Is the rate and extent of pH fall correlated across the muscles in sheep?

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Abstract

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The pattern of pH decline in animals has been related to several factors such as electrical simulation, rapid chilling, and/or meat tenderness. The main objective of this study was to analyse the factors that affects the change in pH in sheep carcasses, and to compare the change among different muscles during the post slaughter period. For this, an experimental trial was carried out using 5 muscles, namely the Longissimus dorsi (LD), Semitendinosus (ST), Gracilis (G), Sternomandibularis (S_tM), and Platysma (P) from 6 sheep carcasses. Muscles from the right-hand side were used for assessing the biochemical characteristics and meat quality. The other side was used to measure the pH and temperature changes. The pH and temperature of the five different muscles on the carcasses was measured at time interval of 90m, 3h, and 24h post-mortem, and off the carcasses was measured at 45m and 48h post slaughter. The rate of decline in pH was correlated to different biochemical characteristics such as muscle fibre typing, lactate content and glycogen content, and meat quality characteristics such as colour, cooking loss, and shear force.

The effect of pH and temperature change, colour, cooking loss, shear force, muscle fibre typing, lactate, and glycogen content across different muscles in sheep was investigated. The study provided evidence to prove that there is a constant decline in muscle pH with Platysma showing the highest and Semitendinosus and Longissimus dorsi muscles showing the lowest pH values. The colour measurements showed that Sternomandibularis had the highest chroma, and Longissimus dorsi muscles hsd the highest hue value. Cooking loss was measured on the 2^{nd} day and 7^{th} day for the muscles, and it was studied that Longissimus dorsi muscles had the highest cooking loss and Gracilis had the lowest. Muscle shear force values was also measured on 2^{nd} and 7^{th} day, which provided evidence to prove that Gracilis had the highest and Semitendinosus and Longissimus dorsi muscles

muscles had the lowest shear force values. However, day 7 measurements showed that G and ST muscles had the highest sheat force whereas LD muscles had the lowest. The muscle fibre typing provided evidence to prove that all the five muscle samples showed the presence of MCH IIx (MHC-1), IIa (MHC- 2), IIb (MHC-4), and MHC-7 (slow/1). The glycogen analysis provided evidence to prove that the concentration of muscle glycogen followed the order P>> ST=G>>SM=LD. This in turn suggested the correlation of glycogen concentration with pH, temperature, and fibre typing.

Keywords: Sheep, pH, biochemical characteristics, meat quality, aging, Longissimus dorsi (LD), Semitendinosus (ST), Gracilis muscle (GC), Sternomandibularis (SM), and Platysma (P)

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Abbreviations

a*	Redness
AA	Amino Acids
ADP	Adenosine-5'-diphosphate
AMG	Amyloglucosidase
ANOVA	Analysis of variance
APS	Ammonium persulfate
ATP	Adenosine triphosphate
ATP	Adenosine-5'-triphosphate
b*	Yellowness
C	Chroma
C*	Colour Saturation
DeoMb	Deoxymyoglobin
DFD	Dark, firm, and dry
G	Gracilis
G-6-P	Glucose-6-phosphate
G6P-DH	Glucose-6-phosphate dehydrogenase
GLM	General linear model
GP	Glycolytic Potential
HA	Hue
HK	Hexokinase
IF	*Infraspinatus*
JML	John Memorial Laboratory
L*	Lightness
LD	Longissimus dorsi
MetMb	Metmyoglobin
MyHC	Myosin heavy chain
$NADP^+$	Nicotinamide-adenine dinucleotide phosphate
NADPH	Nicotinamide-adenine dinucleotide phosphate
OxyMb	Oxymyoglobin
P	Platysma
PCA	Perchloric acid

pH_u	Ultimate pH
PM	Post-mortem
PM	*Psoas Major*
SDS	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SS	Supraspinatus
ST	Semitendinosus
S_TM	Sternomandibularis
TEMED	N, N, N', N'-tetramethylethylenediamine

Chapter 1

Introduction

1.1 Background

Sheep meat is a high value nutritious food product which is a rich source of protein and micronutrients such as vitamins A, B12, B6, D and E, zinc, iron, and selenium (Morán et al., 2013). The rate and extent of fall in pH during the postmortem (PM) period is an important factor which influences the quality of the sheep carcasses (McGeehin, Sheridan, & Butler, 2001). Hopkins, Hegarty, Walker, and Pethick (2006) reported the correlation between objective measures such as pH, pattern of temperature decline in the carcasses, color, cooking loss, shear force, and sensory traits such as flavor, tenderness, juiciness, and overall liking of the meat products. This in turn depends on several factors such as stress, chilling temperature, glycolytic potential, and electrical stimulation (McGeehin et al., 2001).

The longissimus dorsi (LD) muscle along the back is the most studied in Meat Science (Ithurralde et al., 2015). However, the muscle is less likely to be sacrificed for analysing the quality of the carcasses as it is a large valuable muscle. In this research, muscles from different parts of the body which are easily accessible and preferably low value, like the neck muscles will be evaluated to study the pH, temperature, biochemical characteristics, and the meat quality characteristics of the sheep carcasses.

1.2 Objective

The main objective of the present study is to analyse the change in pH of different muscles that are convenient and easy to access, such as the neck muscle in sheep. Their characteristics were evaluated at fixed time intervals and was correlated to the LD muscle.

1.3 Experimental Design

The experiment is designed to analyse the pH change across five different muscles in sheep. This includes two fast glycolytic muscles, namely the Semitendinosus (ST) and the Longissimus (LD) muscles, and two neck muscle, namely the Platysma and the fast glycolytic Sternomandibularis (S_tM), and one other muscle, namely the Gracilis. These five muscles will be taken from the right-hand side, whereas the left-hand side will be left intact in the sheep carcasses after the slaughter of 6 animals. The right-hand muscles will be used for assessing the biochemical characteristics and meat quality. The other side was used to measure the pH and temperature changes. Biochemical characteristics measured will be the muscle fibre types, glycogen content, and lactate content. The meat quality characteristics will be the shear force, colon, and cooking loss.

Chapter 2

Literature Review

2.1 Introduction

Quality of a meat product is often dependent on several intrinsic and extrinsic factors which includes the post-mortem pH decline, buffering capacity, and muscle temperature (Ithurralde et al., 2018). However, there are several factors which affects the extent and rate by which the PM glycolysis changes in lamb carcasses (McGeehin et al., 2001). It is unavoidable that animals experience certain stress level during the time of pre-slaughter. As they get exposed to these pre slaughter stress conditions, they trigger mechanisms by which the animals get through the stress conditions and maintain the homeostasis. However, this can result in a deviation from the normal physiological function, resulting in increased level of cortisol, lactate, and glucose. This in turn causes an increase in pH, and a decrease in redness (a*), and lightness values (L*) (Stempa, Muchenje, Abrahams, & Bradley, 2018). Additionally, the rate and extent of decrease in pH during the PM period is highly dependent on the glycolytic potential (GP). This outlines the significance of anerobic metabolism which plays a crucial role in extending the rate of lactic acid synthesis and subsequently decreasing the pH (Hudson, 2012). The distribution of water in muscles and its mobility highly influence the meat quality characteristics like tenderness, appearance, firmness, and juiciness. During the post slaughter period, as muscles get converted to meat, the myowater content, its mobility and location would change as a function of numerous factors such as muscle, breed, stress level, and post-mortem characteristics such as cooling rate, slaughter process, aging time, and temperature (Pearce, Rosenvold, Andersen, & Hopkins, 2011).

2.2. Post-mortem metabolism in muscle

2.2.1. Energy system in post-mortem metabolism

Myofibres are typed as glycolytic or oxidative based on metabolic pathways. Two metabolic pathways that are used for the generation of Adenosine triphosphate (ATP) in skeletal muscles are the oxidative or aerobic pathway and the anaerobic pathway. The aerobic pathway aids in the oxidation of lipids, ketone bodies, amino acids, glucose, and glycogen in mitochondria. This produces a large amount of energy with high oxygen requirement. The anaerobic pathway enables the conversion of stored glycogen to lactate, in the absence of oxygen (Lefaucheur, 2010).

Skeletal muscle is characterized by energy consumption during physical exercise (Lefaucheur, 2010). Various biochemical process which are involved in maintaining the stability of metabolism have been

governed by sarcoplasmic proteins (Gao, Wu, Ma, Li, & Dai, 2016). During the process of low intensity work, the energy demand of the muscle is met by the aerobic metabolism. But as the intensity of muscle contraction and speed increases, the available oxygen becomes limiting. Therefore, the rapid anaerobic glycolytic pathway becomes essential for supplying energy. As a result, ATP hydrolysis occurs during contraction, which occurs as acto-myosin ATPase activation (Lefaucheur, 2010). According to Hamilton, Miller, Ellis, McKeith, and Wilson (2003) glycolytic potential acts as an indicator of all the compounds in the muscle that can be converted to lactic acid. Additionally, it also acts as an indicator of decrease in muscle pH during the PM period.

2.2.2. pH change during post-mortem metabolism

The rate and extent of decline in pH during the early post-mortem period is an important factor which determines the structural changes happening in muscles during this time (Hughes, Clarke, Purslow, & Warner, 2017). Tenderness of meat is a factor which influence the extent of pH change in muscle during the PM period (McGeehin et al., 2001). Depending on the concentration of glycogen in muscle and its type, there is a drop in the level of muscle pH from 7.0 (live muscle) to pH of 5.5. to 6.2 (meat) (Pearce et al., 2011). The conversion of glycogen into lactate and H^+ ions during the PM period can cause a decrease in the pH of meat. High pH during the early hours of post-mortem can result in cold shortening if muscle temperature is lowered quickly. This in turn increases the toughness of meat (McGeehin et al., 2001). Additionally, the level of glycogen during the time of slaughter is inversely proportional to the ultimate pH value (Shija et al., 2013). Due to the increase in the hydrogen ion concentration, there is a reduction in the myofibrillar electrostatic repulsion. This in turn reduces the repulsion between filaments which causes muscle fibre shrinkage (Pearce et al., 2011).

The combination of pH and temperature during the onset of rigor is an important factor which determines the degree of meat shortening and tenderness. The tendency of the muscle to cold shorten decreases as pH falls below 6.0. Additionally, the toughness of the muscle samples was studied to rise as the ultimate pH increases from 5.5 to 6 and then decrease with a further rise in pH to 7 (Ertbjerg & Puolanne, 2017). According to Hughes, Clarke, Purslow, and Warner (2018) a low pH value can cause the inhibition of mitochondrial oxygen consumption. This is because mitochondrial respiration favoured the conversion of oxymyoglobin (OxyMb) to metmyoglobin (MetMb) or deoxymyoglobin (DeoMb). As a result, a low pH resulted in a stable color, due to the absence of auto oxidation and the strong effects of the MetMb reductase (Jacob et al., 2014).

2.2.3. Temperature change during post-mortem metabolism

Temperature is an important factor which determines the storage life of fresh meat products. Meat held at a temperature of -1.5°C is studied to achieve the maximum storage. Therefore, to attain

maximum shelf life for lamb meat and other chilled meat products, it should be maintained at a temperature of -1.5 °C during storage and extended transport (Rosenvold & Wiklund, 2011). Experimental evidence suggests that, temperature of the meat samples at pH=6 has shown to be an important factor which determines the overall eating quality and tenderness of the meat samples. Additionally, as the temperature at pH=6 increases, the overall liking attribute of the meat also increases such that, an increase in 10° C would result in 4.3 improvement regarding the overall liking score of the product (Hopkins et al., 2011).

An increase in the rigor temperature is studied to have high global brightness and lightness which acts as an indicator of higher light scattering capacity of the muscles compared to muscles with low rigor temperature of 5 to 15 °C (Hughes et al., 2018). An elevated rigor temperature of 35-40 ºC can cause structural changes in the muscle which increases the light scattering capacity. The high rigor temperature of the muscle can cause increased sarcoplasmic and myosin protein degradation. This in turn can cause the myofilament lattice space shrinkage (Rosenvold & Wiklund, 2011). According to Hughes et al. (2018) muscles with high rigor temperature of 35 °C, have sarcomeres which are unstretched and free to shorten. At this temperature, it possesses a structure with long sarcomeres, and high global brightness and longitudinal peak intensity when compared to muscles with rigor temperature as low as 5^{o}C. Additionally, a high rigor temperature is known to cause myosin head denaturation, and this may have occurred during or at full rigor thereby interfering with the myosin and actin crossbridge formation, resulting in the prevention of sarcomere shortening (Hughes et al., 2018).

2.3. Meat quality characteristics and its variation during the post slaughter period

2.3.1. Colour

Meat colour is an important factor which determines the quality and freshness of the product during retail display (Jacob & Thomson, 2012) and is often correlated with consumer acceptability (Hughes et al., 2017). It is determined by quantity of myoglobin present and its oxidative status. Additionally, the structural opacity of the muscle fibres and their light scattering capacity are the other properties which correlate with the colour of the muscle (Jacob & Thomson, 2012). Visual appearance is a sensory property of high importance because the purchase decision of consumers is highly dependent on the appearance of meat. They associate bright red colour to fresh meat with superior quality (Bellés, del Mar Campo, Roncalés, & Beltrán, 2019). Colour of meat is highly dependent on the chemical state and the quantity of myoglobin, the principle pigment present in meat. The conversion of Fe^{2+} to Fe^{3+} results in the conversion of myoglobin to metmyoglobin, which results in the change of color from red to

brown (Bellés et al., 2019). Superior quality of meat is identified by its freshness and cherry red colour (Gao et al., 2016). Pale or dark coloured meat is associated with rejection or low consumer preference compared to bright coloured meat (Hughes et al., 2017). During the retail display, colour of the meat changes from red to brown because of MetMb formation (Jacob & Thomson, 2012). The length of the sarcomere is an important area in the muscle which determines the light scattering property of the meat (Hughes et al., 2018). As mitochondria remains active during the PM period, consumption of oxygen is favoured, and this influence the depth of oxymyoglobin layer formed from the surface. This in turn affects the blooming time and the depth of formation of metmyoglobin from the surface (Jacob & Thomson, 2012). During this time, the purple DeoMb changes to red OxyMb and then to the brown colored MetMb. The stability of meat colour at the end of the display time is a simple method to describe the stability of the meat color which the consumers and producers can relate to (Calnan, Jacob, Pethick, & Gardner, 2014). Additionally, there exists a close link between the color of the meat and the oxidation of lipid, which takes place due to the balance between antioxidant and pro-oxidant substances. Lipid oxidation can result in the deterioration of meat quality which includes the discoloration of meat samples. To this point several mechanisms have been studied to protect the meat from oxidation such as pro-oxidants and endogenous antioxidants (Gao et al., 2016).

Different components involved in imparting the colour of the meat includes relative proportion and distribution of purple deoxymyoglobin (DeoMb), brown oxidized metmyoglobin (MetMb) , the bright red oxygenated oxymyoglobin (OxyMb). The formation of metmyoglobin is identified to be the reason for the discoloration of meat. It can be retarded by the enzymatic, non-enzymatic, and the reducing systems which are present in the mitochondria. As a result, these biochemical factors are important for maintaining the stability of the colour (Gao et al., 2016). Measurement of colour stability is measured by accessing the change in a* value, which is the change in redness of meat, and the rate of brownness formation, which is ratio of oxyMb and metMb on the surface of meat (Ponnampalam, Burnett, Norng, Warner, & Jacobs, 2012). The oxy/met ratio is used for describing the redness:browness ratio on the surface of meat. Evidence from research suggest that it takes about 3 days for the lamb meat to approach the minimum value, which in turn is dependent on temperature and the packaging methods. This is identified to be the blooming period after which the oxidation of myoglobin to MetMb would be progressed and there is a colour change from purple to red. The rate at which the surface color of meat changes from red to brown is determined by i) the rate of diffusion and consumption of oxygen, (ii)auto oxidation rate of myoglobin to metmyoglobin pigment, (iii)rate of reducing metmyoglobin activity (Calnan et al., 2014). Spectrophotometric methods are more

preferred to chemical analysis as it is cheap and easy to perform (Jacob & Thomson, 2012). This is calculated based on the reflectance ratio of 630:580 nm wavelength (Ponnampalam et al., 2012).

