than the Dorper which had (36.39 ± 0.46) and (29.33 ± 0.71) L* and H* values, respectively. Blood glucose was also positively correlated ($P < 0.05$) to blood cortisol ($r = 0.27$), but was negatively correlated ($P < 0.001$) to meat lightness ($r = -0.44$). Blood lactate levels were also positively ($P < 0.001$) correlated to blood cortisol ($r = 0.37$). Blood cortisol was positively correlated ($P < 0.001$) to pH_{45} ($r = 0.34$), pH_{24} ($r = 0.22$), meat yellowness ($r = 0.24$) and chroma ($r = 0.37$), but was negatively correlated to meat lightness ($r = -0.47$). Glycolytic potential was positively correlated ($P < 0.001$)

to muscle glycogen levels (r = 0.66) and muscle lactate (r = 0.71). A positive relationship (P < 0.001) existed between pH_{45} to pH_{24} (r = 0.47), meat redness (r = 0.30), meat redness (r = 0.31) and chroma (r = 0.31). However, a negative relationship (P < 0.05) existed between pH_{45} and meat lightness (r = -0.21). There was a positive relationship (P < 0.01) between pH_3 and pH_{24} (r =0.61), but pH_{24} was negatively correlated to meat redness (r = -0.67), meat yellowness (r = -0.34) and chroma (r = 0.57). Meat lightness was negatively correlated (P < 0.001) to meat redness (r = -0.67) and meat yellowness (r = -0.34). Moreover, there were negative correlations (P < 0.001) between meat lightness and chroma (r = 0.57). Whilst meat redness was positively correlated (P < 0.001) to meat yellowness (r = 0.73). A positive correlation (P < 0.001) also existed between meat redness and chroma (r = 0.95). Meat yellowness was positively correlated (P < 0.001) to hue angle (r = 0.76), chroma (r = 0.86). Furthermore, hue angle was positively correlated (P < 0.001) to meat chroma (r = 0.40). It was therefore concluded that sex and breed differences in response to pre-slaughter stress have an effect on the mutton quality. The results showed that the rams produced meat with better meat quality compared to the ewes, whilst the Dorper sheep produced meat with a darker colour compared to the Merino. Furthermore there were significant relationships amongst blood stress indicators, muscle metabolites, glycolytic potential and meat quality attributes.

Key words: glycolysis, post-mortem, pH

4.1 Introduction

In South Africa (S.A) sheep rearing plays a significant role in the livelihood of farmers and boosting the economy of the country, since they are a source of income, wool and meat (Mapiliyao *et al.*, 2012). The Dorper and the Merino are the most commonly reared breeds in S.A, which are kept for different purposes. The Merino is regarded as the major dual-purpose breed in S.A (Cloete and Oliver, 2010) and it is better suited for intensive farming. Whilst the Doper sheep are regarded

as the major breed for meat production in S.A and are normally farmed in extensive areas (Cloete *et al.,* 2013).

In chapter 3 breed and sex had an effect on the levels of blood glucose, lactate and cortisol in response to pre-slaughter stress. Furthermore, it is important to understand how breed and sex affect muscle metabolites and meat quality in response to pre-slaughter stress. The quality of sheep meat can also be influenced by a number of factors namely pre-slaughter stress (Chulayo and Muchenje, 2015), sex (Dransfield *et al.,* 1990; Sañudo *et al.,* 1998; Tejeda *et al.,* 2008; Hopkins and Mortimer, 2014) and breed (Teixeira *et al.,* 2005; Hopkins and Mortimer, 2014). However, meat acceptability and quality are determined by its physico-chemical characteristics such as colour and pH (Tejeda *et al.,* 2008). Moreover, the pHu of meat is a main cause of mutton quality (Watanabe *et al*., 1996) and is associated to the extent of glycogen breakdown and release lactate before and after slaughter (Kadim *et al.,* 2006).

Sufficient muscle glycogen content pre- and post-mortem will determine whether the desirable pHu of 5.5 at post-mortem will be attained during the transformation of muscle into meat (Tarrant, 1989). In contrast when glycogen levels are low the desirable ultimate pH will not be attained, leading to a meat with a high pHu and dark colour such meat is termed dark firm and dry (DFD) due to exposure to aversive pre-slaughter environment (Immonen *et al.,* 2000). DFD meat is deemed undesirable for human consumption due to its undesirable flavour, susceptibility to microbial spoilage and dark colour, which makes it unacceptable to consumers and hence it is condemned (Shorthose, 1989; Purchas and Aungsupakorn, 1993). DFD meat is unacceptable to consumers because colour is the most important indicator of quality and freshness (Resurreccion, 2003).

The ability of muscle to store glycogen (glycolytic potential) is affected by factors such as sex, breed (King *et al.,* 2006), pre-slaughter stress and the manner in which sheep respond to pre-slaughter stress (O'Neill *et al.,* 2006). Exposure to pre-slaughter stress reduces glycogen content in the muscles through the activation on the nervous and endocrine system due to stress response (Pighin *et al.,* 2014) and thus has a undesirable impact on the quality of meat pH and colour (Muchenje *et al.,* 2009a). Animal characteristics such as sex and breed also affect the manner in which sheep respond to the pre-slaughter environment (Hopkins and Mortimer, 2014). Hence this study aims to determine sex and breed differences in muscle glycogen, lactate, glycolytic potential, meat pH decline, colour and their correlations.

4.2 Materials and Method

4.2.1 Description of the study site and study animals

The study site and management of experimental sheep were as described in Section 3.2.

4.2.2 Blood sample collection and analysis

Blood sample collection and analysis were as described in Section 3.2.

4.2.3 Meat sample preparation

Two different meat samples were collected from the *Muscularis longmissius thoracis et lumborum* (LTL) in each of the carcasses. Meat samples for the measurement of glycogen and lactate content were collected 30 minutes after slaughter. Approximately 50 grams of muscle were collected from each carcass. Fat tissue was removed from each sample using a sterilised knife and the samples were instantaneously frozen in liquid nitrogen at -196 ^{o} C to prevent further glycolysis. The samples were then kept at a -80^{o} C refrigerator until analysis. Pethick and Rowe (1996) reported that, there is little change in the level of muscle glycogen for the first 30 minutes of slaughter in

carcasses that have not been electrically stimulated. Hence the carcasses used in this study were not electrically stimulated. Whilst meat samples to be used for the measurement of colour and pH were collected from the same carcasses 24 hours after slaughter.

