The effect of biochemical parameters on tenderness in beef

Biokjemiske parametere som påvirker mørhet i storfekjøtt

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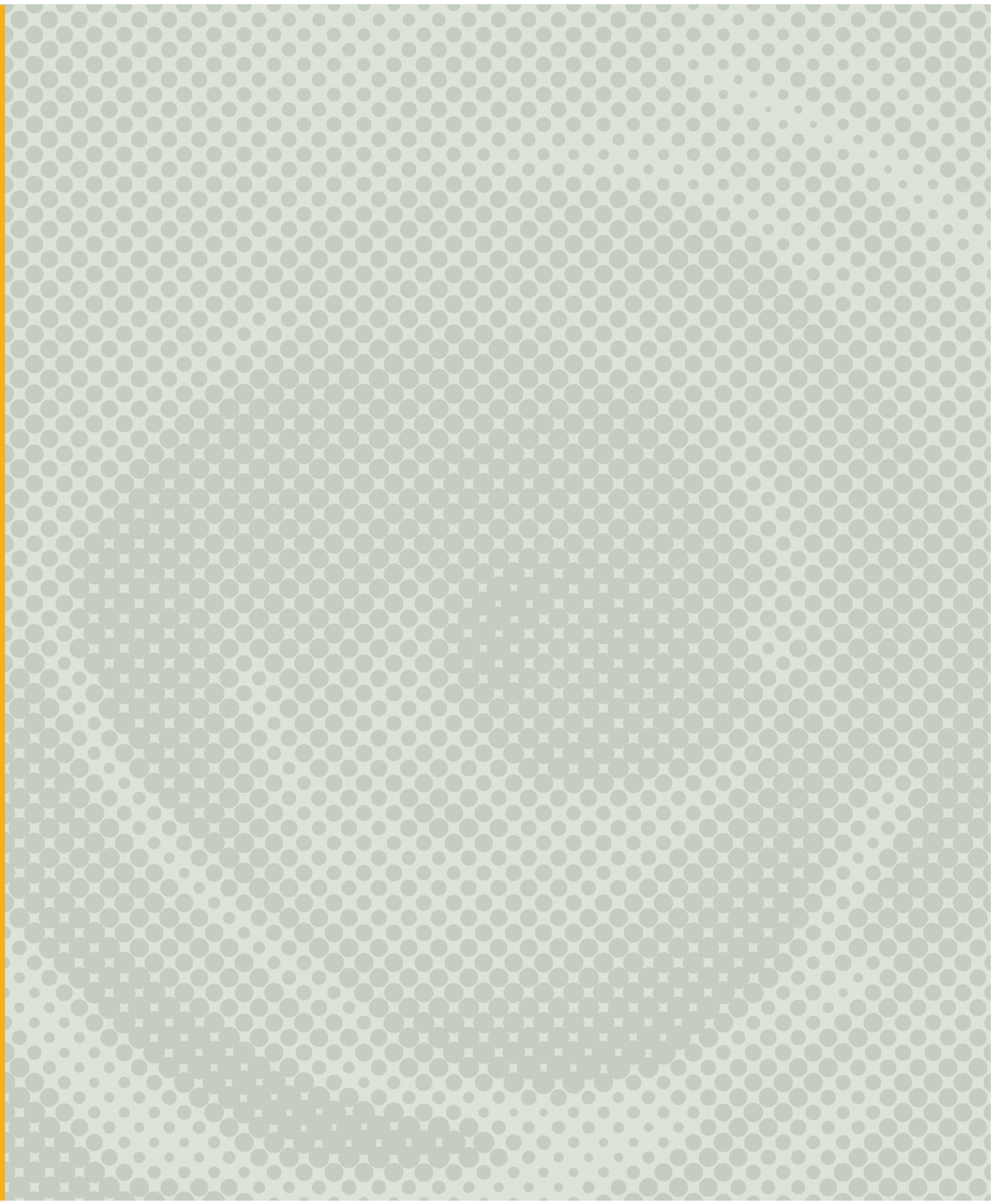

Acknowledgments

After five years of studying food, the time as student is soon to be finished. My interest for meat started summer 2008 when I worked at the laboratory at Nortura Rudshøgda. In collaboration with Laila Aas the subject “The effect of biochemical parameters on tenderness in beef” was prepared. This thesis has been challenging, fun, frustrating and very educational to write.

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Abstract

The tenderness of beef is determined by the degree of myofibrillar toughness and content of insoluble collagen. The myofibrils and connective tissue and their degradation is largely determined by the amount of proteases in the muscle. These includes, respectively, of calpains and metalloproteinases. The design of the experiment makes it possible to study the relative importance of myofibrils and the connective tissues role in tenderness of beef, one topic yet to be resolved in meat science.

The samples were withdrawn from 550 NRF bulls from 25 elite sires in a period from fall 2008 to winter 2012 and slaughtered at Nortura Rudshøgda. The samples analyzed in this thesis were selected on the basis of their values from Warner- Bratzler's tenderness measurements. Preliminary heritability (h^2) of Warner-Bratzler was calculated to be about 0.35 and means that there is a significant variation between the tenderness of the families.

The hydroxyproline content, intramuscular fat and calpain activity was in general more or less the same for all of the samples. The one parameter that stood out was MMP-2 and the greater activity in the group "tender with low standard deviation".