2.3.2. Water holding capacity and cooking loss

Cooking is defined as the process of heating meat muscles to a high temperature which enables the denaturation of proteins. The time and temperature of cooking has profound effect on the physical properties and eating quality of the meat (García-Segovia, Andrés-Bello, & Martínez-Monzó, 2007). The three main factors which affects the cooking loss of meat includes the internal and surface temperature of the meat, and the method of heat transfer. Additionally, the length of time at which the meat was cooked during the post-mortem period and the method of cooking also affects the cooking loss of meat. These factors in turn causes change in the amount of fluid expelled from the meat, and the loss of water binding capacity (Geesink, Sujang, & Koohmaraie, 2011). Additionally, those factors which control the toughness of muscles are the proteins in the connective tissue, elastin, collagen, and the myofibrillar proteins. During the process of heating, the proteins in the meat denatures, resulting in the structural changes in the meat. This includes the damage to cell membrane, aggregation, and formation of sarcoplasmic and myofibrillar proteins, solubilization and shrinkage of connective tissues, and the shrinkage of meat fibres (García-Segovia et al., 2007). The water holding capacity of raw meat is explained by several factors such as electrostatic repulsion between the actin and myosin filaments, capillary force, osmotic pressure, surface area of proteins which interact with sarcoplasm, and the water structure. The water holding mechanism of meat is highly related to the net charge of the myosin and actin filaments resulting in an electrostatic push. It is studied that the net charge in the filament becomes similar on increasing the swelling force. Pre-rigor muscle shortening may result in the release of water from the meat (Ertbjerg & Puolanne, 2017). Cooking loss is also directly related to the filament lattice shrinkage. During the process of cooking, the meat shrinks due to the loss of water and thereby causes changes in the tenderness of meat. The shrinkage of meat during cooking can be described as a 2-Dimensional process. Transverse shrinkage which happens perpendicular to the muscle fibre type starts at a temperature of 35-45 ^{o}C and completes between 60-62°C. The parallel or longitudinal shrinkage which happens in the direction of the muscle fibre starts at 55°C to 65°C and ends by 90°C. Meat shrinkage has been associated with the denaturation of connective tissues and myofibrillar proteins (Vaskoska, Ha, Naqvi, White, & Warner, 2020).

As meat is cooked during post-rigor there is a decrease in the sarcomere length as the cooking temperature increases. If the meat is cooked before rigor it can cause strong contraction of muscles (Ertbjerg & Puolanne, 2017). During the time of cooking, sarcoplasmic proteins and myofibrillar proteins expands and collagen shrink which lead to solubility changes. As a result, there is a change in the water retention, colour, and the mechanical properties of meat samples (Liu et al., 2013). Cooking

LD muscle of the lamb during the rigor period or post rigor and during rigor can result in huge difference in the meat contraction status after cooking. Pre-rigor muscles which are cooked rapidly can result in increased meat tenderness. This is caused due to the tearing and cracking of muscles, due to the shortened cooking process (Ertbjerg & Puolanne, 2017).

2.3.3. Shear Force

Tenderness, juiciness, and flavour are the most important attributes which controls the palatability of meat. Of these, tenderness is ranked to be the most important trait affecting the quality (Schmidt, Scheier, & Hopkins, 2013) and consumers acceptance of meat products (Veiseth, Shackelford, Wheeler, & Koohmaraie, 2004). The rate and extent by which the tenderisation of meat happens depends on both extrinsic and intrinsic factors. Extrinsic factors include chilling temperature, electrical stimulation, and type and duration of storage (Pearce et al., 2011). Aging has a significant effect on Warner–Bratzler Shear force (Toohey, Hopkins, Stanley, & Nielsen, 2008). The method of cooking has large effect on the tenderness of the muscle. The level of muscle contraction can determine the toughness or tenderness of the meat muscle. Muscles which are moderately cold shortened are very tough and severely cold shortened are comparatively tender (Geesink et al., 2011). During the initial post-mortem period, the muscles go through the toughening phase which causes the shortening of sarcomeres during the process of rigor. Following this is the tenderization process, which is mainly caused by the calpain proteolytic system. The μ-Calpain degrades the myofibrils and proteins which results in tenderization of the muscles (Veiseth et al., 2004).

Meat tenderization is mainly attributed to the cytoskeletal and myofibrillar proteins. Tenderization during the post-mortem period causes major changes in the myofibrillar and cytoskeletal protein components owing to the proteolysis of the myofibrillar and associated protein (Pearce et al., 2011). Experimental evidence by Li et al. (2012) indicated that those samples which are incubated at a temperature of 14°C were identified to be more tender during the time of aging. Additionally, other factors determining the tenderness of meat is the tenderizing phase, toughening phase, background toughness. The tenderizing and toughening phase takes place during the aging or the post-mortem storage. Furthermore, the degradation of protein because of aging causes a change in the molecular structure of the proteins (Schmidt et al., 2013).

2.4. Biochemical characteristics of muscles and its variation during the post slaughter period

2.4.1. Muscle Composition and structure

Muscles are organised tissues which are composed of fibres and structured by connective tissues. Each muscle fibre is composed of large number of strands called myofibrils, which in turn are made of myofilaments. These myofilaments are composed of thin actin and thick myosin filaments. Two key regulatory proteins which are associated with actin are tropomysin and troponin (Pearce et al., 2011). Skeletal muscles are composed of 0.5 to 8% lipids, 19% protein, 75% water and 1% glycogen. Additionally, it constitutes several cell types along with intramuscular adipocytes, connective tissues, nervous and vascular tissues (Lefaucheur, 2010). Sarcomeres serve as the machine that produce force for the straited muscle. These muscles are essential to achieve the locomotive task in living muscles. However, a greater proportion of the body heat is produced as a by-product. Physiological turn over and muscle growth results in renewal of sarcomeres. They are formed by assembling large number of protein subunits, to large macromolecular complex. They have an interconnected, but extremely ordered structure (Ertbjerg & Puolanne, 2017). Additionally, a heterogeneous population is represented my myofibrils as they differ structurally, physiologically, and metabolically. Myofibrils can be characterized by their metabolic properties, length, and cross-sectional area. Composition of myofibre type is an important factor which affects the quality of meat (Lefaucheur, 2010). Myofibrils appear as alternating dark and light bands when observed under a light microscope. This is due to the ordered arrangements of the filaments in the sarcomere. The dark band is termed the A band whereas the light band is termed the I-band. The H zone is the center of the A band and M band is identified to be the center of the H band (Ertbjerg & Puolanne, 2017). Lamb meat also contains n-3 polyunsaturated fatty acids which has beneficial effect on the health of consumers. It helps to decrease the mortality, blood pressure, morbidity, and triglyceridemia following tumor growth and cardio-vascular accidents (Guy, Prache, Thomas, Bauchart, & Andueza, 2011).

As muscles get converted to meat, the major biochemical changes are driven for the achievement of rigor mortis (Pearce et al., 2011). The muscle fibres enter rigor mortis individually, resulting in the formation of irreversible association between the thin and thick muscle filaments due to the depletion of ATP. Eventually, the whole muscle enters rigor mortis and becomes inextensible (Gault, Gordon, & Tolland, 2005). Hydrolysis of muscle ATP is the major biochemical change which is necessary to maintain a relaxed sarcomere. As glycolysis progresses during the post-mortem period, there is a drop in the level of glycogen and concentration of ATP in muscles. As this stage continues to progress, the ATP level diminishes, and the muscles shortens to some degree. However, the temperature of muscle during the pre-rigor time determines the extent to which the muscle shortens. As the ATP level in the

muscle becomes critically low, the myosin head bounds permanently to the actin filaments, resulting in the formation of actomyosin complex. This results in the formation of inextensible muscles (Pearce et al., 2011).

Myoblast fusion during fetal development constitutes 75-90% of the muscle volume. The diameter of these myofibrils ranges from 10 to 100 μm and are classified with respect to their metabolic and contractile properties (Lefaucheur, 2010). Myosin heavy chain (MyHC) constitutes around 35% of the total protein and is identified to be the most abundant protein present in the skeletal muscles. The contraction speed of the muscle as determined by the cross-bridge cycling is determined by the ATPase activity of the myosin heavy chain. The straited muscles constitute a minimum of eight different myosin heavy chain genes, which includes two developmental (fetal and embryonic), one α - cardiac, one slow (β – cardiac or Type I), three adult fast type II myosin heavy chain (IIa, IIb, and IIx), and the extraocular myosin heavy chain. These fibre types exhibit different biological characteristics as given in Table 1 (Lefaucheur, 2010). This includes morphological, chemical, physiological, metabolic, and contractile characteristics.

2.4.2. Muscle Fibre typing

Myosin heavy chain is the most abundant protein present in the skeletal muscle and comprises 35% of the total proteins. Each of these fibre type exhibit different morphological, chemical, physiological, metabolic, and contractile characteristics (Lefaucheur, 2010). Muscle fibre types have been classified into three main types based on the activity of acto-myosin ATPase. This includes types I, IIA, and IIB fibres (Lefaucheur, 2010). The type of muscle fibres includes type 1 - oxidative and slow twitch, type IIA, which includes the oxidative, fast twitch, and glycolytic, and type II B which includes the glycolytic and fast twitch (Shija et al., 2013). Figure 2.1 shows the biological characteristics exhibited by different muscle fibre types.The type I fibres have less glycogen content and are rich in myoglobin and triglycerides. They also possess high resistance to fatigue. Additionally, the glucose uptake, hexokinase and GLUT-4 activity, glycogen turnover, the insulin receptors and insulin sensitivity are high in slow twitch type I muscles fibres than in fast twitch type II fibres (Lefaucheur, 2010). Type I fibres are identified to be the smallest in diameter whereas type II is identified to be the largest in diameter. The contraction of muscles during the post-mortem period is dependent on the sarcomere length. As the fibre diameter increase due to the increased myofilament lattice spacing, it deceases the light scattering capacity (Hughes et al., 2018). Each of these fibre types exhibit different morphological, chemical, physiological, and contractile characteristics. Slow-twitch oxidative type I fibres have lower excitation threshold, and they make use of a large amount of energy as they require contractions of low intensity for performing the basic activities. They have high resistance to fatigue, rich in triglycerides and myoglobin, and poor glycogen content. However, the type II fibres exhibit high

ATPase and myofibrillar activity. They can withstand high intensity contractions due to the instant availability of phosphocreatine and the degradation of glycogen via the glycolytic pathway. These fibre types display high threshold for excitation because they do not use a large amount of energy, and they are used only occasionally to sustain vigorous movements of less duration (Lefaucheur, 2010). Muscles of type I fibres had faster rate of pH decline when compared to muscles of type IIb fibres (Daly, Gardner, Ferguson, & Thompson, 2006).

Diversity of muscle fibre plays an important role in the quality of meat. Metabolic and contractile properties are of prime importance for maintaining the heterogeneity of muscle fibre. The skeletal muscles are classified in accordance with their metabolic and contractile properties (Ithurralde et al., 2015). Composition of myofibre type is an important factor which affects the quality of the meat products (Lefaucheur, 2010). Characteristics of these muscle fibres are studied to have a huge impact on the quality of meat. The variation in the quality of the meat from different fibre typing was studied by Ithurralde et al. (2018). Within the fast-glycolytic muscles, those with less oxidative activity and fast glycolytic fibres are associated with lower a* value, higher L* value, lower ultimate pH, and longer sarcomeres. From the intermediate muscles, those with less oxidative activity and fast glycolytic fibres are associated with higher L* values, less meat tenderness, short sarcomeres, and lower ultimate pH. Similarly, from the slow oxidative muscles, less oxidative activity and fast glycolytic fibres were associated with less expressed juice, less meat tenderness, and lower a* values (Ithurralde et al., 2018). Redder meat is usually associated with oxidative muscles. Additionally, these muscles also possess less tenderness and shortened sarcomeres. Therefore, these muscles are benefitted by more aging time and electrical stimulation. Conversely, fast glycolytic muscles are associated with lower ultimate pH, less water holding capacity, and bright coloured meat (Ithurralde et al., 2015). Ithurralde et al. (2015) reported that based on the variables involved in fibre typing, they can be classified as fast glycolytic muscles, such *as Rectus femoris, Caudal Gluteobiceps, Longissimus thoracis, Gluteus medius, Vastuslateralis, Longissimus lumborum,* and *Semitendinosus* muscles. The intermediate muscles include the *Cranial Gluteobiceps, Psoas major, Triceps brachii caput longum, Adductor,* and *Semimembranosus*. The slow oxidative muscles include the *Serratus ventralis, Infraspinatus, Tricepsbrachii caput laterale* and *Supraspinatus* (Ithurralde et al., 2015).

	I	IIa	IIx	IIb
Contraction speed	+	+++	++++	+++++
Myofibrillar ATPase	+	+++	++++	+++++
Oxidative metabolism	+++++	++++, +++++	+, ++	+
Glycolytic metabolism	+	++++	++++	+++++
Hexokinase	+++++	+++	+	+
GLUT-4	+++++	+++	+	+
Phosphocreatine	+	+++++	+++++	+++++
Glycogen	+	+++++	++++	+++++
Triglycérides	+++++	++	+	+
Vascularization	+++++	+++	+, ++	+
Myoglobine	+++++	++++	++	+
Buffering capacity	+	++++	+++++	+++++
Diameter	++	+, ++	++++	+++++
Fatigue resistance	+++++	++++	++	+

[a] +, very low; ++, low; +++, medium; ++++, high; +++++, very high.

Figure 2.1. Biological characteristics exhibited by different types of fibre on muscle.

2.4.3. Glycogen content

2.4.3.1 Glycogen concentration and muscle pH

Energy is stored within the animal muscles in the form of glycogen. During the post slaughter period, glycogen is converted to lactate by the process of anaerobic respiration, which causes a decrease in the muscle pH to a level of pH 5.5. A minimum of 45-55mmol/kg of glycogen is required during the PM period for attaining this PH (Daly et al., 2006). If the glycogen concentration is maintained at a lower level inside the muscle, it results in the production of low level of lactic acid in meat during the time of rigor mortis. This causes the muscles to attain a high level of ultimate pH (pH_u) (Gardner, McIntyre, Tudor, & Pethick, 2001). This results in the formation of dry, firm, and dark meat (Daly et al., 2006). As a result, meat associated with high muscle pH_u is studied to possess low palatability (Gardner et al., 2001). However, if the glycogen level is maintained above this concentration, referred to as residual glycogen, it could be considered as a buffer to high ultimate pH in muscle. This in turn prevent the formation of dry, firm, and dark meat (Daly et al., 2006). Hudson (2012) reported that glycolytic potential is an indicator of compounds in the muscle, that represents the capacity of the muscle for the post-mortem glycolysis and the extent of decline in pH after slaughter. The results obtained suggest that the increased concentration of glycogen in muscle resulted in faster decline in pH. Concentration of glycogen in muscles during the time of slaughter is an important factor which determines the anaerobic glycolysis which happens during the post-mortem period. Suitable concentration of muscle glycogen can lead to adequate pH decline as muscles get converted to meat. This in turn acts as an indicator of the overall acceptability of the product. However, if the

concentration of glycogen in the muscles is lower, it limits the decline of pH, leading to an increased ultimate pH, which is also referred to as dark cutting (Pighin, Brown, Ferguson, Fisher, & Warner, 2014).

2.4.3.1. Glycogen concentration and muscle temperature

The rate of ATP hydrolysis determines the rate of post-mortem glycolysis in lamb muscles. Consequences which arise because of energy metabolism in the muscle during the post-mortem period includes production of metabolic heat, accumulation of H^+ ions, decline in pH. During the post-mortem period, as the energy reserves in the muscle decreases, the muscle enters the state of rigor mortis. This results in the stoppage of blood supply and chilling of meat, which causes a drop in the muscle temperature. Additionally, if the pre rigor temperature is maintained abnormally high, it is bound to produce negative impacts on the quality of meat (Kim, Warner, & Rosenvold, 2014). As the incubation temperature of the muscle increases, it results in fast decline in pH, as a result of impact of temperature on the enzyme activity and thereby affecting the rate of glycolysis. As a result, as the cooling temperature is decreased it results in low pH and increased temperature during the PM period (Jacob & Hopkins, 2014). Addition the increased temperature is bound to make many in direct effect on the quality of the meat product. This is because the high temperature results in low muscle pH, which causes denaturation and thereby results in the loss of functionality of the protein. This influences the quality if the meat as it causes the shortening of sarcomere, early browning during retail market, pale colour, reduced tenderization, and reduced water holding capacity (Jacob & Hopkins, 2014).

2.4.3.2. Stress and Glycolysis

The level of glycogen during the time of slaughter is determined by the pre slaughter stress conditions and palatability of the muscle. However, it is also studied that the pre slaughter stress can reduce the level of glycogen stored in the muscles (Daly et al., 2006). The stress conditions include physiological, physical or the combination of both. This includes fasting climatic extremes, dehydration fear and pain, disruption of social structure, and unfamiliar surroundings (Daly et al., 2006). Experimental evidence suggest that the elevated level of lactate, glucose, and cortisol is related to high pH values, lower redness, and lightness values, which in turn indicates that the quality of the meat is highly affected by blood stress indicators (Stempa et al., 2018). As animals get exposed to pre slaughter stress conditions, it results in the release of nor- epinephrine and epinephrine, which would result in a series of events such as tachycardia, higher body temperature, and rise in metabolic rate. The release of Catecholamines causes glycogenolysis which results in the mobilization of muscle glycogen, causing elevation in blood lactate and glucose concentration (Gruber et al., 2010). Dark cutting meat has less desirable shelf life, tenderness, overall acceptability, and flavour. The rate of decline in pH during the

post-mortem period depends on diverse factors such as composition of muscle fibre, exercise, diet, temperature of muscle, and stress (Zhu, Ruusunen, Gusella, Zhou, & Puolanne, 2011).

Chapter 3

Materials and Methods

3.1. Experimental Design

The experiment was carried out using 5 different muscles, each taken from 6 different animals. These five muscles include two fast glycolytic muscles, namely the Semitendinosus (ST) and the Longissimus (LD) muscles and two neck muscles, namely the Platysma, and the fast glycolytic Sternomandibularis (S_tM), and the fifth muscle, the Gracilis. Figure 3.1 shows the diagrammatic representation of the different muscles in sheep carcasses. Each muscle was studied for analysing the pH, temperature, color, cooking loss, shear force, muscle fibre typing, and glycogen content.