4.2.4 Determination of muscle glycogen

Glycogen level was measured using the enzymatic method of Chan and Exton (1976). Muscle (0.5 grams) were homogenised using a Polytron (PT 2500 E) homogeniser in 100 µl of water placed on ice. The homogenates were then boiled for five minutes for enzyme inactivation. The samples were then centrifuged at 13,000 x g for five minutes in order to separate the insoluble material. The soluble material was then pipetted into labelled sterile pre-chilled tubes and 2 µl of hydrolysis enzyme mix was added.

The colorimetric assay method (Sigma Aldrich, MAK016) was used to determine the level of glycogen in the muscles. The samples (50 µl) were then pipetted into the microtiter plates and the master reaction mix (50 µl) was then added. The samples plate was then covered with foil to avoid light exposure and later mixed with a microtiter plate shaker (Orbit ^{TM} P2 S2020 P2-B) and incubated for 30 minutes. The samples were then inserted in the microtiter plate reader (BioTek Multi-Mode Synergy 2). Absorbance values were then read at 570 nm and a standard curve was established for the assay (Figure 4.1).

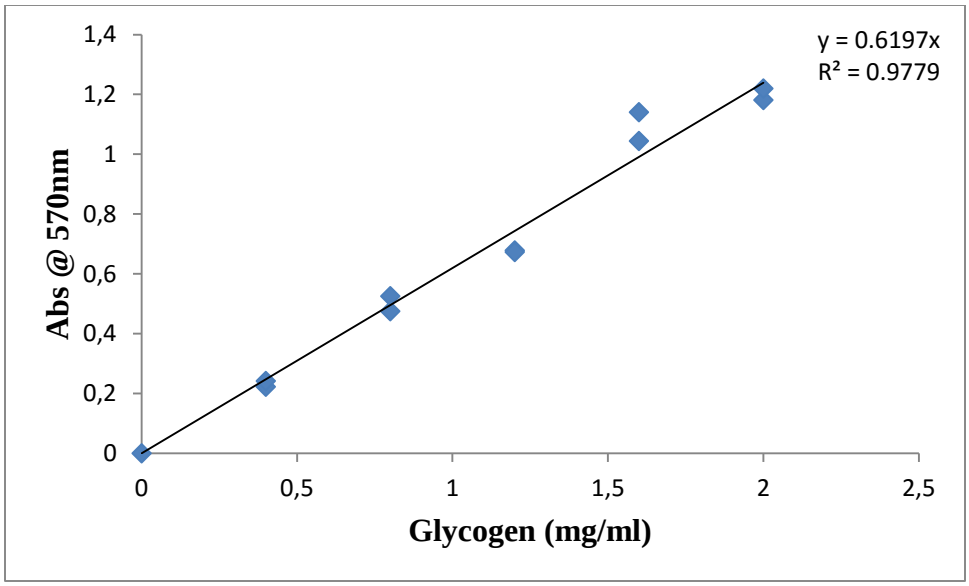

Figure 4.1: Glycogen standard curve

Whilst the concentration was calculated using the following formulae:

Concentration of glycogen

Sa/Sv = C

Sa = Amount of glycogen in unknown sample (mg) from standard curve

Sv = Sample volume (mL) added into the wells

C = Concentration of glycogen in sample

Sample Calculation

Amount of glycogen (Sa) = 1.60 mg (from standard curve)

Sample volume (Sv) = 50 mL

Concentration of glycogen in sample

1.60 mg/50 mL = 0.32 mg/mL

4.2.5 Determination of muscle glucose + Glucose-6-phosphate

Glucose content was determined after hydrolysing 10 µl of the muscle homogenate in 0.1 M HCL for 2 hours at 100^{o} C. The pH of the samples was later adjusted between 6.5 and 7.5 (Lowry and Passoneau, 1973). Glucose was then determined using a Sigma Aldrich, MAK064 assay kit as described above.

4.2.6 Determination of muscle lactate

Muscle (0.5 grams) were homogenised using a Polytron (PT 2500 E) homogeniser in 4 volumes of lactate buffer assay placed on ice. The samples were then centrifuged at 13,000 x g for 10

minutes in order to separate the insoluble material. The samples were then deproteinised with 10 kDa molecular weight cut-off spin filter 15 ml (Amicon Ultra-15 centrifugal filter devices). The soluble fraction was then pipetted into labelled sterile pre-chilled tubes.

The colorimetric assay method (Sigma Aldrich, MAK064) was used to determine the level of lactate in the muscles. The samples (50 µl) were then pipetted into the microtiter plates and the master reaction mix (50 µl) was then added. The samples plate was then covered with foil to avoid light exposure and later mixed with a microtiter plate shaker (Orbit ^{TM} P2 S2020 P2-B) and incubated for 30 minutes. The samples were then inserted in the microtiter plate reader (BioTek Multi-Mode Synergy 2). Absorbance values were then read at 570 nm and a standard curve was established for the assay (Figure 4. 2). The concentration was calculated using the following formulae:

Concentration of Lactate

$Sa/Sv = C$

Sa = Amount of lactate acid in unknown sample (nmole) from standard curve

Sv = Sample volume (mL) added into the wells.