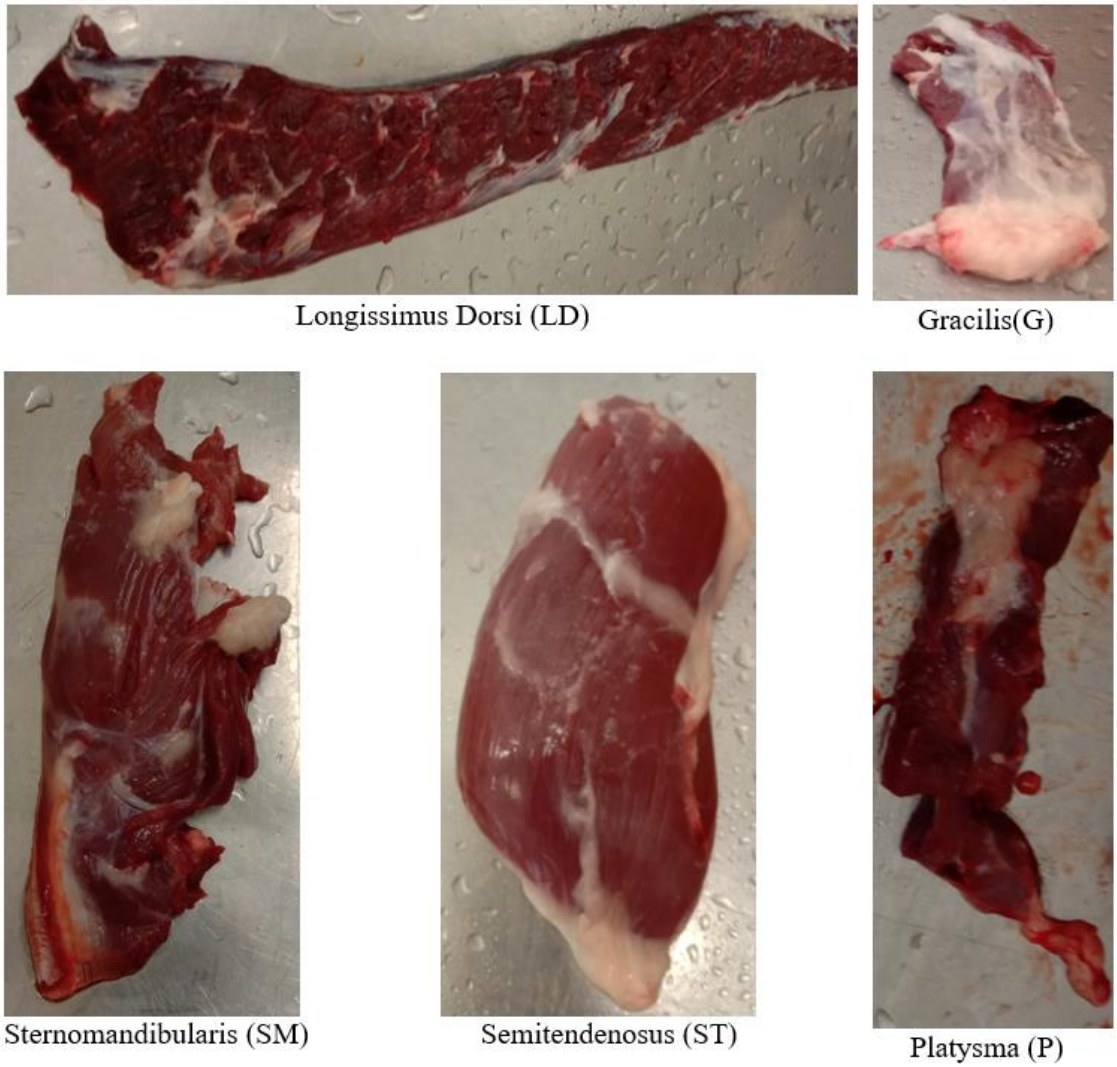

Figure 3.1 Pictorial view of the five different muscles taken from sheep carcasses

Figure 3.2 Five muscles placed on 120cm long benchtop for size representation (1. SM; 2. P; 3.LD; 4. ST; 5.G)

3.2. Slaughter process

Slaughtering of six animals were done at Johnstone Memorial Laboratory (JML) abattoir, Lincoln. The carcasses were dressed and were normally hung on cold room which were set for fast temperature decline possessing an ambient temperature of -4^{o}C. Upon entry to the room, the muscle pH and temperature of the muscle samples on the carcasses were measured.

3.3. Sampling

Muscles were collected soon after slaughter and stored in separate zip lock covers. They were stored in refrigerator and maintained at -4^{o}C for checking the meat quality characteristics. Muscles for analysing the biochemical characteristics were also taken from 6 different sheep carcasses (n=6), each of them weighing around 5g, at the time of slaughter. Each of the collected samples were frozen immediately in liquid nitrogen, to inhibit further glycolysis. These samples were then stored frozen at -20 °C for 1 month until the glycogen content was determined.

3.4. pH and Temperature measurements

The pH and temperature measurements of meat samples were measured at 1.5h, 3h, 24h, and 48h for the muscles on the carcasses and 45m, 24h, and 48h for the muscles taken off the carcasses. The measurement was made using a Hanna HI 9025 pH meter directly on the muscle of interest as suggested by Stempa et al. (2018) with slight modifications. The meter was standardized before making the measurements using the standard solutions of pH 7 prior to starting the measurements. The pH and the temperature can be measured simultaneously using the electrode and the mental probe in the pH meter by inserting the prob into the muscle of interest, namely ST, S_tM, LD, G, and P.

3.5. Meat Quality Characteristic

3.5.1. Color

Samples were taken from all six carcasses soon after slaughter at 48h after slaughter and allowed to bloom. They were later stored under refrigerator conditions until the color measurements were done. Color of the raw meat sample was measured at five random spots using a Minolta CR-400 Chromometer (Minolta Camera co., Japan). The color coordinates which were determined corresponds to b* (yellowness), a* (redness) and L*(lightness), Hue (H*), and color saturation (C*) after blooming for 1 hour (Immonen, Ruusunen, & Puolanne, 2000). The surface color of the muscle was determined for each muscle samples. The psychometric hue angle, which indicates the angle at which the vector diverges into yellow and red quadrants. The hue angle (HA) was calculated as: HA = $\tan^{-1}$ (b^* / a^*) and the color saturation or the psychometric Chroma (C) was calculated as C = (a^{*2}+ b^{*2})$^{1/2}$ (Stempa et al., 2018). The instrument was standardized using a black and white reference tile, provided by the company prior to its use (Rosenvold & Wiklund, 2011).

3.5.2. Cooking Loss

The cooking loss was determined for the three muscles LD, ST, G aged 3d and 7d, for the muscles taken on the carcasses and off the carcasses, from all six biological replicates using the procedure of Liu et al. (2013) with slight modifications. The samples were carefully placed in plastic bags and cooked by immersing them in a water bath which is maintained at a temperature of 80°C, until the internal temperature of the meat samples reaches about 75°C. Following this the samples were cooled until it attains the room temperature. The cooled meat samples were carefully removed from the package and dried using a blotting paper and weighed immediately. Thermocouples were used to monitor the central temperature of the sample during the cooking process. Cooking loss was determined in terms of percentage by using the below formulae:

$$Cooking\ loss\ (\%) = \frac{[(weight\ before\ cooking - weight\ after\ cooking)]}{weight\ before\ cooking} * 100\%$$

3.5.3. Shear Force

Shear force was measured for the muscles aged 2d and 7d for each of the five muscle samples, taken off the carcasses and on the carcasses. The muscles were cooked, and the shear force was measured using the method adapted from Thompson et al. (2005). Ten sub-samples of size 1cm^2 was cut, and these samples were tested using MIRINZ tenderometer. The machine had a vee-shaped blade for cutting through the samples. The peak force values (kPa) obtained by the MIRINZ Tenderometer was converted to Newton (N), using the equation:

$$Shear\ force\ (N) = [\text{shear force}(kPa) \times 0.216) - 2.030] \times 9.8$$

3.6. Biochemical Characteristics

Samples for the determination of biochemical characteristics was immediately frozen in liquid nitrogen and stored at -20 ^{o}C, until the analysis was carried out.

3.6.1. Muscle Fibre Typing

Separation of myosin heavy chain isomers were performed using the SDS PAGE gel electrophoresis according to the method of Picard, Barboiron, Chadeyron, and Jurie, (2011) with modifications. Extraction of myofibrillar proteins was done from 400mg of muscles using a 10 ml of buffer containing 0.5M NaCl, 50mM Tris, 20mM Sodium pyrophosphate, 1mM EDTA and 1mM freshly prepared dithiothreitol. The prepared samples were homogenized using FJ200 high speed homogenizer at 23,000 rpm for 30s. Following homogenization, the samples were centrifuged at 2500g for 10 min at 4 °C. Supernatant was collected and was diluted in 1:1 v/v with glycerol. The samples were further diluted with distilled water at 1:1 ratio, and vortexed thoroughly. To the prepared sample mixture, equal volume of basic 2X Laemmli buffer containing, 125mM Tris (pH 6.8), 4% w/v SDS, 20% v/v Glycerol, 10% v/v b-mercaptoethanol and 0.04% w/v bromophenol blue was added and was incubated at room temperature for 10 minutes. Followed this the prepared solution was heated at 70°C for 10 minutes. The separating gel consisted of 9% w/v acrylamide-Bis, 35% v/v glycerol, 230mM Tris (pH 8.8), 0.4% w/v SDS and 115mM glycine. The stacking gel consisted of 6%w/v acrylamide-Bis, 47% v/v glycerol, 110mM Tris (pH 6.8), 6mM EDTA and 0.4% w/v SDS. Polymerization was initiated by using 0.1% w/v ammonium persulfate and 0.05% v/v TEMED. Separate lower and upper running buffers were prepared with the lower buffer having twice the concentration of the upper buffer. The lower running buffer contained 50mM Tris (base), 0.05% w/v SDS, and 75mM glycine and the upper buffer had 1.25mM glycine, 0.1% w/v SDS, 100mM Tris and 0.07% v/v b-mercaptoethanol. 7µl of dye was mixed with 12µl of protein and the mixture was loaded onto separate wells in the 0.75 mm thick gels set up on the MiniProtean II Dual slab cell electrophoretic system (Bio- Rad). The electrophoresis unit was set up in the chilling room at 4°C and was carried out at 70 V for 25h. After complete separation of the bands, the gel was fixed in a solution of 15% (w/v) trichloroacetic acid for 30m and was washed thrice in distilled water for 5m each. Following this the gel was stained in a solution of Gelcode for 1h at room temperature, gently shaking on the rocker. The stained gel was washed using distilled water and was scanned using Geldoc (Bio-rad). Table 3.1 provides information regarding the preparation of stacking and separating gel for casting four gels of 0.75mm thickness. TEMED and APS was added toward the end, prior to pouring the separating and stacking gel into the tray.

Table 3.1 Preparation of 6% stacking and 9% separating gel from stock solution for SDS-PAGE

Stock solution	Separating gel (ml)	Stacking gel (ml)
100% Glycerol	7	6.2
30% Acrylamide: bis[1] (50:1)	6	2.6
1.5M Tris (pH 8.8)	3.06	-
0.5M Tris (pH 6.7)	-	2.93
1M Glycine	2.3	-
100mM EDTA pH 7.0	-	0.8
10% SDS	0.8	0.533
Distilled water	0.626	-
TEMED[2]	0.006	0.006
10% Ammonium persulfate (APS)	0.2	0.133

[1]Acrylamide-bis: acrylamide-N, N'-methylene-bis-acrylamide
[2]TEMED: N, N, N', N'-tetramethylethylenediamine

3.6.2 Glycogen Concentration

The glycogen concentration of the five different muscles, ST, LD, G, P, and S_tM was determined by analysing muscle glucose concentrations in hydrolysed and non-hydrolysed muscle extracts with the D- Glucose HK Assay kit (Megazyme, Ireland). The muscle samples were prepared according to the method of Hammelman et al. (2003), with slight modifications. Muscle samples were collected by scrapping them down using a sharp knife. 0.3g of each of the muscle sample were homogenized using a Polytron in 10 ml of 0.5 M PCA (perchloric acid) for 30-40 sec in a fume hood. The muscle samples were kept on ice box during the process of homogenization.

250µl of homogenised muscle sample was collected to which 575µl of water was added. The prepared samples were centrifuged at 10 000g for 15mins at 4^{o}C. Supernatant were collected carefully into an eppendorf tube and stored under refrigeration condition (Non-hydrolysed muscle extracts). Another 250µl of homogenised muscle sample went into the hydrolysis process by adding 25µl of 30% KOH and 500µl of amyloglucosidase (AMG) (Megazyme, Ireland), and incubated in a water bath set at 37^{o}C for 3h. The hydrolysis was stopped by adding 3M PCA. The samples were then centrifuged at 10,000g for 15m. Supernatant was collected and stored under refrigeration condition (Hydrolysed muscle extracts).

The hydrolysed and non-hydrolysed muscle extracts were subjected to the microplate assay procedure as described in the manual Megazyme 2020, D-Glucose - HK Assay Procedure (HK/G6P-DH Format). The blank samples were prepared with water to replace to the muscle extracts. The 96 well microplate was loaded with blank, standards, hydrolysed and non-hydrolysed muscle extracts in a pre-set manner. The glucose standards were prepared in the concentrations of 0.075, 0.05, 0.025, 0.0125, 0.00625 mg/ml (S1, S2, S3, S4, and S5 respectively). The experimental procedure was carried out in a set of three 96 well plates, each containing a blank, the set of standards, and the hydrolysed and the

non-hydrolysed samples from 2 sheep (n=2) for all the five muscle extracts. 50µl of each of the five different muscle sample from all the six biological replicates (n=6) were loaded in triplicates using the three 96 well plate. Similarly, 50µl of non-hydrolysed muscle samples from all the five different muscles from 6 different sheep were also loaded in the same manner. To all the samples, including the water, the blank, hydrolysed, and the non-hydrolysed muscle extracts, 10µl of buffer and 10µl of $NADP^+$/ATP was added and was allowed to wait for 3m before the absorbance was measured at 340nm (OD_1, Control/Background noise). Following this, 2µl of HK/G-6-PDH suspension was added and the mixture was allowed to wait for 5m for the enzyme reaction to complete, before taking absorbance at 340nm (OD_2). Final absorbance values associated with muscle glucose were determined by subtracting OD_1 from OD_2.

Muscle glycogen concentration was determined by subtracting the concentration of glucose in non-hydrolysed from hydrolysed muscle extracts. The standard graph was plotter with Absorbance at 340nm Vs Concentration. The concentration of glucose in the LD, ST, S_tM, G, and P muscle sample extracts were calculated using the standard equation linear equation, obtained from the graph. The results obtained were calculated using Microsoft® Excel® for Office 365, version 2010 (Microsoft Corporation, Richmond, USA) for determining the concentration of glycogen in the muscle extracts.

3.7. Statistical data Analysis

Mean and Standard deviation corresponding to the pH and temperature changes and the meat quality characteristics for the 6 different sheep replicates was calculated. Statistical analysis was carried out using the analysis of variance (ANOVA) using a general linear model (GLM). Mean values that are significantly different from each other ($P \leq 0.05$) was identified using post-hoc Tukey's honestly Significantly Different (HSD) test. Minitab® (Minitab, LLC, Pennsylvania, USA) version 18.1 was used for carrying out all the statistical analysis.

Chapter 4

Results

The experimental design involved five different muscle samples, namely, ST, S_tM, LD, G, and P. The muscle samples were analysed for its pH, temperature, meat quality characteristics, and biochemical characteristics. pH, temperature, and meat quality characteristics were measured on the muscles on the carcasses and off the carcasses. The measurements on the carcasses was done at time interval of 90m, 3h, 24h, and 48h, and off the carcasses measurements was done at 45m, 24h, and 48h. The biochemical characteristics were studied using the frozen samples collected at the time of slaughter.

4.1. Weight of carcasses

The head, fleece, and viscera of six sheep were removed soon after slaughter and the carcasses weights were recorded under Table 4.1.

Table 4.1 Mean±SD values of weight of six sheep carcasses (n=6)

Carcasses	1	2	3	4	5	6	Mean ± SD
Weight (kg)	38.7	30.3	39.6	34.6	39.7	29.2	35.35±4.73

4.2. pH measurements

For all the five muscle samples, on the carcasses and off the carcasses, the pH values decreased with time (Table 4.2 and Table 4.3, respectively).

The mean values of the pH measured on Gracilis (G) , LD and ST muscle on-the carcasses showed a decline during the first 24h post-mortem (PM) (Table 4.2). The results showed no further significant drop in the muscle pH from 24 to 48h, which implies that the muscles have reached an ultimate pH at 5.59±0.05 during the first 24h PM. At 1.5hPM, the G had 7.25 pH, which is significantly high compared to LD and ST muscles which had an average pH of 6.5 (Table 4.3). At 3hrs PM period, the pH of G muscle (6.55) decreased closely to the pH of LD and ST muscle (pH=6.4). For the Gracilis muscle from the 1.5h PM to 3h PM time there was a significant 10% drop in pH , while the muscle pH from the LD and ST remained unchanged during this time interval. By the end of 24hPM period there was a total of 14% decline in pH for the G and LD muscle, and a 12% decline for the ST muscle in compared to the pH measured at 1.5 PM.

The mean values of the pH measured on P, S_tM, G, ST, and LD muscles prepared off-the carcasses showed a sharp decline from 45m (0.75hr) to 48h as shown in Table 4. At 0.75hr PM period, there

were a range of a pH in all five muscle between 7.5 and 6.6. Platysma, the neck muscle and G had significantly high pH at 7.47 and 7.25 respectively, followed by 6.79 of another neck muscle S_tM. while ST and LD muscle had the lowest at 6.6. At the 24h PM, there was a total of ~20% decline in pH for the muscle P and G, whereas a 12% decline for the muscle S_tM, and 15% decline for the muscle ST and LD. There were no significant changes in the pH of all five muscle samples at the 48hrs PM after 24h PM, suggested they reached mean ultimate pH of 5.69±0.182.

Table 4.2 Kinetics of pH decline of the sheep muscles G, LD, and ST (n=6) on the carcasses during the first 48h Post-mortem. Mean values which does not share the same letters are significantly different (P<0.05) during the post-slaughter period.

Time (h)	G	LD	ST
1.5	7.25 ± 0.43^{A}	6.58 ± 0.038^{B}	6.4 ± 0.41^{B}
3	6.55±0.34	6.41±0.39	6.4±0.37
24	5.63±0.08	5.53±0.12	5.61±0.05
48	5.49±0.09	5.44±0.11	5.5±0.1

Table 4.3 Kinetics of pH decline of the sheep muscles P, StM, G, ST, LD (n=6) taken off the carcasses, during the 48h. Mean values which does not share the same letter are significantly different (P<0.05) during the post slaughter period.

Time (h)	P	SM	G	ST	LD
0.75	7.47 ± 0.63^{A}	6.79 ± 0.18^{AB}	7.25 ± 0.43^{AB}	6.6 ± 0.35^{B}	6.65 ± 0.38^{B}
24	6.00 ± 0.16^{A}	5.96 ± 0.004^{A}	5.68 ± 0.06^{B}	5.6 ± 0.07^{B}	5.56 ± 0.05^{B}
48	5.89 ± 0.12^{A}	5.87 ± 0.07^{A}	5.67 ± 0.06^{B}	5.58 ± 0.08^{B}	5.47 ± 0.04^{B}

From Figure 4.1 it is studied that there is a sharp decline in pH for the muscles ST, LD, and G muscles on the carcasses during 24h time and thereafter continues to reach a constant pH of 5.5. Figure 4.2 provides information regarding the kinetics of pH for the muscles LD, ST, G, P, and S_tM taken off the carcasses. There is a sharp decline in muscle pH during 24h of PM, and thereafter the muscles attain a constant pH of 5.5.

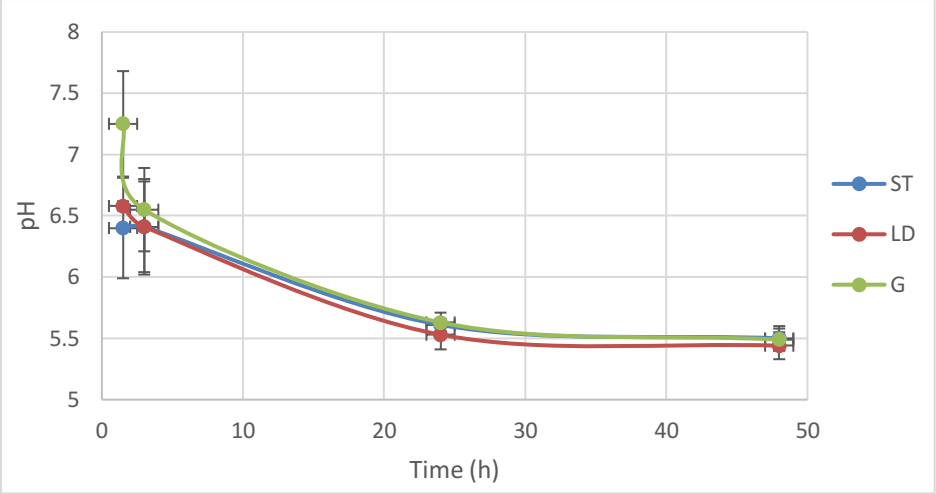

Figure 4.1 Kinetics of pH decline of the muscles G, LD, and ST (n=6) on the sheep carcasses during 48h of post-mortem.

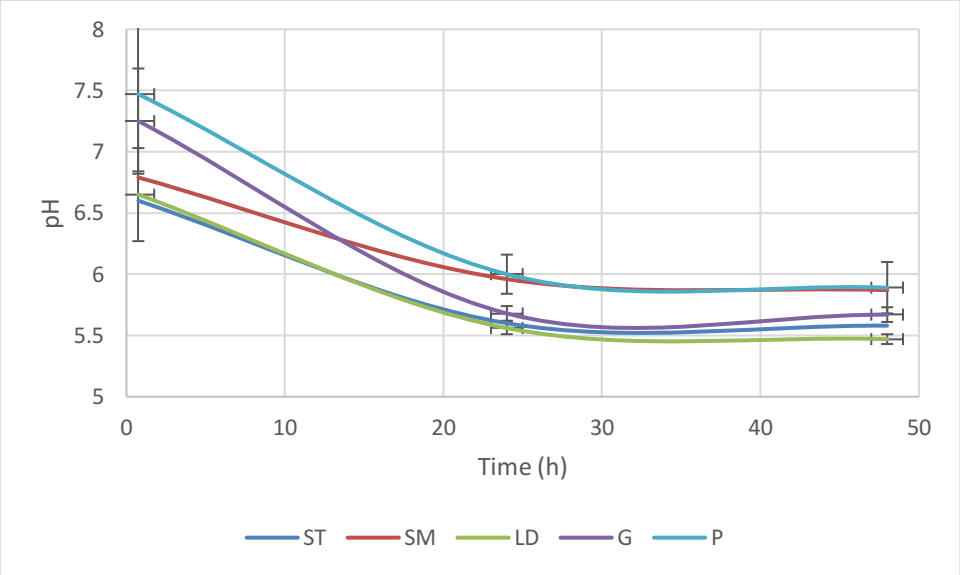

Figure 4.2 Kinetics of pH decline of the muscles G, LD, ST, SM, and P (n=6) taken off the sheep carcasses during 48h of post-mortem.

4.3. Temperature measurements

For all the five muscle samples, on the carcasses and off the carcasses, the temperature values decreased with time (Table 4.4 and Table 4.5, respectively).

The mean values of the temperature measured on G , LD and ST muscle on-the carcasses showed a decline from 1.5h to 24h during the PM (Table 4.4). LD muscle showed the highest temperature reading of 24^{o}C at 1.5h, followed by G and ST muscles. However, there was no significant difference in temperature for the three muscle samples LD, ST, and G at 1.5h. Additionally, the results showed that there was a significant drop in the muscle temperature from 1.5 to 3h, which further continued to drop till 24h. However, there was no significant drop in the temperature of the muscle from 24h to

48h. This implies that the muscles have reached a constant temperature of 3.79±0.05 during 24h of PM.

At 1.5h PM, the ST, LD, and G muscles had a significantly similar temperature at 1.5h, 3h, and 48h of PM. However, the ST muscle showed a significantly higher value at 24h compared to LD and G muscles. Additionally, the results obtained suggest ~70% drop in the temperature measurements for the LD and G muscle at 24h and about 50% drop for the ST muscle during the same time interval. Additionally, it was studied that there was no significant temperature drop occurred after 24h time.

The mean values corresponding to the temperature measurements taken for different muscle samples P, S_tM, G, ST, LD taken off the carcasses is shown in Table 4.5. Over the 24h, during the post slaughter period, there was a sharp decline in temperature measurements from 23°C to 6°C. At 0.75m, of PM period, there was a range of pH measurements of which LD muscle had a significantly higher temperature , followed by ST and G, and then by the S_tM, and the P muscle. The values at this time ranged between 17 to 24 ^{o}C.

Table 4.4 Kinetics of temperature decline of the muscles G, LD, and ST (n=6) on the sheep carcasses during the first 48h Post-mortem.

Time (h)	ST	LD	G
1.5	19.37±1.93	24.16±4.54	22.66±3.69
3	12.1±3.35	13.87±3.71	13.6±2.7
24	5.61±0.07	4.05±0.44	3.81±0.21
48	3.83±0.13	3.73±0.08	3.81±0.4

*All values are statistically similar (P ≥ 0.05)

Table 4.5 Kinetics of temperature decline of the muscles G, LD, and ST (n=6) taken off the sheep carcasses during the first 48h post-mortem.

Time (h)	ST	S_tM	LD	G	P
0.75	21.96±2.81	17.71±1.75	23.86±3.02	19.62±2.14	16.96±0.75
24	5.6±0.07	6.86±2.53	5.66±0.42	6.95±1.4	10.2±1
48	6.83±1.96	8.75±1.16	6.01±0.59	8.63±1.96	10.72±2.83

*All values are statistically similar (P ≥ 0.05)

From Figure 4.3 and 4.4, it is studied that there is a sharp fall in temperature measurements for all the muscle samples on the carcasses and those taken off the carcasses from the time of slaughter. For the sample set of samples, it was observed that a time interval of 24h is required to attain a steady temperature range.

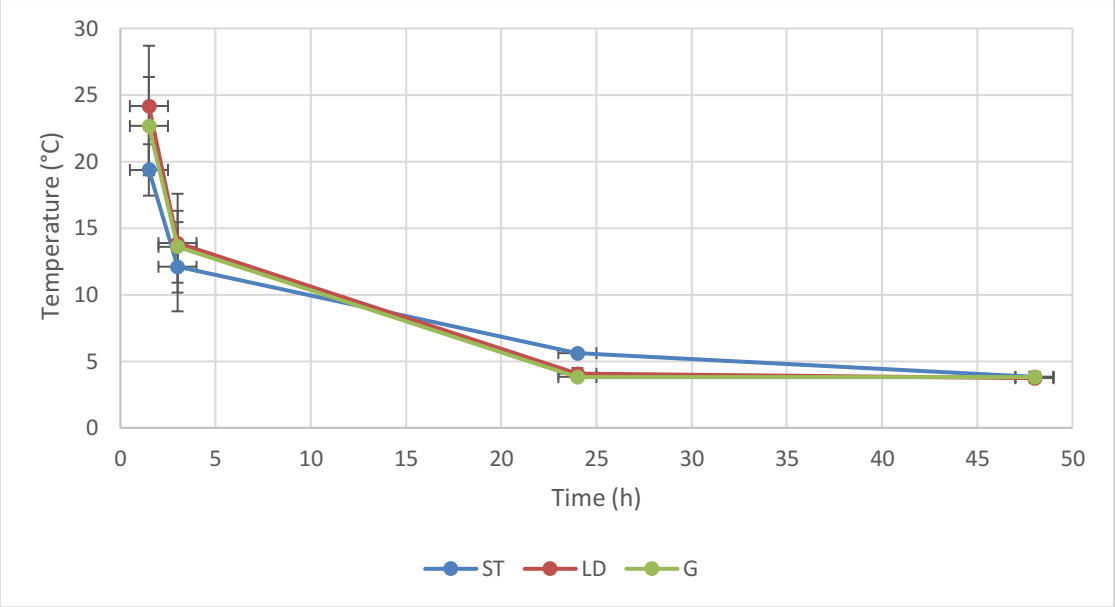

Figure 4.3 Kinetics of temperature decline of the muscles LD, G, and ST (n=6) on the sheep carcasses

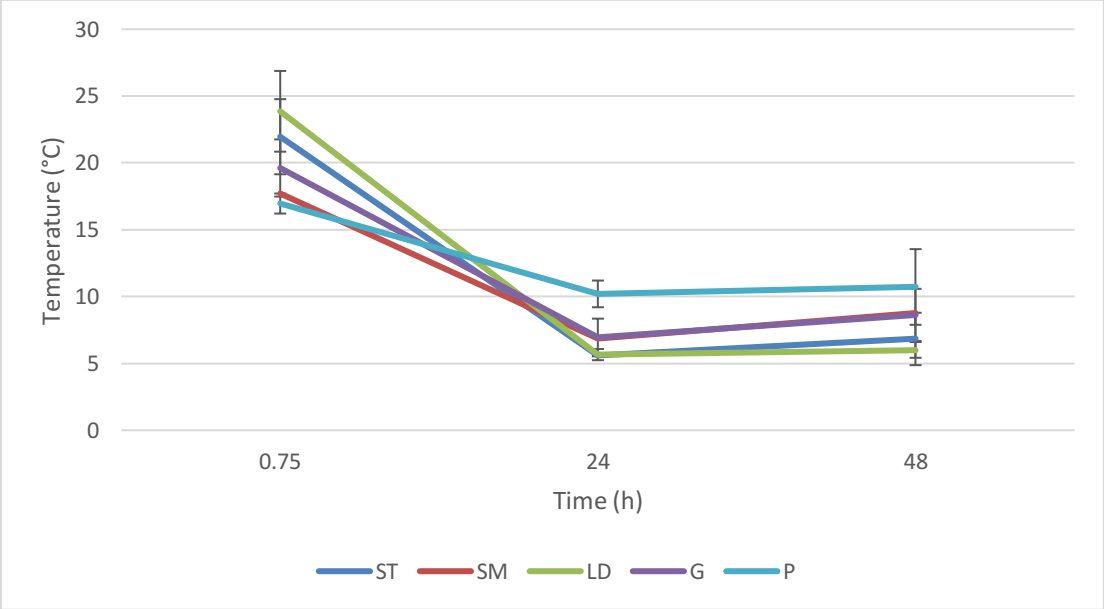

Figure 4.4 Kinetics of temperature decline of the muscles ST, StM, LD, G, and P (n=6) taken off the sheep carcasses

4.4. Colour Measurements

The colour measurements were taken for five and three different muscle samples on the carcasses and off the carcasses respectively from 6 different sheep (n=6). All the readings were noted at 48h of post slaughter. Table 4.6 shows the lightness, redness, and the yellowness values for each of the five different muscle samples taken off the carcasses from six different sheep (n=6). The results obtained showed that, ST muscle had the highest lightness value (L*), followed by P, G, and LD which were

significantly similar. The S_tM muscle showed the lowest lightness measurements. The redness value (a*) obtained showed that, S_tM muscle had the highest value, followed by P, which was statistically similar with LD, S_tM, G, and ST muscles. The LD, S_tM, G, and ST muscles had the lowest redness measurements. The yellowness values (b*) obtained showed that LD and ST muscles had the highest values and S_tM and P had the lowest value. G was found to be statistically similar in b* value compared to all other muscles in the present study.

Table 4.6 Colour measurements of the muscle samples LD, StM, G, P, and ST taken off the carcasses from six sheep (n=6).

Muscle	L* (Lightness)	a* (Redness)	b* (Yellowness)	C (Chroma)	HA (Hue)
LD	37.39 ± 4.09^{ab}	15.22 ± 2.69^{b}	1.88 ± 1.72^{a}	15.33 ± 3.19^{b}	0.1208 ± 0.56^{a}
S_tM	36.15 ± 2.93^{b}	18.45 ± 2.36^{a}	-0.22 ± 1.53^{b}	18.45 ± 2.81^{a}	-0.0051 ± 0.57^{a}
G	39.32 ± 3.01^{ab}	16.21 ± 2.46^{b}	1.11 ± 2.52^{ab}	16.24 ± 3.52^{b}	0.054 ± 0.79^{b}
P	38.42 ± 4.98^{ab}	16.77 ± 3.19^{ab}	-0.28 ± 1.59^{b}	16.77 ± 3.56^{ab}	-0.023 ± 0.46^{b}
ST	39.89 ± 1.99^{a}	16.19 ± 1.47^{b}	1.85 ± 2.00^{a}	16.29 ± 2.48^{b}	0.11 ± 0.93^{a}

*Mean values which share the same letters are statistically similar (P≥0.05)

Table 4.7 provides information on the lightness, redness, and yellowness values for the three different muscles LD, G, and ST on the carcasses. The results obtained showed that there was significant difference in the L*, a* and b* values for the three different muscles, LD, G and ST. For the muscle samples on the carcasses, the L*values showed that the LD and G muscle samples had significantly similar values. Tukeys test showed that ST muscle had a higher mean value compared to LD and G. The a* value obtained showed that G and ST muscles had statistically similar values, whereas the mean value of LD muscle was significantly higher than G and ST muscle when compared using Tukeys test. The b* values obtained showed that, LD and ST muscles were similar. The chroma value obtained showed that LD muscles had a significantly higher mean value when compared to G and ST which have statistically similar values. This variation in the colour parameter can be due to pre slaughter stress conditions (Bekhit, Geesink, Morton, & Bickerstaffe, 2001).