C = Concentration of lactate acid in sample

Lactate molecular weight: 89.07 g/mole

Sample Calculation

Amount of Lactate (Sa) = 5.07 nmole

Sample volume (Sv) = 50 mL

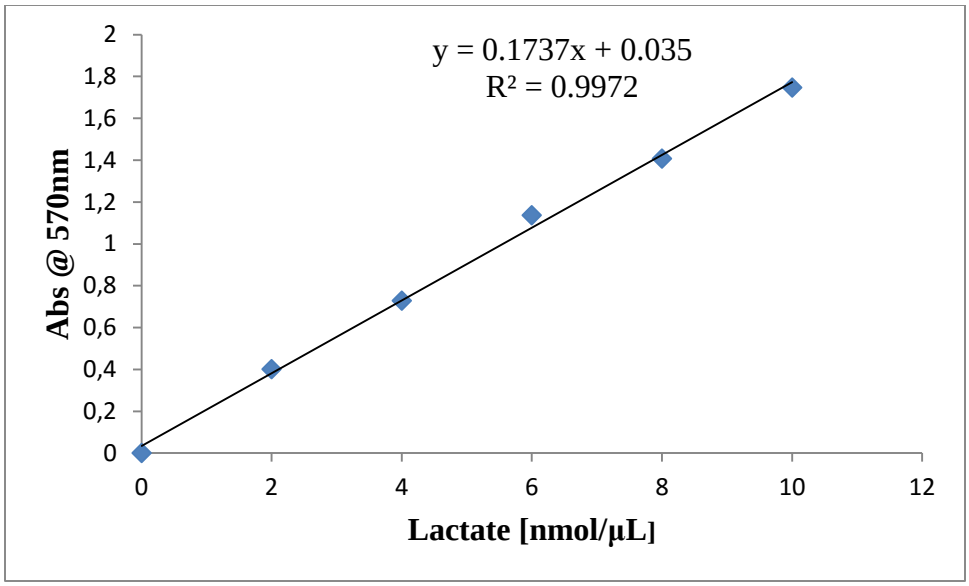

Figure 4.2: Lactate standard curve

Concentration of lactate in sample

5.07 nmole/50 mL = 0.101 nmole/mL

0.101 nmole/mL ´ 89.07 ng/nmole= 9.0 ng/mL

4.2.6 Glycolytic potential calculation

The glycolytic potential calculation was done according to Monin and Sellier (1985), Glycolytic Potential = [lactate] + 2 ([glycogen] + [glucose-6-phosphate] + [glucose] the results were expressed in nmol/g.

4.2.7 Meat quality measurements

4.2.7.1 pH measurements

The pH of the meat was measured at 45 minutes, 3 hours and 24 hours post mortem using a portable pH meter (Crison pH 25, instruments, S.A., Alella, Spain). The pH meter was calibrated once before the readings were taken with pH 4, pH 7 and pH 9 standard solutions and readings were recorded into a data sheet accordingly.

4.2.7.2 Meat colour measurement

The colour variables L* (lightness), a* (redness), b* (yellowness), were also measured from each of the obtained meat samples using a Minolta colour-guide 45/0 BYK- Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-day light, 10º observation angle . The machine was calibrated before taking the measurements using the green, black and white standard colour samples provided for this purpose. The readings were obtained by rotating the colour guide 90° between measurements in order to attain the average value for the colour (Commision International De L' Eclairage, 1976).

The psychometric hue angle which is an indication of the angle at which a vector radiates into the red yellow quadrant will be calculated as: Hue angle = $\tan^{-1}(b^*) / (a^*)$; and psychometric chroma which is a measure of colour saturation will be calculated as: Chroma = $(a^{*2} + b^{*2})^{1/2}$ (Wyszcecki and Stiles, 1982; Minolta, 1993).

4.6.8 Statistical analysis

Generalised Linear Model procedure of SAS (2009) was used to determine the effect of sex and breed on muscle glycolytic potential, glycogen, lactate levels, pH decline and mutton colour (L^*, a^*, b^*, H* and C*). The following model was used:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} = Response variable (glycolytic potential, muscle glycogen and lactate content, pH, L*, a*, b*, hue angle and chroma)

μ = Overall mean

α_i = i^{th} effect of sex (Rams and Ewes)

β_j = j^{th} effect of breed (Dorper and Merino)

$(\alpha\beta)_{ij}$ = Interaction between sex and breed

ε_{ijk} = Random error

The strength of relationships between blood metabolites (glucose, lactate and cortisol), muscle metabolites (glycogen, lactate), glycolytic potential, pH and colour were determined using Pearson`s correlation coefficient (SAS, 2009). Significant differences among group means were

tested using Least Significant Differences (LSD) and differences at $P < 0.05$ were considered to be statistically significant. Interactions between sex and breed were tested.

4.3 Results and Discussion

Effect of sex on muscle glycolytic potential, glycogen and lactate from rams and ewes

The results for the effect of sex on glycolytic potential, muscle glycogen and lactate are shown in Table 4.1. There were no interactions between sex and breed. Furthermore, no significant ($P > 0.05$) sex differences between the glycolytic potential in muscles from rams and ewes (8.1 ± 1.04 nmol/g and 8.7 ± 1.04 nmol/g, respectively). Moreover, statistical analysis showed no significant ($P > 0.05$) differences between rams and ewes for the levels of muscle glycogen (2.8 ± 0.37 nmol/g and 2.6 ± 0.36 nmol/g). The results also indicated no significant differences ($P > 0.05$) in the levels of muscle lactate between the rams and ewes (1.7 ± 0.39 nmol/g and 3.4 ± 1.71 nmol/g, respectively). These results concur with those reported by Lowe *et al.* (2004) and Bertol *et al.* (2011) who also found no significant sex differences on muscle glycogen, lactate and glycolytic potential.

Table 4.1: Effect of sex on muscle glycolytic potential (nmol/g), glycogen (nmol/g) and lactate (nmol/g) from intact rams and non-pregnant ewes (LSMeans ± standard error of the mean)

	Sex		
Muscle metabolites	**Rams**	**Ewes**	**P value**
	n =50	*n* =50	
Glycolytic potential	$33.2^{a} \pm 1.04$	$31.6^{a} \pm 1.04$	0.7109
Muscle glycogen	$5.5^{a} \pm 0.37$	$5.3^{a} \pm 0.36$	0.5945
Muscle lactate	$11.1^{a} \pm 0.39$	$10.5^{a} \pm 0.39$	0.9882

Means in the same row with different superscripts are significantly different at $P < 0.05$.