Table 4.7 Colour measurements of the muscle samples LD, G, and ST taken on the carcasses from six sheep (n=6).

Muscle	L*	a*	b*	C	HA
LD	33.94 ± 2.09^{b}	19.93 ± 1.80^{a}	1.12 ± 1.74^{a}	19.96 ± 2.50^{a}	0.013 ± 0.76^{b}
G	34.84 ± 3.79^{b}	15.38 ± 2.31^{b}	-1.93 ± 1.58^{b}	15.44 ± 2.79^{b}	0.043 ± 0.59^{a}
ST	38.22 ± 6.66^{a}	16.22 ± 3.14^{b}	0.31 ± 1.80^{a}	16.22 ± 3.61^{b}	0.025 ± 0.51^{ab}

*Mean values which share the same letters are statistically similar (P≥0.05)

4.5. Cooking loss

Figure 4.5 and Figure 4.6 showed the percentage of cooking loss with time for the three different muscles ST, LD, and G on the carcasses and those taken off the carcasses, respectively . The results obtained showed that LD muscles had the highest and G had the lowest cooking loss (%) on the 3rd day and on the 7th day. Additionally, the results obtained showed that ST and LD muscles showed significantly similar behaviour on 2nd day for the muscles on the carcasses and those taken off the carcasses. Whereas on day 7, LD muscle had the highest cooking loss for the muscles on the carcasses, followed by ST and G. Whereas for the muscles taken off the carcasses, LD and ST muscles had the highest cooking loss on the 7th day followed by G.

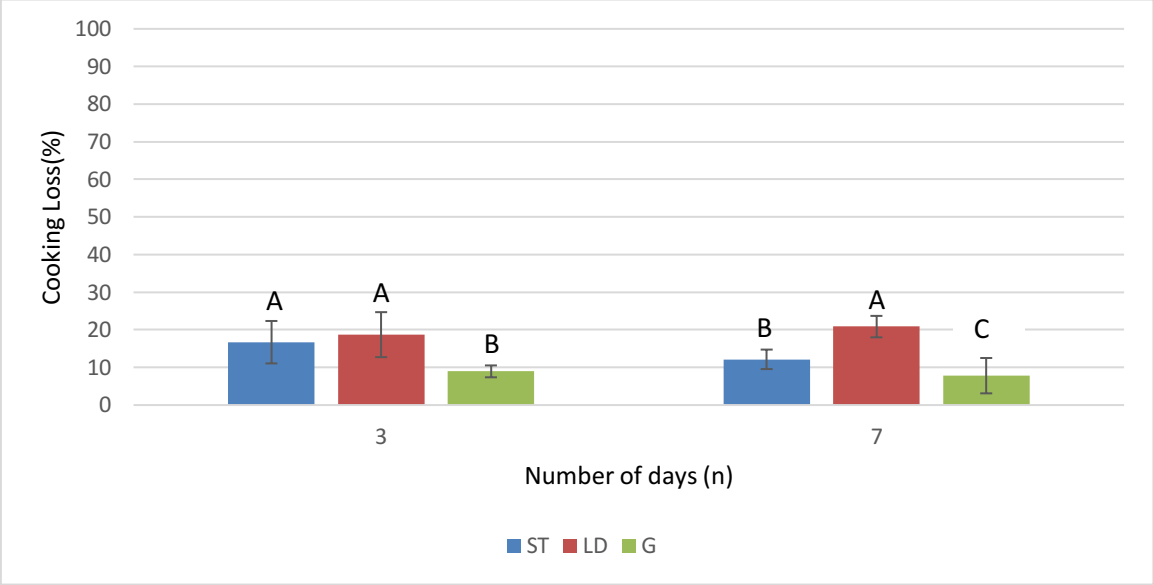

Figure 4.5 Mean±SD values of cooking loss (%) calculated on the 3rd day and 7th day of the lamb muscles ST, LD, and G on the carcasses. *Mean values which share the same letters are statistically similar (P≥0.05)

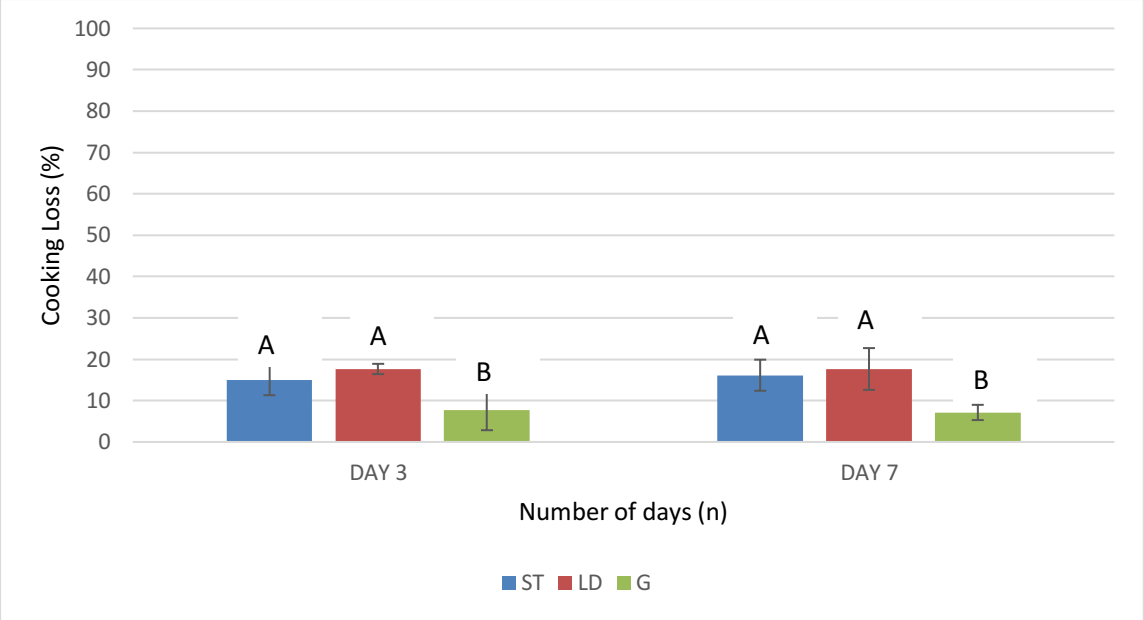

Figure 4.6 Mean±SD values of cooking loss (%) calculated on the 3rd day and 7th day of the sheep muscles ST, LD, and G (n=6) taken off the carcasses. *Mean values which share the same letters are statistically similar (P≥0.05)

4.6. Shear Force

Figure 4.7 showed the change in parameters associated with shear force with time for the three different muscles ST, LD, and G muscles on the carcasses. The results obtained showed that there was a decrease in shear force for all the three muscle samples from day 3 to day 7. G had the highest shear force compared to LD and ST which had statistically similar values on the 3rd day. Furthermore, the results obtained showed that the shear force calculated on 7th day for the muscles G and ST muscles had a comparatively higher mean value compared to LD muscles. This in turn suggest that aging has a significant effect on shear force of meat (Toohey et al., 2008). Additionally, another possible implication would be the rate of chilling which decreased the tenderness of meat (Starkey, Geesink, Oddy, & Hopkins, 2015).

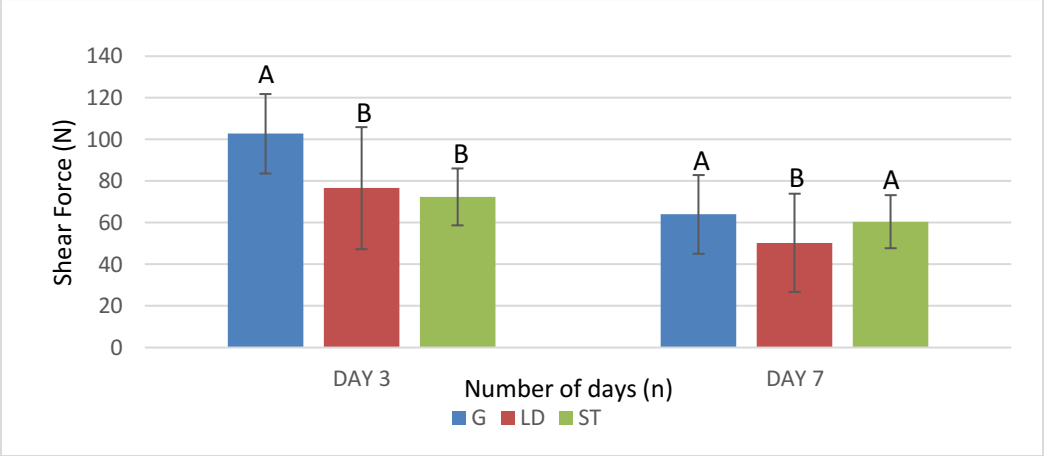

Figure 4.7 Mean±SD values of shear force calculated on the 3rd day and 7th day of the sheep muscles (n=6) ST, LD, and G on the carcasses. *Mean values which share the same letters are statistically similar (P≥0.05)

Figure 4.8 showed the change in parameters associated with shear force with time for the three different muscles ST, LD, and G muscles on the carcasses. The results obtained showed that G had the highest shear force followed by ST and LD on the 3rd day. Statistical analysis showed that the three muscle samples were significantly different from each other. Furthermore, the results of shear force calculated on 7th day showed that G had a comparatively high mean value compared to LD and ST muscles which were significantly similar.

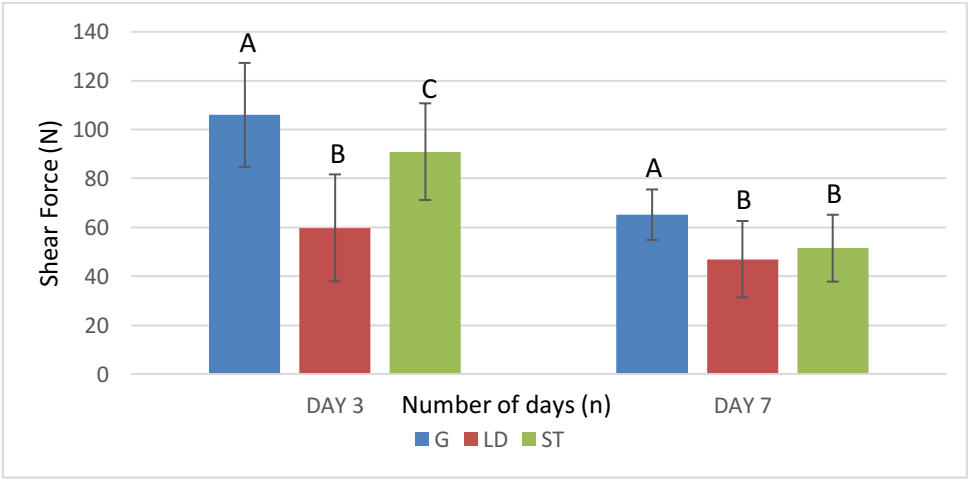

Figure 4.8 Mean±SD values of shear force calculated on the 3rd day and 7th day of the sheep muscles (n=6) ST, LD, and G taken off the carcasses. *Mean values which share the same letters are statistically similar (P≥0.05)

4.7. Muscle Fibre Typing

The myosin heavy chain isoforms were identified according to the molecular mass for five different lamb muscles namely LD, ST, S_tM, G, and P by performing sodium dodecyl sulfate-polyacrylamide gel

electrophoresis (SDS PAGE). It was observed that, an electrophoresis time of 25h or above can result in the separation of MyHC isoforms which lies approximately to 250 kDa. The procedure was carried out by varying different conditions such as electrophoresis running time, sample storage time, and the volume of sample used for running the gel.

Figure 4.9 shows the electrophoretic image obtained by running the gel for 24h at 70V using the freshly prepared samples. The volume of the muscle sample loaded corresponds to 6µl. The figure clearly shows the presence of MHC bands which lies approximately to 250 kDa for the muscles samples which were freshly prepared. According to the results obtained by Kim (2014) the separation of MHC by SDS PAGE shows the identification of four different isoforms which includes, three MHC type II and one MHC type I. This includes MCH IIx (MHC-1), IIa (MHC- 2), IIb (MHC-4), and MHC-7 (slow/1). The theoretical molecular weight of these heavy chain isoforms is 223.9kDa for MHC-1 (II-x), 224.23 kDa for MHC-2 (II-a), 223.86 kDa for MHC-4 (II-b), and 224.02 kDa for MHC-7(slow/1). The results obtained for the freshly prepared muscle samples in the present study was in line with the above findings. However, it was difficult to differentiate between the different isoforms as their MWs are nearly same.

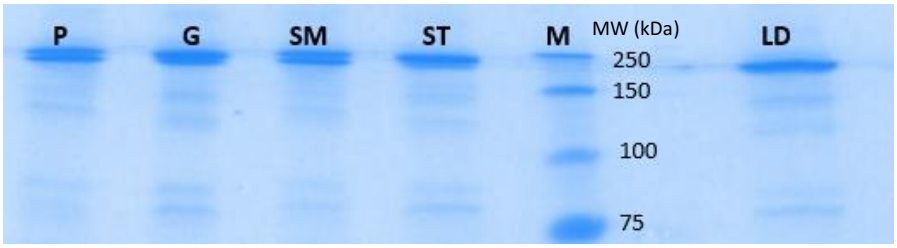

Figure 4.9 Electrophoretic separation of MHC isoforms of sheep muscle (n=1) P, G, S_tM, ST, and LD. (M : Molecular marker)

Figure 4.10a and 4.10b represents images obtained by running the gel using extracted muscle samples stored for 30days prior to running the gel. The electrophoresis was run for 30h. Figure 4.11 corresponds to the image obtained for the muscle samples which were extracted and stored for 30d, and the electrophoresis was performed for 36h at varying voltages. The electrophoretic unit was run at 70V for the first 30h and was increased to 120V for the next 6h. Additionally, the results obtained from Figure 4.10a, 4.10b, and 4.11 identified that the bands obtained had comparatively higher molecular weight (>250kDa) compared to the freshly prepared sample.

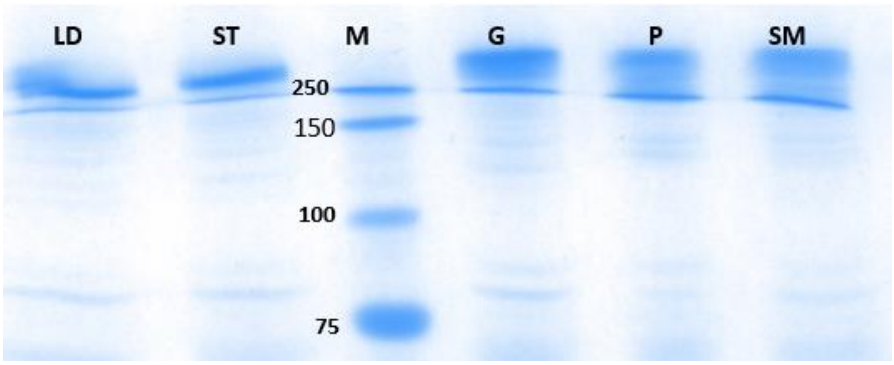

Figure 4.10a Separation of MHC isoforms of sheep muscles (n=1) P, G, S_tM, ST, LD, and G containing 6µl of the sample. (M : Molecular marker)

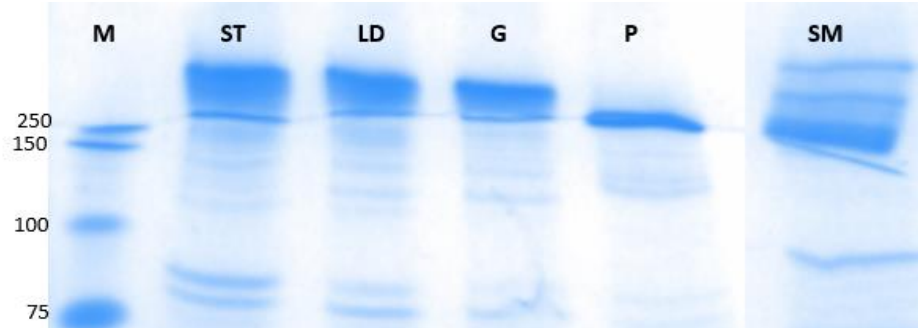

Figure 4.10 b Separation of MHC isoforms of sheep muscles (n=1) P, G, S_tM, ST, LD, and G containing 6µl of the sample. (M : Molecular marker)

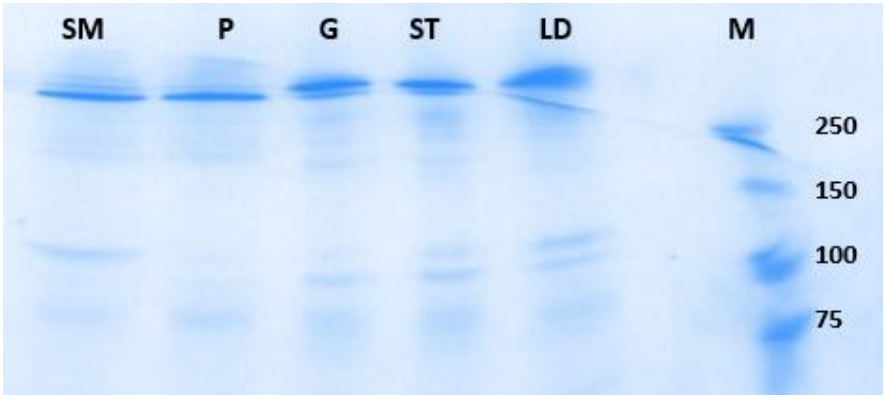

Figure 4.11 Separation of MHC isoforms of sheep muscles (n=1) P, G, S_tM, ST, LD, and G containing 6µl of the samples. The electrophoresis was run for 30h at 70V and for 6h at 120v. (M : Molecular marker)

4.8. Glycogen Content

The concentration of muscle glycogen in the five different muscles from 6 biological replicates (n=6) were estimated by the subtracting glucose concentration in non-hydrolysed from hydrolysed muscle extracts (Table 4.8). Statistical evaluation of the glycogen concentration of the different muscle samples revels that the values were statistically similar, and the values ranged between 14 to 40 µmoles/g. The concentration of glycogen for the different muscles followed the order P>> ST=G>> S_tM =LD. The mean values of the non-hydrolysed samples followed the order ST>>G>> S_tM >>LD>>P. Tukeys comparative test showed that LD, S_tM, and G were statistically similar (P≥0.05), whereas ST had a significantly higher value and P had a significantly low value. From the mean values obtained it is studied that the concentration of the hydrolysed samples was highest for P>>ST>>G>>LD= S_tM. However, the statistical evaluation showed that there was no significant difference in the hydrolysed muscle concentration for the different muscle samples.

Table 4.8 Mean±SD values for the concentration of muscle glucose in hydrolysed and non-hydrolysed muscle extracts and estimated muscle glycogen concentration from 6 biological replicates (n=6).

Muscle	Concentration of Hydrolysed muscle extracts (µmoles/g)	Concentration of Non-Hydrolysed muscle extracts (µmoles/g)	Glycogen concentration (µmoles/g)
LD	14.1±12.09	2.9±1.035^{ab}	13.8±10.92
ST	22.8±17.38	4.5±1.3418^{a}	18.2±17.16
S_tM	14.8±8.57	3.0±0.9138^{ab}	12.0±8.37
P	40.1±31.93	1.9±1.29^{b}	38.7±31.72
G	21.8±12.96	3.2±1.87^{ab}	18.4±14.68

*Mean values which share the same letters are statistically similar (P≥0.05)

Chapter 5

Discussion

5.1. pH and temperature decline

The results from the present study indicated that, there was a sharp decline in pH values for the muscles G, LD, and ST from 1.5h to 24h for the muscles on the carcasses and from 45m to 24h for the muscles P, S_tM, G, ST, and LD taken off the carcasses. Similar findings were indicated by Shija et al. (2013) who reported that there was a sharp decline in pH measurements for the muscles in sheep at 45m, 3h, 6h, 12h, and 24h. A possible implication for this decrease in pH values as suggested by Vestergaard, Oksbjerg, and Henckel (2000) is the conversion of glycogen into lactate and H^+ during the post-mortem period, which results in low meat pH. Additionally, the glycogen level at the time of slaughter is inversely related to pH_u value.

For the muscles on the carcasses, the pH value ranged from 6.4 to 7.2 and for the muscles off the carcasses, it ranged from 6.6 to 7.4 after 45m. This is similar to the results obtained by McGeehin et al. (2001) which indicated that the extent of pH ranges between 6.0 to 6.8 after 45m of animal death. This in turn is an appropriate way to measure the extent of glycolysis in sheep muscles. A possible explanation for this change in pH includes chilling temperature and pre slaughter stress conditions (McGeehin et al., 2001). At 24h, after the post slaughter period, it is studied that the pH values for the muscles on the carcasses ranged from 5.54 to 5.63 and for those off the carcasses the mean value ranged from 5.5 to 6.0. These results were comparable to those obtained Shija et al. (2013), who suggested that the mean pH_u values were ≤ 6. Additionally, it can be suggested that the depletion of the glycogen level in the sheep carcasses can be a possible explanation for the decrease in the pH during the post slaughter period. As the pH falls in the range of 5.8-6.2, the meat appears to be dark, firm and dry (DFD) and it tends to be tougher as it reaches pH of 6.2. The change in meat to the DFD form can cause huge financial loss for the cattle and sheep industries.

The pH measurements showed that G had the highest pH value for the muscles on the carcasses, and for those taken off the carcasses. There was no significant difference in the LD and ST muscles during 1.5h for the muscles on the carcasses. Additionally, there was a significant decline in pH from 3h to 24h. According to Knight et al. (2019), this change in pH influence the colour of the meat, which is an important factor which affects the consumers decision in purchasing a product. Additionally, the study suggest that a low pH value indicates a decrease in the water holding capacity and a light colour whereas a high pH value indicates meat with dark colour and poor quality. There, taken together both these results suggest that muscles in the said pH range are superior in tenderness and uniformity.

Moreover, the pH range attained after 1.5h of slaughter is ideal for consumption and is regarded superior in meat quality.

According to Gault et al. (2005) the low pH values of ST and LD muscles for both on the carcasses and those taken off the carcasses, was due to the reason that, these muscles were close to entering rigor. However, there was no difference in the pH measurements for the three muscle samples LD, ST, and G at 3h, 24h, and 48h. The time taken to reach a pH of 6.2 and the temperature of the muscle attained at this temperature are indicators of the metabolic process which takes place in the muscle. This in turn directly impacts the meat tenderization and the muscle shortening process.

There was a sharp decline in temperature for the muscles ST, LD, and G on the carcasses and the muscles ST, LD, S_tM, G, and P taken off the carcasses. However, the pattern of temperature drop for the different muscle was different. As suggested by Shija et al. (2013), the difference in the decrease in temperature for the different muscle is probably due to the difference in the size, percentage of fat coverage, and the location of the muscle (surface or internal). Muscles of smaller size would probably dissipate heat at a faster rate compared to the muscles of bigger size which are associated with slower temperature decline (Shija et al., 2013). For the muscles on the carcasses there was no significant difference in the temperature for the muscles LD, G and ST at 1.5h, 3h, and 48h. However, at 24h it was identified that ST muscle had a significantly high temperature compared to LD and G. Therefore, it can be attributed that the low temperature drop for the ST muscle may be attributed to the greater thickness of the muscle.

5.2. Color Measurements

The rate at which the meat surface colour changes from red to brown is dependent on different factors. This includes the oxygen consumption and diffusion rate, the auto oxidation rate of myoglobin to metmyoglobin pigment, and the rate of metmyoglobin reducing activity (Calnan et al., 2014). Literature evidence suggest a strong correlation between the pH and color of the meat. A high pH could reduce the amount of oxygen which can penetrate the surface of the meat. Elevated pH measurements can cause more utilization of oxygen by mitochondria, thereby reducing the availability of oxygen form red oxymyoglobin pigment (Calnan et al., 2014). Therefore, the findings suggest that pH is an important carcasses trait which needs to be reduced for preventing the deterioration of meat color. The lightness value was highest for ST and the lowest for the S_tM. Additionally, the muscles G, P, and LD showed significantly similar characteristics of ST and S_tM. The results obtained was in accordance with that of Vestergaard et al. (2000), who reported that the lightness of meat is inversely related to its pH measurements. The redness value was lowest for LD and highest for S_tM. A possible implication for this as suggested by Vestergaard et al. (2000) is that the concentration of the heme

pigment was highest for the LD and ST muscle. This was related to the change in the oxidative capacity and muscle fibre typing. Additionally, pigmentation is related to the a* value. The difference is the level of pigmentation can be related to the level of physical activity involved. The yellowness value was highest for ST and lowest for S_tM. High values of yellowness, lightness and hue can be explained by the denaturation of proteins which is caused by the low pH values at high-pre rigor temperature. This reduces the stability of metmyoglobin reduction, thereby resulting in reduced color stability (Rosenvold & Wiklund, 2011).

5.3 Cooking Loss

The cooking loss of meat is mainly caused by the denaturation of proteins due to heat and reduced water holding capacity (Vaskoska et al., 2020). The loss of mass during the process of cooking includes water, vitamins, soluble proteins, and high valued nutrients (Rant et al., 2019). Information regarding the mechanism associated with the water holding capacity in meat is limited. The results from the present study provide evidence to prove that LD and ST muscles had the highest cooking loss on the 3rd day and the 7th day. The results obtained suggest that, cooking loss was related to the time at which the meat was cooked during the post-mortem period. Major theory associated with cooking loss is the electrostatic repulsion between the actin and myosin filaments, the capillary and osmotic forces, and the protein surface area which interacts with the water surface and the sarcoplasm (Ertbjerg & Puolanne, 2017). The highest cooking loss was observed at day 3 compared to day 7. According to (Panea, Sanudo, Olleta, & Civit, 2008) (Geesink et al., 2011), the three main factors which influence the cooking loss includes internal and surface temperature of meat, and the method of heat transfer. However, these factors have directly influenced the extent of connective tissue shrinkage and decreased the water binding capacity. The water holding capacity assumes that as the net charge in the filament becomes the same, the swelling force increases which in turn cause an increase in the water holding capacity. Therefore, it can be implied that there is an increased water holding capacity for the Gracilis muscle as compared to the LD and ST muscle. Additionally, the rate of cooking loss is affected by many factors such as method of heat transfer, and internal and surface temperature of meat (Shija et al., 2013). A possible explanation for these factors as given by the early findings of Lawrie (1991) from Shija et al. (2013) is that these factors cause the denaturation of proteins in the muscle which in turn lowers the water binding capacity of meat resulting in the forced expulsion of fluid from meat (Shija et al., 2013). Cooking of pre rigor meat is studied to have shortest sarcomeres followed by the rigor and the post rigor meat. However, the cooking method did not have any effect on the sarcomere length (Geesink et al., 2011). The results obtained showed that the cooking loss was high for ST and LD compared G. A possible reason for this as suggested by Vaskoska et al. (2020) is

that the low water holding capacity of ST muscle is mainly attributed to the presence of type IIB fibres, which has reduced water retaining capacity as compared to type I muscle. The main difference mainly attributes to the difference in the elastin and collagen content (Vaskoska et al., 2020).

The space between the myofilaments is an important factor which determines the water holding capacity of muscles. As a result, the shrinkage of the interfilamental lattice would cause a reduction in the water holding capacity of muscles during the postmortem period. Therefore, the decrease in the water holding capacity may be due to the denaturation of the myofibrillar proteins. However, this is in contrary to the denaturation of sarcoplasmic proteins, as the denatured protein forms a covering layer which act as a protection layer for myosin from denaturation by heat treatment (Liu, Arner, Puolanne, & Ertbjerg, 2016). As a result, the denaturation of sarcoplasmic proteins increases the hydrophobicity of the muscle surface. Therefore, it can be concluded that cooking loss is dependent on the time after slaughter, at which the meat was cooked. The results obtained suggest that the highest cooking loss was at day 7. Additionally, there is also a relationship between pH and cooking loss, with minimal cooking loss at increased pH. There is a strong influence on the Warner–Bratzler shear force and the cooking temperature. The solubilization and tenderization of connective tissue at 60ºC can cause tenderization of meat, whereas myofibrillar protein denaturation takes place at 40to 55 ºC and toughening of muscles at 65 ºC to 72 ºC (Vaskoska et al., 2020).

5.4. Shear Force

The conversion of muscle to meat involves several metabolic and structural changes which in turn affects the tenderness of meat due to the change in the muscle fibre and connective tissue properties. This in turn increase the rate of meat tenderization (Shija et al., 2013). Shear force values obtained from the present study suggest that G had the highest value for the muscles on the carcasses and off the carcasses, both on the 2^{nd} day as well as the 7^{th} day. Early findings of Gaili and Ali (1985) as cited by Shija et al. (2013) reported that, the thick muscle fibre could be a possible reason for the toughness of muscles. Additionally, the shear force value also depends on factors like slaughter age, breed, pH values, cooking time and temperature etc. Furthermore, the tenderness of the meat also reflects the size of their muscle fibre.

From figure 4.7 and 4.8, it can be inferred that there was a decrease in the shear force value as aging proceeds. This infers that there is tenderization of meat as they are kept longer. The difference in the force required for shortening meat was related to the collagen content of the muscle. Additionally, the sarcomere length and the fibre type also affect the extent of muscle shrinkage. Tenderization of meat due to aging is studied to cause disruption of protein structure due to the proteolysis of myofibrillar protein such as nebulin, titin, and desmin. The longitudinal shrinkage was related to the

muscle type, aging temperature, and the type of muscle (Vaskoska et al., 2020). Additionally, the shear force was affected by the cooking method and the time of cooking. It was studied that there was large variation in the shear force, which the highest shear force obtained for the rigor cooked meat samples. A large decrease in shear force was probably obtained for the muscles cooked on 7^{th} day compared to that of the 3^{rd} day. This may probably be attributed to the tenderization of the meat sample due to the solubilization of the connective tissues, which causes a decrease in shear force (Geesink et al., 2011). According to the studies by Geesink et al. (2011), the toughness of meat which was subjected to cooking at 70°C cannot be resolved by cooking them for an increased time. Additionally, as the cooking time increased it can result in tenderization resulting in the solubilization of connective tissues. Results from Hopkins et al. (2006) suggest that sheep meat with a shear force value between 40 to 49N can be considered 3-star rating and therefore good for everyday consumption. Additionally, the results also suggest that, to obtain a mean overall liking score 63, a shear force of 27N is required, which indicates that a threshold lower than 49N may be suitable in accordance with the requirements of Australian consumers.

Therefore, the study provides evidence to prove that there exists a strong relationship between tenderness and cooking loss. This comparable effect was evident from the results obtained by cooking the meat samples on the 2^{nd} day and 7^{th} day. Therefore, the results from the present study provide evidence to prove that aging to be an effective option for improving the tenderness of muscles like LD, ST, and S_tM as used in the present study. Additionally, the preparation of pre rigor meat is likely to produce tough meat unless it is prepared soon after the slaughtering process. The toughness of meat during the early post-mortem period is due to muscle shortening which happens during the time of heating process (Geesink et al., 2011).

5.5. Muscle Fibre Typing

The MyHC (myosin heavy chain) isoforms were considered as the major factor for the determination of meat quality (Kim, Jeong, Yang, & Joo, 2013). These isoforms were identified according to the molecular mass as indicated on the gel. The polymorphism of MyHC is that each isoform encodes a single gene. The contraction speed of the isoforms is ranked in the order I < IIa < IIx < IIb (Lefaucheur, 2010). Type 1 MHC isoforms which includes the slow twitch type 1 can sustain the motor activity, which is slow contractile in nature, whereas type II MHC isoforms includes fibres which are fast contracting in nature. As type I fibres sustains greater contractions, they have greater oxidative capacity. However, type IIb fibres are glycolytic in nature, and uses glycogen rapidly for short series of activity. Type IIx and IIa are intermediate to that of type IIb, and type I fibres. The skeletal muscles are

identified to be dynamic tissues which adjusts to the external environment by changing its contractile ability and metabolic nature (Park, Gunawan, Scheffler, Grant, & Gerrard, 2009).

Figure 4.9 shows the separation of the bands which lies next to 250kDa which are indicated as band 1, and 2 in the figure. However, it could be implied that more than one MHC should be present in each of these bands. This is because the molecular weight of these heavy chain isoforms is nearly same. Experimental evidence by Kim (2014) reported that the molecular weight of MHC-1 (II-x) is 22.39kDa, MHC- 2 (II-a) is 22.42kDa, MHC-4 (II b) is 22.38kDa and MHC -7(slow /I) is 22.40kDa. Therefore, taken together, both these results suggest that the MHC-IIx, MHC-IIb, and MHC-I has nearly same MW and so they cluster together and appears as one single band represented by band 2 in the picture, and the MHC IIa which had a comparatively higher MW of 22.42kDa is represented as band 1. However, there was no clear separation of the bands observed in the LD muscles. Additionally, several bands have appeared in the range of 150 to 50 kDa which underlines the presence of other proteins in the muscle samples.

The quality of meat is influenced by both the contractile and metabolic nature of the fibres. According to Choe et al. (2008), the fast twitch glycolytic fibre muscle proportion can cause an increase in the extent of pH decline in pigs during the post-mortem period. Additionally, it can cause a decrease in the water holding and protein denaturation capacity, cooking loss, paleness, and the lightness (Lefaucheur, 2010). Therefore, it can be attributed that the presence of MHC type IIa, b, and x in all the muscles plays an important role in maintain the quality of the muscle. The early findings (Hämäläinen & Pette, 1995, as cited in Picard and Cassar-Malek, 2009, p. 30) hypothesized that MHC-IIb is unlikely to be expressed in large mammals due to their slow movements. However, the results from Lefaucheur (2010) reported that the Myosin heavy chain IIb is found in almost all the mammals. Additionally, the study also suggested the occurrence of MyHC IIb in the LD and ST muscles of mammals. These results are consistent with the findings from the present study as the results obtained by SDS PAGE confirms the presence of MHC-IIb in ST, S_tM, LD, G, and P muscles of sheep. However, these results were contradicting to that of Picard and Cassar-Malek (2009) and a possible explanation for this is that the different fast MHC isoforms differ from each other based on their actin binding site which is the N-terminal region of loop 2. Based on the substitutions in this region, it causes change in the mATPase activity. This in turn made it possible to distinguish between the different fast fibres based on their ATPase activity.