Effect of breed on muscle glycolytic potential, glycogen and lactate from Dorper and Merino sheep

The results for the effect of breed on glycolytic potential, muscle glycogen and lactate are shown in Table 4.2. There were no significant ($P > 0.05$) breed differences between the glycolytic potential in muscles from Dorper and Merino (30.7 ± 1.02 nmol/g and 34.6 ± 1.06 nmol/g, respectively). Moreover, statistical analysis showed no significant ($P > 0.05$) differences between Dorper and Merino for the levels of muscle glycogen (5.1 ± 0.35 nmol/g and 5.8 ± 0.37 nmol/g). The results indicated no significant differences ($P > 0.05$) in the levels of muscle lactate between the rams and ewes (10.1 ± 0.38 nmol/g and 11.5 ± 0.40 nmol/g, respectively). These results are in contrast with those reported by Gardner *et al.* (1999) who reported that Merino sheep were more likely to lose muscle under commercial slaughter conditions.

Table 4.2: Effect of breed on muscle glycolytic potential (nmol/g), glycogen (nmol/g) and lactate (nmol/g) from Dorper and Merino sheep (LSMeans ± standard error of the mean)

	Breed		
Muscle metabolites	**Dorper**	**Merino**	**P value**
	n = 52	*n* = 48	
Glycolytic potential	$30.7^{a} \pm 1.02$	$34.6^{a} \pm 1.06$	0.1443
Muscle glycogen	$5.1^{a} \pm 0.35$	$5.8^{a} \pm 0.37$	0.1639
Muscle lactate	$10.1^{a} \pm 0.38$	$11.5^{a} \pm 0.40$	0.5257

Means in the same row with different superscripts are significantly different at $P < 0.05$

Effect of sex on pH decline and colour in *Muscularis longissimus thoracious et lumborum* samples from Dorper and Merino sheep

The results for the effect of sex on mutton quality attributes are shown in Table 4.3. There was a significant difference ($P < 0.001$) on meat pH at 45 minutes, between the rams and ewes (6.4 ± 0.04 and 7.1 ± 0.04, respectively). Where, the rams had higher values of pH at 45 minutes compared to the ewes. Sheep with a faster working metabolism at pre-slaughter are more likely to continue glycogen metabolism at a quicker rate. This may result in faster glycolysis thus a lower initial pH (McGeehin *et al.,* 2001). Furthermore, this may be an indication that the rams experienced had a faster glycolytic rate compared to the ewes as indicated by the high pH at 45 minutes. These results contradict those reported by Okeudo (1994) and McGeehin *et al.* (2001) who found no significant sex differences in early pH (pH at 45 minutes) at post-mortem. However, these results concur with results by other authors (Chrystall and Hagyard, 1976; Solomon *et al.,* 1986; Johnson *et al.,* 1989; McGeehin *et al.,* 1999), who also reported pH at 45 minutes to range from 6.4 to 7.00.

Significant sex differences ($P < 0.001$) were observed on meat pH at 3 hours between the rams and ewes (6.1 ± 0.04 and 6.5 ± 0.04, respectively). These results contradict those reported by McGeehin *et al.* (2001), who reported pH values for ewes were lower than males at 4 hours. These pH values are in accordance with those reported by Sheridan *et al.* (1998), where the pH values ranged between 6.02 to 6.27. Furthermore, statistical analysis showed significant differences ($P < 0.001$) between rams and ewes (5.9 ± 0.03 and 6.0 ± 0.03, respectively) on the value of pH at 24 hours. These results contradict those reported by McGeehin *et al.* (2001); Tejeda *et al.* (2008) where he found no significant sex differences in the pH_{24} of sheep.

Table 4.3: Effect of sex on pH decline and colour in *Muscularis longissimus thoracious et lumborum* samples from intact rams and non-pregnant ewes (LSMeans ± standard error of the mean)

	Sex		
Meat quality	**Rams**	**Ewes**	**P value**
	n = 50	*n* = 50	
pH_{45} minutes	$6.4^{b} \pm 0.04$	$7.1^{a} \pm 0.04$	0.0001
pH_3 hours	$6.1^{a} \pm 0.04$	$6.5^{b} \pm 0.04$	0.0001
pH_{24} hours	$5.9^{a} \pm 0.03$	$6.0^{b} \pm 0.03$	0.0001
L*	$38.1^{a} \pm 0.47$	$36.4^{a} \pm 0.47$	0.0090
a *	$12.3^{a} \pm 0.33$	$14.3^{b} \pm 0.33$	0.0001
b *	$7.0^{a} \pm 0.29$	$8.8^{a} \pm 0.29$	0.0001
H *	$29.4^{a} \pm 0.73$	$31.5^{b} \pm 0.73$	0.0431
C *	$14.2^{a} \pm 0.42$	$16.8^{b} \pm 0.42$	0.0001

Means in the same row with different superscripts are significantly different at $P < 0.05$, NS- not significant, pH_{45} minutes = pH at 45 minutes, pH_3 hours = pH at 3 hours, pH_{24} hours = pH at 24 hours, L* = Lightness, a*= Yellowness, b*= Redness, H*= Hue angle, C*= Chroma

In this study the ewes had a higher pH_{24} (6.00 ± 0.03) than the rams (5.88 ± 0.03).The pH values after 24 hours ranged from 5.88 to 6.00. These values are within normal pH range (Devine *et al.,* 1993). However the ewes had a higher pH_{24} than the rams, this could have been attributed by rams and ewes being placed in lairages together. The rams could have been excited and attempted sexual activity (Ferguson *et al.,* 2001; Johnson *et al.,* 2005; Cloete *et al.,* 2012) by doing so stressing the ewes. The pH_{24} of meat could also have been influenced by the amount and rate of glycogen breakdown for lactate production in the muscle to lower the meat pHu at post mortem (Kadim *et al.,* 2006). This could have also been influenced by differences in glycogen storage and muscle physiology (Gregory, 1998). These results indicate that the ewes experienced a higher degree of stress than the rams hence less glycogen was available in the muscle to produce lactic acid in order to acidify and lower the pH to the recommended ultimate pH at 24 hours. Restless animals tend to have reduced levels of glycogen hence a high ultimate pH (Warriss, 2010). When there are low glycogen reserves at slaughter lactate production at after slaughter will be limited thus a pH greater than 6.00 will result dark cutting may result (Coombes *et al.,* 2014). A higher pH_{24} negatively affects the transformation of mtton to meat because of low glycogen storage thus little lactic acid in muscles at post-mortem (Ferguson and Warner, 2008; Rodríguez *et al*., 2011). The pH of meat at 24 hours is a very important determinant of the quality of meat (Chulayo and Muchenje, 2013). Meat with a high pH_{24} is also prone to microbial spoilage hence low shelf-life, reduced flavour and foul smell (Young *et al*., 1993). Although pH_{24} of ewes was 6.00 there were no cases of DFD since the L* values were within the recommended range. It can therefore be concluded that, the speed of pH decline is affected by exposure to stress (Apple *et al.,* 1995), animal related factors such as species (Varnham and Sutherland, 1995), breed (Sanz *et al.,* 1996) and sex (Brown *et al.,* 1990).