Results from the present study provides evidence to prove the presence of MHC IIb in five different muscles. According to Picard and Cassar-Malek, (2009), MHC IIb is reported to be the most glycolytic muscles compared to all the MHC isoforms, which implies its presence in fast glycolytic muscles only. However, the present study reported the presence of MHC IIb in the five different muscles. This

difference in the composition of muscle fibres was explained by Vestergaard et al. (2000) as the fibre type is dependent on the mode of exercise practiced by different animals grown under extensive production system. This directly influences the change in the composition of the fibre type. Additionally, the study also suggests that the muscle fibres are mutable, and they can change from one fibre type to another depending on the type of physical activity, and other forms of stimuli. The SDS-PAGE of the stored muscle samples revealed the presence of MHC isoforms with increased MW compared to the freshly prepared samples. A possible implication for this as suggested by Ooizumi and Xiong (2006) is the cross-linking of polymers which may have occurred due to the oxidation of myosin during the aggregation process. In the present study the muscle samples were extracted by the homogenization process, which may have rendered the proteins present in them susceptible to oxidative damage. As a result, intermolecular cross linking of the disulfide bonds may have occurred which have led to the intermolecular aggregation (Ooizumi & Xiong, 2006). This process may have resulted in increased molecular weight of the protein which appeared in the form of high molecular weight bands in the SDS PAGE image.

5.6. Glycogen Analysis

The biochemical changes which happen in the animal muscle after the death of an animal play an important role for the conversion of the muscle to meat. Glucose can be assayed, and this requires the hydrolysis of glycogen to glucose, and then measuring the concentration of the glucose. The depletion of glycogen during the post-mortem period results in the build-up of lactic acid which results in the decline in muscle pH (Choe et al., 2008). In the presence of adenosine-5'-triphosphate (ATP) and hexokinase (HK), the phosphorylation of D- Glucose to glucose-6-phosphate (G-6-P) takes place, simultaneously resulting in the formation of adenosine-5'-diphosphate (ADP). The addition of the enzyme glucose-6-phosphate dehydrogenase (G6P-DH) results in the oxidation of G-6-P by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to gluconate-6-phosphate along with nicotinamide-adenine dinucleotide phosphate (NADPH). The amount of D- Glucose formed is stochiometric with the formation of NADPH. Therefore, the measure of the NAPH at an absorbance of 340nm estimates the amount of D-Glucose present in the samples (Choe et al., 2008). The present study evaluates the amount of muscle glycogen concentration for the different muscle LD, ST, S_tM, G, and P muscles. The study provide evidence to prove that the concentration of glycogen was highest for P>> ST=G>> S_tM =LD. The high glycogen concentration in Platysma directly relates to the post-mortem metabolic rates of the muscle (Choe et al., 2008). Additionally, it was observed that the muscle samples had turned more viscous and opaquer in colour on storage which may have contributed to the huge variation in glycogen concentration for the 6 biological replicates.

According to Pighin et al. (2008) adequate glycogen level of the muscle is required for the effective decline in meat pH. This is essential for the conversion of muscle to meat which in turn relates to the overall quality of the meat product. If the level of muscle glycogen is lower, it may limit the decline in pH. This increases the ultimate pH of the meat or can cause colour changes, which is referred to as dark cutting. The present study provide evidence that the meat quality of muscles followed the order P>> ST=G>> S_tM =LD. However statistical analysis found that there was no significant difference in glycogen among the different muscles. This possibly is due to the high standard deviation which arises due to the high variability among the 6 different biological replicates. However, taking the mean values in to consideration gives us the information that glycogen concentration of the muscle was in the order P>> ST=G>> S_tM =LD. A possible implication for this may be that LD and ST muscles are fast glycolytic muscles, and so are able to hydrolyse ATP quickly compared to the other muscles. As a result, in fast glycolytic muscles, there is a dramatic fall in ATP. This might result in the unavailability of ATP which is required for the conversion of PFK to fructose 1,6-bisphsophate. Phosphofructokinase (PFK) which helps in the conversion of fructose-6-phosphate to fructose 1,6 bisphosphate could also act as a rate limiting enzyme during the post-mortem metabolism period (Hammelman et al., 2003). A possible implication for this is that glycogen phosphorylase, which is a glycolytic enzyme capable of cleaving the glycogen residues in order to release the glucose molecules may be a responsible factor for the accumulation of G6P in muscles (Hammelman et al., 2003).

There is an inverse correlation between the muscle glycogen level and the body temperature. It is suggested that the increased body temperature indicates an increased stress condition of the animal prior to slaughter. This negatively affects the quality of the meat (Hammelman et al., 2003). This implies that lower glycogen level of the muscle in turn suggest the pre slaughter stress condition of the animals and the poor quality of the meat (Pighin et al., 2014). According to Sitthigripong, Sethakul, and Chaosap (2013) the fast glycolytic muscle fibres, which includes the type IIA and IIB are studied to have high glycogen content compared to the type I slow oxidative fibres. Research evidence suggest that in ovine muscle samples there was an increased glycolytic activity for the LD muscle compared to the *Psoas Major (*PM), *Supraspinatus* (SS), and *Infraspinatus* (IF). This was closely associated with the pH values of the muscle samples, as it was reported that the muscles with high glycogen content was related to low pH values. Taken together the results obtained from the present study provides a close relationship between the muscle glycogen concentration, pH, temperature, and the sarcomere length.

Chapter 6

Conclusion

The present study provide evidence to prove that pH and temperature of the sheep muscles play an important role in determining the quality of the meat. The results obtained revealed that there was a sharp decline in the muscle pH with time until the muscles attain an ultimate pH of 5.6 in 24h. Meat with dark cutting properties implies that there is a great impact of stress on the biochemistry of the meat, which in turn affects the body temperature. The results from the present study suggest that a correlation exist between the glycogen level of the muscle and the pH and body temperature. As a result, monitoring the pH and temperature of the muscle acts as an effective method to predict the glycogen content in the muscle. This could in turn propose the changes in animal handing procedure prior to slaughter for improving the quality of the meat. However, further research regarding the enzyme responsible for increasing the glycogen rate in the muscle is required for determining the biochemical parameters which supports the muscle metabolism. Furthermore, the experiment provide evidence to prove that there existed an inverse relationship between the shear force and the cooking loss. Also, there was a decrease in the shear force value as aging proceeds, which correlates to the tenderization of meat during aging. Additionally, the study suggest that a low pH value indicates a decrease in the water holding capacity and a light colour whereas a high pH value provides a darker colour and meat of poor quality.

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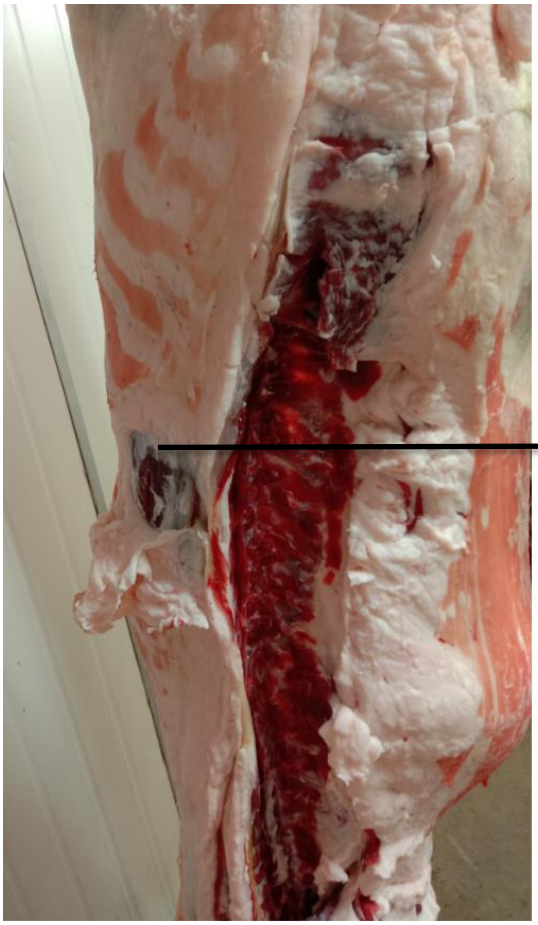
Appendix A

Pictures of animal slaughter

A.1 Whole view o f the six sheep carcasses

A.2 **Position of five muscles in the sheep carcasses**


Longissimus dorsi


Semitendinosus

Appendix B

Raw Data

B.1 pH measurements of muscles after slaughter

Muscle	Carcasses	pH - 90m	pH - 3h	pH - 24h	pH - 48h	pH -45m	pH - 24h	pH - 48hrs
ST	1	5.81	5.71	5.65	5.44	6.5	5.69	5.65
ST	2	6.37	6.6	5.59	5.59	6.26	5.62	5.66
ST	3	6.41	6.62	5.67	5.63	6.71	5.52	5.49
ST	4	6.3	6.27	5.48	5.49	6.43	5.53	5.58
ST	5	6.41	6.59	5.63	5.54	6.43	5.59	5.46
ST	6	7.12	6.67	5.68	5.32	7.27	5.67	5.64
SM	1	-	-	-	-	6.62	5.96	5.91
SM	2	-	-	-	-	6.96	5.95	5.85
SM	3	-	-	-	-	7.04	5.93	5.78
SM	4	-	-	-	-	6.62	6.03	5.99
SM	5	-	-	-	-	6.83	5.99	5.82
SM	6	-	-	-	-	6.68	5.9	5.92
LD	1	5.89	5.81	5.43	5.35	6.38	5.5	5.4
LD	2	6.76	7.01	5.56	5.55	7.15	5.55	5.53
LD	3	6.58	6.46	5.51	5.47	6.86	5.56	5.44
LD	4	6.50	6.20	5.35	5.5	6.15	5.58	5.42
LD	5	6.74	6.55	5.65	5.54	6.93	5.66	5.53
LD	6	7.02	6.46	5.69	5.25	6.48	5.56	5.52
G	1	7.95	6.12	5.64	5.4	7.95	5.65	5.57
G	2	7.35	7.02	5.74	5.54	7.35	5.65	5.73

G	3	7.19	6.57	5.65	5.49	7.19	5.64	5.68
G	4	7.16	6.22	5.63	5.63	7.16	5.7	5.72
G	5	7.27	6.6	5.47	5.39	7.27	5.68	5.63
G	6	6.58	6.82	5.66	5.52	6.58	5.81	5.7
P	1	-	-	5.56	5.55	7.78	5.9	5.82
P	2	-	-	5.53	5.47	7.63	5.8	5.82
P	3	-	-	5.58	5.53	7.8	5.96	5.93
P	4	-	-	5.52	5.57	6.35	6.13	6.3
P	5	-	-	5.66	5.58	7.4	6.13	6.1
P	6	-	-	5.64	5.79	7.83	6.14	5.9

B.2 Temperature measurements of muscle after slaughter

Muscle	Carcasses	Temp - 90m	Temp - 3h	Temp - 24h	Temp - 48h	Temp -45m	Temp - 24h	Temp - 48hrs
ST	1	19.76	14.1	5.65	3.8	17.5	5.69	5.1
ST	2	19.4	10.7	5.59	3.8	22.4	5.62	8.5
ST	3	20.3	12.2	5.67	3.7	22.9	5.52	6.6
ST	4	20.1	11.3	5.48	3.9	24.6	5.53	7.8
ST	5	21.1	17.1	5.63	4	24.6	5.59	5.8
ST	6	15.6	7.2	5.68	3.8	19.8	5.67	7.2
SM	1	-	-	-	-	17.3	4.9	6.6
SM	2	-	-	-	-	15.7	5.3	9.9
SM	3	-	-	-	-	19.4	6.7	9.4
SM	4	-	-	-	-	20.2	5.7	8.4
SM	5	-	-	-	-	16.3	11.8	9.2
SM	6	-	-	-	-	17.4	6.8	9
LD	1	26	18.3	4.5	3.8	22.1	5.6	6.4
LD	2	21	12.4	3.6	3.6	23	5.2	5.7

LD	3	29.5	17.7	3.8	3.7	21.4	5.4	5.8
LD	4	27.1	11.56	3.8	3.8	29.6	6.4	5.3
LD	5	24.5	14.6	4.7	3.8	24.7	5.5	5.9
LD	6	16.9	8.7	3.9	3.7	22.4	5.9	7
G	1	16.9	17.7	3.9	4.6	15.7	5.2	5.9
G	2	23.5	12.4	3.6	3.5	20.1	7.2	10.1
G	3	28.4	11.4	3.7	3.7	19.1	6.3	9.5
G	4	21.7	11.9	3.7	3.6	19.6	8.2	6
G	5	23.2	16.4	4.2	3.9	18.8	5.9	8.6
G	6	22.3	11.8	3.8	3.6	22.3	8.9	10.1
P	1	-	-	3.3	3.2	16.8	10.2	10.7
P	2	-	-	3.5	3.3	16.3	10.8	12.6
P	3	-	-	3.7	3.5	16.2	8.6	13.5
P	4	-	-	4.7	3.4	17.7	10.6	7.4
P	5	-	-	3.7	3.4	16.9	10.2	10.7
P	6	-	-	3.7	3.5	17.8	10.8	9.4

B.3. **Colour measurements of the muscles on the carcasses**

		Muscles off the carcasses			**Muscles on the carcasses**		
Muscle	Carcasses	L*	a*	b*	L*	a*	b*
		22.67	11.21	-1.71	36.74	24.6	6.64
		35.8	15.57	3.26	35.02	19.91	0.72
		35.96	18.89	1.46	38.22	15.83	0.47
		32.27	17.59	1.77	35.41	20.94	1.3
		36.6	14.41	0.03	33.89	16.39	-2.74
LD	1	34.86	17.69	2.18	40.63	17.22	2.18

		39.33	16.02	3.2	31.51	21.82	1.46
		40.24	16.74	5.25	32.48	20.45	0.2
		38.55	14.7	2.63	33.59	22.88	2.61
		40.42	12.95	1.81	33.75	18.44	-0.3
		38.11	17.2	3.03	36.38	18.68	1.1
	2	38.42	16.01	4.24	32.15	18.2	-1.55
		33.61	15.67	3.04	29.87	22.35	0.24
		33.41	12.39	-1.19	32.42	25.17	4.18
		37.65	13.62	2.76	30.25	21.45	0.97
		37.03	15.6	4.28	32.02	23.07	2.67
		33.45	14.14	2.88	31.51	20.99	1.17
	3	35.29	18.12	4.17	33.96	21.92	2.85
		35.71	13.45	-0.31	31.26	19.49	-0.47
		34.46	20.16	2.9	33.59	18.77	0.14
		34.81	14.73	0.59	34.41	16.37	-0.98
		31.35	19.06	-1.09	38.32	15.78	1.69
		43.31	11.02	4.01	30.84	19.78	-1.66
	4	33.25	18.17	1.62	32.63	20.78	0.72
		39.61	15.2	0.4	38.15	20.04	5.08
		37.48	17.51	2.97	36.78	18.5	2.53
		50.4	9.4	0	33.6	18.11	-0.32
		36.39	14.61	2.73	36.68	18.86	1.65
		43.03	13.18	1.96	40.66	19.02	4.23
	5	36.8	18.03	1.07	35.56	19.47	3.07
		36.53	15.69	-0.14	37.73	18.88	3.05
		40.81	12.65	1.45	34.93	18.27	1.13
		36.72	15.55	3.94	35.39	18.89	2.93
		47.38	11.1	0.11	33.42	21.76	2.97
		33.48	18.82	-1.42	32.58	19.94	0.83
	6	51.06	11.08	3.86	34.38	18.28	-0.07

		37.42	20.03	4.45			
		45.52	16.55	7.55			
		38.55	22.96	4.23	-	-	-
		41.64	17.43	-0.18	-	-	-
		37.68	17.31	-2.46	-	-	-
SM	1	41.3	15.86	1.85	-	-	-
		33.04	19.04	-2.14	-	-	-
		33.97	22.01	-0.29	-	-	-
		39.69	14.45	0.19	-	-	-
		33.74	18.76	-1.48	-	-	-
		33.33	21.39	-0.68	-	-	-
	2	32.98	19.89	-1.85	-	-	-
		31.16	21.43	-2.75	-	-	-
		30.11	23.8	-1.8	-	-	-
		31.85	21.61	-1.24	-	-	-
		30.19	22.78	-0.64	-	-	-
		30.99	22.44	-1.33	-	-	-
	3	32.26	18.37	-2.32	-	-	-
		32.55	19.08	-2.16	-	-	-
		33.16	20.58	-1.28	-	-	-
		33.81	20.13	-1.35	-	-	-
		32.63	19.28	-1.46	-	-	-
		34.28	19.8	-1.02	-	-	-
	4	33.6	21.45	-0.65	-	-	-
		37.44	16.13	0.11	-	-	-
		36.97	21.41	1.34	-	-	-
		54.4	12.99	6.58	-	-	-
		40.21	14.48	0.68	-	-	-
		55.82	9.88	4.18	-	-	-
	5	36.52	18.61	-1.03	-	-	-

		35.15	15.81	-1.54	-	-	-
		34.37	16.16	-1.82	-	-	-
		35.49	11.86	-2.67	-	-	-
		34.58	16.44	-1.25	-	-	-
		31.97	16.64	-2.41	-	-	-
	6	33.36	17.61	-1.39	-	-	-
		39.92	17.67	3.96	35.09	16.33	-3.51
		43.27	11.16	0.45	38.26	11.91	-3.68
		36.22	17.98	1.36	36.12	14.6	-3.87
		41.75	17.94	3.2	31.05	18.3	-2.02
		38.4	16.81	3.02	29.14	18.12	-3.36
	1	40.89	12.86	-2.09	31.18	17.22	-2.38
		41.42	14.55	1.49	35.74	14.79	-4
		39.33	15.3	-1.82	30.09	18.64	-3.74
		42.25	13.63	-0.47	35.57	15.72	0.41
		50.09	8.82	0.47	36.72	14.1	-1.82
		42.89	11.97	-1.17	35.03	15.94	-2.96
	2	37.41	16.69	-1.19	35.05	16.3	-1.48
		44.72	10.48	-2.41	28.89	12.46	-5.08
		34.67	19.32	1.54	38.43	9.96	-3.38
		36.16	15.36	-2.23	39.28	12.35	-0.89
		38.48	17.75	3.58	29.99	13.24	-4.37
		34.65	19.45	0.99	34.48	10.93	-3.58
	3	36.54	19.63	3.68	30.82	11.73	-5.44
		38.33	15.34	1.95	31.76	18.13	-1.49
		37.3	16.27	-0.95	37.82	14.56	1.27
		41.82	11.92	1.37	33.19	19.87	1.29
		36.52	16.72	-3.17	36.88	15.19	1.3
		37.39	18.42	3.56	31.56	19.13	-0.8
G	4	37.52	16.2	-0.99	37.04	16.5	0.99

		34.85	18.24	-1.45	34.42	17.46	0.36
		37.5	18.76	1.75	44.77	10.11	-1.87
		38.15	18.56	4.55	35.02	14.69	-5.15
		36.33	15.98	-2.78	32.8	17.26	-2.44
		37.48	20.25	3.56	34.7	18.01	0.94
	5	44.74	22.53	9.19	36.26	14.61	-2.61
		38.92	16.52	0.11	34.64	17.28	0.47
		38.51	15.88	-1.45	35.48	13.63	-4.15
		44.13	14.48	1.65	34.69	19.08	-0.04
		39.46	15.66	3.28	31.69	17.52	-2.18
		38.58	17.34	3.2	34.07	18.39	1.32
	6	39.01	17.45	4.46	46.73	9.76	-1.78
		37.93	18.08	-1.29	-	-	-
		42.65	14.02	-1.14	-	-	-
		46.37	22.6	7.95	-	-	-
		52.58	10.57	2.28	-	-	-
		39.9	12.71	-0.74	-	-	-
	1	45.64	14.46	1.65	-	-	-
		31.46	15.1	-4.11	-	-	-
		34.29	12.76	-3.4	-	-	-
		29.06	17.81	-3.91	-	-	-
		30.94	15.62	-3.65	-	-	-
		29.5	17.83	-3.42	-	-	-
	2	30.07	18.39	-3.15	-	-	-
		35.76	16.69	0.95	-	-	-
		36.85	21.75	0.99	-	-	-
		54.05	13.78	2.88	-	-	-
		33.41	19.62	0.23	-	-	-
		44.93	17.52	1.04	-	-	-
P	3	36.22	21.77	1.65	-	-	-

		37.36	17.59	4.36	34.39	17.67	0.5
		36.69	20.61	4.33	45.98	9.75	-0.07
		34.65	19.45	2.34	34.89	15.19	-0.85
		34.6	18.52	0.32	37.25	15.57	-0.35
		40.42	16.21	1.7	37.22	19.12	2.86
	1	36.89	18.68	3.07	43.48	11.7	-0.75
		39.72	16.33	0.04	78.62	4.71	2.67
		38.75	16.33	0	45.47	15.13	4.01
		36.36	16.33	-2.05	52.91	9.71	3.47
		38.74	15.44	1.4	45.11	12.28	0.9
		41.31	13.18	-0.59	42.36	14.78	0.86
	2	38.53	16.97	2.06	34.6	16.53	-3.51
		33.47	18.22	-2.29	54.02	7.99	0.08
		39.86	17.58	0.68	42.53	11.05	-0.82
		38.75	16.49	2.01	32.87	16.23	-3.65
		39.65	15.63	0.81	38.53	13.17	0.33
		39.46	18.36	3.98	30.88	18.12	-3.13
	3	40.76	18.34	3.83	34.77	15.12	-2.64
		39.36	17.71	2.82	32.72	21	-0.19
		40.57	17.39	4.07	35.17	20.31	0.73
		36.5	18.08	0.51	33.23	20.72	-0.94
		42.57	14.98	6.33	45.99	10.44	-0.11
		43.94	11.87	-1.45	38.87	19.96	3.4
	4	42.56	15.46	1.34	36.42	19.44	1.72
		41.29	15.6	2.56	35.99	21.61	4.11
		45.43	13.54	2.73	34.17	20.41	1.04
		41.27	15.99	2.78	34.42	19.83	0.57
		40.46	16.59	2.11	39.36	18.16	1.8
		42.46	15.82	4.55	43.06	16	2.63
ST	5	40.02	16.97	2.28	37.34	19.2	1.75

		41.67	13.67	0	33.91	18.1	-0.72
		43.68	12.06	-1.48	31.26	16.72	-2.89
		42.45	13.6	2.78	40.38	18.85	3.68
		42.48	13.48	1.64	37.77	15.79	-0.13
		42.64	16.04	7.12	35.25	18.5	-0.65
	6	40.94	13.77	-0.07	34.1	18.61	0.03

B.4 **Cooking loss calculated on muscles aged 2 days**

	Muscles on the carcasses			**Muscles off the carcasses**		
Muscle	Weight before cooking	Weight after cooking	Weight lost (%)	Weight before cooking	Weight after cooking	Weight lost (%)
	68.78	52.64	16.14	38.4	29.22	9.18
	64.63	46.7	17.93	68.2	52.03	16.17
	54.84	43.73	11.11	77.69	57.72	19.97
	80.43	59.36	21.07	73.32	56.79	16.53
	84.44	60.11	24.33	52.65	36.88	15.77
ST	40.77	31.08	9.69	47.33	34.82	12.51
	62.53	47.82	14.71	68.36	51.95	16.41
	76.3	60.19	16.11	88.5	71.59	16.91
	88.01	62.46	25.55	72.84	56.33	16.51
	99.18	72.01	27.17	85.34	65.96	19.38
	73.3	58.1	15.2	71.18	52.4	18.78
LD	63.9	50.28	13.62	71.41	53.46	17.95
	37.78	29.31	8.47	65.27	48.19	17.08
	38.62	31.65	6.97	30.6	24.64	5.96
G	46.8	38.48	8.32	37.51	34.33	3.18

	45.93	37.66	8.27	29.66	23.4	6.26
	41.46	30.79	10.67	25.52	17.75	7.77
	63.98	52.91	11.07	26.9	21.1	5.8

B.5. **Cooking Loss calculated on muscles aged 7 days**

		Muscles on the carcasses			**Muscles off the carcasses**		
Muscle	Carcasses	Weight before cooking	Weight after cooking	Weight lost	Weight before cooking	Weight after cooking	Weight lost
	1	38.13	30.04	8.09	62.58	45.18	17.4
	2	41.11	30.53	10.58	75.87	55.82	20.05
	3	54.98	40.96	14.02	42.43	32.75	9.68
	4	60.14	46.6	13.54	63.77	44.95	18.82
	5	59.18	44.02	15.16	59.06	42.18	16.88
ST	6	46.39	34.77	11.62	43.56	29.55	14.01
	1	81.75	58.82	22.93	96.12	69.12	27
	2	74.15	54.9	19.25	57.45	45.35	12.1
	3	59.39	43.32	16.07	73.15	56.68	16.47
	4	87.3	65.91	21.39	71.32	52.62	18.7
	5	75.38	51.35	24.03	61.47	46.18	15.29
LD	6	70.74	49.2	21.54	57.48	41.1	16.38
	1	66.63	49.73	16.9	23.65	17.9	5.75
	2	17.4	13.45	3.95	26.65	17.55	9.1
	3	30.16	22.78	7.38	41.3	31.5	9.8
	4	13.31	9.01	4.3	23.32	17.45	5.87
	5	20.34	13.17	7.17	23.37	16.61	6.76
G	6	23.85	16.66	7.19	35.1	29.5	5.6

B.6 Shear force calculated on muscles aged 2nd days

Muscle	Carcasses	Number of Trial	On the carcasses	Off the carcasses
LD	1	1	40	30
		2	36	30
		3	30	35
		4	34	35
		5	34	30
		6	35	38
		7	38	38
		8	33	34
		9	45	34
		10	38	33
	2	1	39	35
		2	39	40
		3	34	40
		4	36	35
		5	33	37
		6	35	45
		7	33	-
		8	33	-
		9	32	-
		10	30	-
	3	1	33	30
		2	33	35
		3	32	30
		4	37	30
		5	31	30
		6	35	28
		7	34	34

		8	30	30
		9	36	31
		10	34	31
	4	1	60	32
		2	55	35
		3	50	30
		4	60	33
		5	65	39
		6	50	35
		7	55	30
		8	40	35
		9	53	30
		10	50	34
	5	1	35	30
		2	50	39
		3	50	39
		4	42	36
		5	42	30
		6	55	32
		7	50	35
		8	47	30
		9	50	35
		10	53	30
	6	1	87	65
		2	77	60
		3	55	45
		4	59	54
		5	72	65
		6	55	62
		7	65	65

		8	68	60
		9	68	55
		10	75	30
ST	1	1	40	55
		2	40	45
		3	56	60
		4	52	50
		5	60	40
		6	35	50
		7	39	-
		8	42	-
		9	-	-
		10	-	-
	2	1	39	52
		2	40	40
		3	45	43
		4	40	46
		5	42	57
		6	40	55
		7	40	43
		8	39	-
		9	39	-
		10	-	-
	3	1	55	50
		2	47	50
		3	40	40
		4	47	40
		5	45	42
		6	55	40
		7	55	45

		8	45	-
		9	47	-
		10	50	-
	4	1	40	47
		2	40	55
		3	37	45
		4	37	56
		5	37	50
		6	36	55
		7	36	50
		8	36	55
		9	42	48
		10	-	-
	5	1	39	68
		2	39	68
		3	39	70
		4	43	78
		5	40	65
		6	39	74
		7	42	
		8	43	
		9	42	
		10	40	
	6	1	45	55
		2	44	52
		3	52	50
		4	55	50
		5	55	55
		6	55	58
		7	-	50

		8	-	55
		9	-	55
		10	-	50
G	1	1	57	80
		2	67	69
		3	58	72
		4	55	75
		5	68	55
		6	57	55
		7	72	69
		8	-	62
		9	-	55
		10	-	55
	2	1	54	65
		2	54	55
		3	53	50
		4	56	55
		5	44	57
		6	35	55
		7	52	50
		8	50	-
		9	54	-
		10	55	-
	3	1	47	58
		2	52	53
		3	43	65
		4	54	55
		5	53	45
		6	54	43
		7	50	55

		8	54	-
		9	52	-
		10	52	-
	4	1	60	55
		2	60	78
		3	70	40
		4	67	55
		5	76	60
		6	90	60
		7	57	-
		8	60	-
		9	58	-
		10	54	-
	5	1	65	70
		2	60	70
		3	70	65
		4	74	55
		5	62	65
		6	62	-
		7	66	-
		8	53	-
		9	52	-
		10	67	-
	6	1	65	51
		2	58	80
		3	53	65
		4	55	42
		5	57	-
		6	56	-
		7	47	-

		8	60	-
		9	-	-
		10	-	-

B.7 **Shear force calculated on muscles aged 7 days**

Muscle	Carcasses	Number of Trial	On the carcasses	Off the carcasses
LD	1	1	32	25
		2	37	20
		3	25	30
		4	45	30
		5	40	25
		6	45	25
		7	45	20
		8	38	20
		9	-	25
		10	-	-
	2	1	30	35
		2	27	30
		3	32	30
		4	27	30
		5	32	35
		6	36	25
		7	35	32
		8	32	25
		9	-	-
		10	-	-
	3	1	30	25
		2	28	30

		3	30	20
		4	28	30
		5	25	25
		6	25	25
		7	27	30
		8	28	-
		9	-	-
		10	-	-
	4	1	25	30
		2	23	40
		3	23	30
		4	24	32
		5	25	35
		6	25	30
		7	22	35
		8	23	30
		9	22	-
		10	-	-
	5	1	25	35
		2	28	32
		3	26	35
		4	25	30
		5	25	35
		6	27	35
		7	26	25
		8	25	25
		9	-	30
		10	-	-
	6	1	60	43
		2	50	42

		3	50	45
		4	60	50
		5	65	45
		6	55	45
		7	55	47
		8	-	-
		9	-	-
		10	-	-
ST	1	1	35	35
		2	50	35
		3	35	30
		4	40	40
		5	42	30
		6	-	30
		7	-	-
		8	-	-
		9	-	-
		10	-	-
	2	1	30	35
		2	35	45
		3	30	37
		4	40	40
		5	30	30
		6	-	30
		7	-	30
		8	-	30
		9	-	30
		10	-	-
	3	1	35	30
		2	35	30

		3	33	40
		4	35	35
		5	38	35
		6	40	30
		7	35	27
		8	-	-
		9	-	-
		10	-	-
	4	1	35	30
		2	33	27
		3	35	35
		4	40	30
		5	42	30
		6	30	30
		7	25	30
		8	40	35
		9	40	-
		10	-	-
	5	1	37	35
		2	40	25
		3	35	30
		4	35	30
		5	35	25
		6	35	30
		7	40	30
		8	33	-
		9	-	-
		10	-	-
	6	1	40	50
		2	45	45

		3	50	40
		4	55	40
		5	40	55
		6	42	-
		7	45	-
		8	38	-
		9	50	-
		10	-	-
G	1	1	30	40
		2	35	40
		3	30	37
		4	30	37
		5	32	42
		6	35	42
		7	33	35
		8	35	37
		9	37	-
		10	-	-
	2	1	35	45
		2	35	41
		3	35	38
		4	35	38
		5	-	50
		6	-	45
		7	-	-
		8	-	-
		9	-	-
		10	-	-
	3	1	50	40
		2	55	40

		3	45	40
		4	45	45
		5	45	45
		6	-	30
		7	-	42
		8	-	42
		9	-	40
		10	-	-
	4	1	30	30
		2	35	30
		3	30	40
		4	30	40
		5	-	28
		6	-	37
		7	-	30
		8	-	-
		9	-	-
		10	-	-
	5	1	55	42
		2	45	45
		3	-	45
		4	-	45
		5	-	45
		6	-	44
		7	-	-
		8	-	-
		9	-	-
		10	-	-
	6	1	50	42
		2	60	45

		3	45	43
		4	40	40
		5	52	42
		6	45	45
		7	-	37
		8	-	42
		9	-	-
		10	-	-

B.8 Determination of muscle glycogen

B.8.1. Concentration of the hydrolysed muscle samples from six biological replicates

Muscle	1	2	3	4	5	6	Mean	SD
LD	4.249263	15.38353	0	28.64436	27.91799	8.467292	14.11041	12.09708
ST	5.471928	29.67469	13.89845	54.05505	21.3448	12.52036	22.82754	17.38634
SM	6.173458	8.104333	26.07537		21.3448	12.52036	14.84366	8.579115
P	14.89581	39.91033		85.29165	20.55024		40.16201	31.93614
G	9.190034		40.83125	21.27971	27.24783	10.77473	21.86471	12.96809

B.8.2. Concentration of the non-hydrolysed muscle samples from six biological replicates

Muscle	1	2	3	4	5	6	Mean	SD
LD	2.851215	3.510987	2.09374	4.626168	1.769704	2.624459	2.9	1.035173
ST	4.50649	4.770399	6.166563	4.965155	2.066661	4.630927	4.5	1.341891
SM	2.649108	3.303869	3.86511	3.973121	2.191062	1.725562	3.0	0.913804
P	1.312862	1.105744	4.506526	1.749768	1.300191	1.484786	1.9	1.290248
G	6.544266	2.435309	1.974098	2.725185	1.444657	4.245685	3.2	1.879738

B.8.2. Concentration of the glycogen in muscle samples for the six biological replicates

Muscle	1	2	3	4	5	6	Mean	SD
LD	1.398048	11.87255		24.01819	26.14828	5.842833	13.85598	10.92836
ST	0.965438	24.90429	7.731883	49.08989	10.09855	16.90248	18.28209	17.16404
SM	3.524349	4.800465	22.21026		19.15374	10.79479	12.09672	8.374083
P	13.58294	38.80459		83.54189	19.25005		38.79487	31.72768
G	2.645768		38.85715	18.55452	25.80317	6.529045	18.47793	14.68342