There was a significant difference ($P < 0.05$) between the rams and ewes on the level of meat lightness (38.1 ±0.47 and 36.4 ± 0.47, respectively). In this study the ewes had a lower L* value than the rams. The L* value is an important indication of the quality of meat since it is influenced by pH_{24} therefore determining whether the meat is DFD (Wiklund *et al*., 2003; Rodríguez *et al*., 2011). However in this study there were no cases of DFD because the pH_{24} values for both rams and ewes were less than 6.2 and the L* values were greater than 33, indicating that they were within the expected ranges (Lawrie, 1974; Diaz *et al.,* 2006) that would not result in dark firm dry (DFD) meat. These results are contrary to results observed by Cloete *et al.* (2012), who reported DFD meat in ewes. Teixeira *et al.* (2005) also reported that rams had higher lightness values than the ewes this means the ewes had darker meat than the rams because of their greater alertness during the pre-slaughter period (Thompson *et al.*, 1979). This is an indication that the ewes were more stressed than the rams, since stress predisposes meat to becoming darker in colour (Klont *et al.,* 2000; Kadim *et al.,* 2006), but L* the value for the ewes was not below 34 which is deemed acceptable by consumers (Khlij *et al.,* 2010). These results contradict those reported by Zhong *et al.* (2011), where meat with a high pHu was reported to have a lower value for meat lightness. In this study meat from ewes had a higher pHu and low levels of lightness and the rams which had a low pHu had a higher value of lightness than the ewes.

Significant sex ($P < 0.001$) differences were observed for meat redness between the rams and ewes (12.3 ± 0.33 and 14.3 ± 0.33, respectively). The ewes had a higher value (14.31 ± 0.33) for redness than the rams, similarly to results observed by Johnson *et al.* (2005) and Cloete *et al.* (2012). These results contradict those by Vergara *et al.* (1999) where the results showed that females had slightly lighter meat and lower redness values than males. Females have been reported to produce redder meat than rams due to their higher alertness during the pre-slaughter period (Tejeda *et al.,* 2008).

Statistical analysis showed significant differences ($P < 0.001$) for meat yellowness between rams and ewes (7.0 ± 0.29 and 8.8 ± 0.29, respectively). These results concur with those reported by Johnson *et al.,* 2005. There was also a significant sex difference ($P < 0.001$) between the rams and ewes for hue angle (29.4 ± 0.73 and 31.5± 0.73, respectively). Furthermore, there was significant sex difference between the rams and ewes on chroma (14.2 ± 0.42 and 16.8 ± 0.42, respectively). The ewes had a more intense colour compared to the rams. Meat colour is an important indicator of red meat quality and it is the mostly used indicator that consumers use to judge the quality for making purchasing decisions (Rani *et al*., 2013).

Effect of breed on pH decline and colour in *Muscularis longissimus thoracious et lumborum* samples from Dorper and Merino sheep

The results for the effect of breed on mutton quality attributes are shown in Table 4.4. The results indicate that there were no significant differences ($P > 0.05$) in pH from Dorper and Merino sheep measured at 45 minutes (6.17 ± 0.06 and 6.78 ± 0.06, respectively), 3 hours (6.26 ± 0.04 and 6.31 ± 0.04, respectively) and 24 hours (5.93 ± 0.03 and 5.98 ± 0.03, respectively) post-slaughter. Although there were no statistical breed differences for pH values, the Merino has slightly higher values compared to the Dorper. These results are in accordance with those reported by Hopkins and Mortimer (2014), where Merinos were seen to be more prone to producing meat with a higher pH (Hopkins *et al.,* 2011). It has also been reported that under stressful pre-slaughter conditions, Merino sheep loose muscle glycogen more rapidly than compared to other breeds (Gardner *et al.,* 1999; Hopkins *et al.,* 2007) but under non- stressful conditions they have a similar meat pH as other breeds (Hopkins *et al*., 2005).

Table 4.4: Effect of breed on pH decline and colour in *Muscularis longissimus thoracious et lumborum* muscle samples from Dorper and Merino sheep (LSMeans ± standard error of the mean)

	Breed		
Meat quality	**Dorper**	**Merino**	**P value**
	n = 52	*n* = 48	
pH_{45} minutes	$6.7^{a} \pm 0.06$	$6.8^{a} \pm 0.06$	0.4299
pH_3 hours	$6.3^{a} \pm 0.04$	$6.3^{a} \pm 0.04$	0.3617
pH_{24} hours	$5.9^{a} \pm 0.03$	$5.0^{a} \pm 0.03$	0.2596
L*	$36.4^{a} \pm 0.46$	$38.2^{b} \pm 0.48$	0.0090
a*	$13.5^{a} \pm 0.35$	$13.0^{a} \pm 0.37$	0.3549
b*	$7.8^{a} \pm 0.31$	$8.1^{a} \pm 0.32$	0.4908
H*	$29.3^{a} \pm 0.71$	$31.6^{b} \pm 0.74$	0.0304
C*	$15.6^{a} \pm 0.45$	$15.2^{a} \pm 0.47$	0.5395

Means in the same row with different superscripts are significantly different at $P < 0.05$, pH_{45} minutes = pH at 45 minutes, pH_3 hours = pH at 3 hours, pH_{24} hours = pH at 24 hours, L* = Lightness, a*=Yellowness, b*= Redness, H*= Hue angle, C*= Chroma

However, there was a significant difference ($P < 0.05$) between the Dorper and Merino on the level of meat lightness (36.4 ± 0.46 and 38.2 ± 0.48, respectively). The Merino had higher values of lightness compared to the Dorper sheep. Furthermore, there were no significant sex ($P > 0.05$) differences were observed for meat redness between the Dorper and Merino (13.5 ± 0.35 and 13.0 ± 0.37, respectively). In Dorper meat was darker than Merino, contrary to results by Cloete *et al.* (2005) who reported that Merino was more prone to stress and had lower meat quality. Breed differences in meat quality are known to be a result of selection for production and economic traits (Hansen *et al.,* 2001). The Dorper breed is selected mainly for meat production whilst the Merino is a dual-breed selected for the production of both meat and wool, but surprisingly in this study the Dorper has better meat quality compared to the Merino.

Even though Merino sheep produced higher meat pH the fresh meat produced is not dark. This may be caused by the low phenotypic correlation between pH and L* values in Merino sheep (Menzies and Hopkins, 1996). Statistical analysis showed no significant differences ($P > 0.05$) for meat yellowness between the Dorper and Merino (7.8 ± 0.31 and 8.1 ± 0.32, respectively). The results indicate that there were significant differences ($P < 0.05$) in hue angle from Dorper and Merino sheep (29.33 ± 0.71 and 31.59 ± 0.74, respectively). The Dorper sheep had lower hue angle values compared to the Merino sheep. Furthermore, there was no significant breed difference between the Dorper and Merino on meat chroma (15.6 ± 0.45 and 15.2 ± 0.47, respectively). These results contradict those reported by O'Neil *et al.* (2006), who reported that breed had an effect on meat colour. In this study Merino sheep had higher values L*, b* and H* compared to the Dorper, similarly to results by Martínez-Cerezo *et al.* (2005). This could be attributed to the adaptability of the Merino breed to exercise in traditional production systems (Tejeda *et al.,* 2008).

Pearson's correlations coefficients (*r*) blood glucose, lactate, cortisol, glycolytic potential, muscle glycogen, lactate, pH and colour from Dorper and Merino sheep of both sexes

In Table 4.5 Pearson's correlations are shown. They were run to assess the relationships between blood glucose, cortisol, muscle glycogen, lactate levels, glycolytic potential, meat pH and colour in intact rams and non-pregnant ewes from both Dorper and Merino breeds. Most muscle metabolites and meat quality traits were not significantly correlated ($P < 0.05$). However, blood metabolites were positively correlated to each other, where glucose levels were positively correlated ($P < 0.01$) to blood lactate ($r = 0.32$). Blood glucose was also positively correlated ($P < 0.05$) to blood cortisol ($r = 0.27$). Blood lactate levels were also positively ($P < 0.001$) correlated to blood cortisol ($r = 0.37$). However, blood glucose was negatively correlated ($P < 0.001$) to meat lightness ($r = -0.44$). The relationships between the levels of blood cortisol, glucose and lactate levels in response to pre-slaughter stress has been well established (Warriss *et al*., 1994; Hambrecht *et al*., 2004; Hambrecht *et al*., 2005; Terlouw *et al*., 2008; Edwards *et al*., 2010). Blood cortisol was positively correlated ($P < 0.001$) to pH at 45 minutes post mortem ($r = 0.34$). There were positive correlations ($P < 0.05$) between blood cortisol and pH at 24 hours post-mortem. However, a negative relationship ($P < 0.001$) existed between blood cortisol and meat lightness ($r = -0.47$). There were positive correlations ($P < 0.01$) between blood cortisol and meat yellowness ($r = 0.24$). Furthermore, there were positive correlations between blood cortisol and chroma ($r = 0.37$). During pre-slaughter stress response, sheep respond by activating the hypothalamic-pituitary-adrenocortical axis thus resulting to an increase in blood cortisol levels (Minton, 1994).

Table 4.5: Pearson's correlations coefficients (*r*) blood glucose, lactate, cortisol, glycolytic potential, muscle glycogen, lactate, pH and colour from Dorper and Merino sheep of both sexes

Blood, muscle metabolites, pH and colour	B.Lac	B.Cor	G.P	M.Gly	M. Lac	pH_{45}	pH_3	pH_{24}	L*	a*	b *	H*	C*
B.Glu	0.31***	0.27**	-0.09^{ns}	-0.04^{ns}	-0.08^{ns}	-0.12^{ns}	-0.11^{ns}	-0.03^{ns}	-0.15^{ns}	0.01^{ns}	-0.04^{ns}	-0.01	0.04^{ns}
B.Lac	-	0.37***	-0.14^{ns}	-0.13^{ns}	-0.06^{ns}	0.15^{ns}	0.10^{ns}	0.10^{ns}	-0.44***	0.24^{ns}	0.09^{ns}	-0.11^{ns}	0.19^{ns}
B.Cor		-	-0.16^{ns}	-0.11^{ns}	-0.10^{ns}	0.34***	0.15^{ns}	0.22*	-0.47***	0.42***	0.24**	-0.03^{ns}	0.37***
G.P			-	0.66***	0.71***	-0.13^{ns}	-0.04^{ns}	-0.04^{ns}	0.15^{ns}	-0.14^{ns}	-0.11^{ns}	-0.07^{ns}	-0.16^{ns}
M.Gly				-	-0.05^{ns}	-0.14^{ns}	-0.01^{ns}	-0.00^{ns}	0.11^{ns}	-0.08^{ns}	0.09^{ns}	-0.09^{ns}	-0.13^{ns}
M.Lac					-	-0.05^{ns}	-0.04^{ns}	0.06^{ns}	0.09^{ns}	-0.11^{ns}	-0.07^{ns}	-0.08^{ns}	-0.09^{ns}
pH_{45}						-	0.76***	0.47***	-0.21*	0.30**	0.31***	0.19^{ns}	0.31***
pH_3							-	0.61**	-0.13^{ns}	0.13^{ns}	0.18^{ns}	0.17^{ns}	0.13^{ns}
pH_{24}								-	0.01^{ns}	-0.30^{ns}	0.11^{ns}	0.17^{ns}	0.02^{ns}
L*									-	-0.67***	-0.34***	0.01^{ns}	-0.57***
a*										-	0.73***	0.18^{ns}	0.95***
b*											-	0.76***	0.86***
H*												-	0.40***
C*													-

Significantly correlated at $^{*}P < 0.05$, $^{**}P < 0.01$,$^{***}P < 0.001$, ns= Not significant ,B.Glu = Blood glucose, B.Lac = Blood Lactate, B.Cor = Blood Cortisol, G.P = Glycolytic potential, M.Gly = Muscle glycogen, M.Lac = Muscle Lactate, pH_{45} = pH at 45 minutes, pH_3 = pH at 3 hours, pH_{24} = pH at 24 hours, L^{*} = Lightness, a*=Yellowness, b*=Redness, H*= Hue angle, C*= Chroma

The significant relationships between blood cortisol and mutton quality are evident because, circulating levels of cortisol tend to recover less rapidly and slower compared to other stress indicators. Hence high cortisol levels have an impact on the mutton quality which reflects the pre-slaughter period (Warriss *et al.,* 1994).

Glycolytic potential was positively correlated ($P < 0.001$) to muscle glycogen levels ($r = 0.66$). Glycolytic potential was also positively correlated ($P < 0.001$) to muscle lactate ($r = 0.71$). The positive relationship between glycolytic potential, muscle glycogen and lactate has been well established, because when glycolytic potential is reduced muscle glycogen and lactate levels are diminished (Kannan *et al.,* 2002). This means meat produced will have a higher ultimate pH because there is insufficient glycogen to produce lactate which is responsible for acidifying the meat and lowering ultimate pH. This relationship is very important in the conversion of muscle to meat (Purchas *et al.,* 1999).

There was a positive correlation ($P < 0.001$) between pH_{45} at 45 minutes post-mortem and pH_3 at three hours post-mortem (r = 0.76). A positive relationship ($P < 0.001$) existed between pH_{45} at 45 minutes and pH_{24} at 24 hours post-mortem ($r = 0.47$). There was a positive relationship ($P < 0.01$) between pH_3 at 3 hours and pH_{24} at 24 hours post-mortem (r =0.61). These results concur with those by Edwards *et al.* (2010). The level of pH decline from the initial to the final pH is influenced by glycogen content at prior to slaughter. When glycogen content prior to slaughter is not optimal a pH of 5.8 and less will not be reached in the muscle at post mortem (Scheffler *et al.,* 2015).

However, a negative relationship ($P < 0.05$) existed between pH at 45 minutes post-mortem and meat lightness (r = -0.21). A positive relationship ($P < 0.01$) exists between pH_{45} at 45 minutes and meat redness ($r = 0.30$). There was a positive relationship ($P < 0.001$) between pH_{45} minutes and meat redness ($r = 0.31$). A positive correlation ($P < 0.001$) existed between

pH_{45} minutes and chroma (r = 0.31).These results contradict those reported by Dokmanivic *et al.* (2015) who found negative correlations between pH at 45 minutes post-mortem, meat redness and yellowness in pigs. A negative relationship (P < 0.001) existed between pH_{24} at 24 hours and meat redness (r = -0.67). There was a negative correlation (P < 0.001) between pH_{24} at 24 hours and meat yellowness (r = -0.34). Furthermore, there was also a negative correlation (P < 0.001) between pH_{24} at 24 hours and chroma (r = 0.57). A strong relationship between meat pH and the colour has been previously reported (Muchenje *et al.,* 2009). This is because the colour of meat is dependent on the rate and extent of post-mortem pH decline (Honikel, 1997). Furthermore, meat with high pH values greater than 6.0 has been reported to have a dark colour (Bartos *et al.,* 1993; Kreikemeier *et al.,* 1998; Mounier *et al.,* 2006). Meat with a dark colour and high ultimate pH is aesthetically unacceptable since it is susceptible to microbial spoilage which results in the formation of slime and off odours (Gardner *et al.,* 2001; Gallo *et al.,* 2003).

A negative correlation (P< 0.001) existed between meat lightness and meat redness (r = -0.67). There were also negative correlations (P < 0.001) between meat lightness and meat yellowness (r = -0.34).These results concur to those reported by McCann *et al.* (2008) and Gajana *et al.* (2013). Moreover, there were negative correlations (P < 0.001) between meat lightness and chroma (r = 0.57). Whilst meat redness was positively correlated (P < 0.001) with meat yellowness (r = 0.73). A positive correlation (P < 0.001) also existed between meat redness and chroma (r = 0.95). There were positive correlations (P < 0.001) between yellowness and hue angle (r = 0.76). A positive correlation (P < 0.001) also existed between meat yellowness and chroma (r = 0.86). Furthermore, hue angle was positively correlated (P < 0.001) to meat chroma (r = 0.40). The three colorimeter values (L^*, a^* and b^*) were all negatively correlated to each other. These results are in contrast with those previously demonstrated results on these relationships available in the literature where, the coloritemer values have been reported to be

positively correlated (Joo *et al.* 1995; Van Laack *et al.* 1996; Muchenje *et al.*, 2008). These results are similar to those reported by Diess *et al.* (2009).

4.4 Conclusion

It can therefore be concluded that sex and breed have an effect on meat quality attributes of Dorper and Merino sheep but not on the muscle glycogen, lactate and glycolytic potential. Meat from the ewes had a high pH, low L* and high a*, b*, C*, H* values compared to the rams. However there were no cases of DFD meat since both the pH and L* values were within the normal ranges. Breed also had an effect on meat L* and H*. The results indicate that the Merino had higher values of L* than the Dorper. However there were no breed effects on muscle glycogen, lactate and glycolytic potential. There were significant positive and negative correlations amongst blood metabolites, glycogen and lactate levels, meat pH decline and colour.

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Chapter 5: General Discussion, Conclusion and Recommendation

5.1 General Discussion

Introduction

During exposure to stress prior to slaughter glycogen reserves are significantly depleted due to the release of stress indicators and catecholamines into the blood stream. When glycogen reserves are depleted prior to slaughter the muscle is unable to produce the required amounts of lactic acid in the muscle to allow the muscle acidification to occur thus the desirable ultimate pH will not be achieved and meat quality will be reduced. However, work done in ruminants is still quite limited, and it does not take into account sex and breed differences that occur in response to stress (Gardner *et al.*, 2014).

Hence, the main objective of the study was to determine the levels of blood metabolites collected at exsanguination (cortisol, glucose and lactate), glycolytic potential and mutton quality from Dorper and Merino sheep of both sexes. In Chapter 3, sex and breed effects on the levels of blood glucose, lactate, cortisol and their correlations were determined. Effects of sex and breed on muscle glycolytic potential, lactate, glycogen levels and mutton quality were determined in Chapter 4. This study was conducted following normal abattoir slaughter conditions using the both intact males and non-pregnant females from Dorper and Merino breeds.

As shown in Chapter 3, blood lactate and cortisol levels between sexes indicated that the pre-slaughter period was a stressful experience, but glucose was not affected by sex differences. However, the levels of blood glucose indicate that both the Dorper and Merino sheep were exposed to stress, since the glucose levels were higher than the normal range (2.36 ± 0.07 mmol/L) (AL-Hadithy and Badawi, 2015).

Ewes were more responsive to psychological and physical stress compared to the rams, as shown by the increased levels of blood lactate and cortisol at exsanguination. This was attributed to the fact that ewes perceive the slaughter processes more stressful than rams (Boissy and Boissou, 1994; Taylor *et al.,* 2001). However breed only affected the levels of blood lactate and did not affect glucose and cortisol. Furthermore, the Dorper had higher levels of lactate than the Merino. These results indicate that the Dorper experienced more stressed than Merino sheep during the pre-slaughter period. Behaviourally reactive animals tend to have higher levels of blood lactate (Coombes *et al.,* 2014). Positive correlations were observed for blood glucose, lactate and cortisol.

In chapter 4, breed and sex effects were observed on mutton quality attributes but not on muscle glycolytic potential, glycogen and lactate levels. Meat samples collected from ewes had higher pH values (pH_{45} minutes, pH_3 and pH_{24} hours), low lightness (L*), higher redness (a*), yellowness (b*), chroma (C*) and hue angle (H*) values than the rams. Ewes experienced a higher degree of stress than the rams hence less glycogen was available in the muscle to produce lactic acid in order to acidify and lower the pH thus a darker colour was produced (Warriss, 2010). Dorper breed produced darker meat than the Merino. This is an indication that the Dorper experience more stress compared to the Merino thereby producing lower values of lightness (L*). These results contradict those reported by Hopkins and Mortimer (2014), where Merinos were seen to be more prone to pre-slaughter stress (Hopkins *et al.,* 2011). Where Merino sheep were reported to lose muscle glycogen more rapidly than compared to other breeds (Gardner *et al.,* 1999; Hopkins *et al.,* 2007) but under non- stressful conditions they have a similar meat quality as breeds (Hopkins *et al*., 2005).

Significant correlations were observed between ewes and rams on pH_{45} minutes, pH_3 hours and pH_{24} hours and meat colour (L*, a*, b*, H* and C*). Ewes had higher levels of pH_{45} min, pH_3, pH_{24} hours, a*, b*, H* and C* than the rams which had lower pH_{45}, pH_3, pH_{24} hours, a*, b*, H* and C* values. Merino sheep had higher levels of L*and H*than the Dorper which had and L* and H* values, respectively.Significant correlations were observed amongst blood stress indicators (glucose, lactate and cortisol), muscle metabolites (glycogen and lactate levels), glycolytic potential, meat pH and colour. These relationships are an indication that there is a link between blood stress indicators and muscle metabolites thus affecting the meat quality in response to pre-slaughter stress responsiveness (Dokmanovic *et al.,* 2015).

5.2 Conclusion, Recommendations and Future research

It can therefore be concluded that sex and breed have an effect on blood lactate, cortisol levels meat colour (L*, a*, b*, C* and H*) and pH decline (pH_{45} minutes, pH_3 and pH_{24} hours) of Dorper and Merino sheep but not on blood glucose, muscle glycogen, lactate and glycolytic potential. Blood lactate and cortisol levels collected at exsanguination reveal that the ewes were more reactive compared than the rams to the pre-slaughter period. However, more studies should be designed to investigate sex differences in response to the pre-slaughter period, where the sheep are placed on lairages according to sex. Since the mixing of rams and ewes might influence the results. Meat samples measured for meat quality attributes revealed also that ewes were more reactive compared to the rams to the pre-slaughter period. Furthermore, meat from the ewes had a high pH, low L* and high a*, b*, C*, H* values. However there were no cases of DFD meat since both the pH and L* values were within the normal ranges. The results also indicate that the Dorper breed had higher blood lactate levels than the Merino. However there were no breed effects on blood glucose and plasma cortisol. Blood lactate is mainly known as an indicator of physical stress, from the results it can be concluded that the Dorper sheep experienced more physical stress compared to the Merino. There were significant correlations

positive amongst blood stress indicators (glucose, lactate and cortisol), meaning when sheep are exposed to stress glucose, lactate and cortisol levels will increase in the blood. Breed also had an effect on meat L* and H*. The results indicate that the Merino had high values of L* than the Dorper. This could be an indication that the Dorper sheep were more agitated during the pre-slaughter period. However there were no breed effects on muscle glycogen, lactate and glycolytic potential. Based on these results it can be seen that sex and breed effects are evident on blood stress indicators and meat quality. However, there were no sex and breed effects on the muscle glycogen, lactate and glycolytic potential. There were significant positive and negative correlations amongst blood metabolites, glycogen and lactate levels, meat pH decline and colour.

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