Sammendrag- Norsk

Mørhet i storfekjøtt er bestemt av graden myofibrillær seighet og innhold av uløselig bindevev. Myofibrillenes og bindevevest grad av nedbrytning post mortem (mørning) avgjøres i stor grad av mengden proteaser i musklene. Disse omfattes henholdsvis av calpainer og metalloproteinaser. Forsøksdesignet gjorde det mulig å studere den relative betydningen av myofibrillenes og bindevevet rolle for mørhet i storfekjøtt, et forstsatt uavklart spørsmål innen kjøttforskning.

I prosjektet ble det slaktet 550 NRF okser fra 25 elite fedre fra og med høsten 2008 til vinteren 2012. Alle oksene ble slaktet ved Nortura Rudshøgda. I denne masteroppgave ble prøvene valgt ut på bakgrunn av Warner-Bratzler målingene. Foreløpig nedarvingsgrad, h^2, for Warner- Bratzler ble beregnet til å være ca 0,35, og betyr at det er en betydelig variasjon mellom mørheten til familiene

Innholdet av hydroxyproline, intramuskulært fett og calpain aktivitet var mer eller mindre likt i alle prøvene. Den parameteren som skilte seg mest ut var MMP-2. Den høyeste aktiviteten ble målt i gruppen ”mør med lavt standardavvik”.

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1 Introduction

Meat has been a major part of the human diet throughout history. Meat has a good digestibility and a balanced composition of essential amino acids and is a great source of proteins, vitamins and minerals. In a relative small serving the daily requirement of protein and amino acids can be reached (Kim, 2001).

The tenderness in meat is ranked as the most important factor by the meat eating public (Veiseth et al., 2003, Koohmaraie & Geesink, 2006). A study done by Wheeler, Shackelford and Koohmaraie (2004) showed that untrained consumers could accurately and repeatedly detect differences in meat tenderness under controlled conditions. Due to inconsistency of meat tenderness and higher quality demands from the consumer, the adoption of new technologies in the industry has accelerated (Koohmaraie et al., 2002).

When muscle turns into meat, there are multiple biochemical and physical changes occurring in the muscle. The muscle is flexible early in the slaughter process while at rigor it shortens and gets rigid (Greaser, 2001). 8-15 hours post mortem the animal will go into rigor mortis due to lack of energy and hence leakage of calcium ions that amplifies the contraction of myofibrils. The extent of this contraction is dependant on many things, such as pre-slaughter settings (Archile- Contreras, Mandell & Purslow, 2010), and the properties in the muscle itself (Ree et al., 2004). In total the toughness in meat is dependant of connective tissue, intramuscular fat and sarcomeres length (Kemp et al. 2009). It is believed that the most important system for degradation of the myofibrils are the cytoplasm located protease enzymes calpains and their inhibitor, calpastatin (Veiseth & Koohmaraie, 2000). While matrix metalloproteinases (MMPs) plays a part in muscle growth, atrophy and remodelling of tissue in live animals (Balcerzak et al., 2001).

Norwegian Red (NRF) is the largest meat producing breed in Norway. The breed was established in 1935 through careful breeding of traditional Norwegian cattle and imported breeds (Solem, 2007, Nyhus, 1990). The breeding goal for NRF is determined by the members of Geno, and is affected by the differences in the price of meat and milk, and price

of dry matter in milk. Today's main breeding goals decreasingly are health and fertility, milk production, functional traits and beef (Geno, 2009).

Due to great differences in toughness in beef even after careful handling of the animals before slaughter, low voltage stimulation and tender stretch, the project "Mer og Bedre Biff" (More and better beef) was started. It was a continued project from the completed project "Arv mør" from 2007. To combine the results from Warner- Bratzler measurements, pH, colour, IMF, calcium, iron and enzyme activity tests, the aim of this thesis was to determine whether there were differences between MMP-2 activity of different groups and if it affects the tenderness in beef. Hopefully will the results give an indication on what can affect tenderness and in the long run be a part of a breeding plan for more tender beef from Norwegian Red Cattle (NRF).

2 Theory

2.1 From muscle to meat

To produce good food, the raw products have to be good. To implement this into the meat industry can be done by only slaughtering healthy and low-stressed animal. The animal welfare and handling prior to slaughtering is of importance for the consumer, the meat quality and most of all; it is the right thing do (Grandin, 2001).

To limit the occurrence of stress in the animals, the transportation time in Norway is set at maximum 8 hours Lovdata (2005). Stress can also be inflicted by extreme temperatures (both low and high), improper handling and shortage of food and water over a longer period (Schwartzkopf-Genswein et al., 2012). An experiment done by Villarroel et al. (2002) found that the transportation time of 3 hours had a positive significant effect ($P<0.05$) on tenderness and overall liking compared to transportation time of 30 minutes and 6 hours. When the animals arrive at the slaughterhouse, a combination of quiet handling and proper stunning will contribute to improve meat quality, better reputation among the public as well as it is the right thing to do. A high focus on ante mortem handling will also increase the safety of the employees by reducing injuries. (Grandin, 2001).

2.1.1 Slaughter process

The most common **stunning** method for cattle is captive bolt gun. To achieve a good stunning the animal must become completely insensible. The captive bolt gun has to be placed in the middle of the forehead at the cross –section between the eyes and horns (Fig 2.1). After stunning, the head usually goes into spasms for a few seconds. To assess insensibility the animal should drop instantly to the floor with the tongue limp and floppy and the back is straighten. If the tongue is stiff and curled and the eyes are blinking, indicates that the animal is likely to be conscious. Poor stunning can lead to the cosmetic defect of blood-splash such as red spots or bruises, in meat. A quick sticking will reduce blood- splash by reducing blood pressure and prevent small capillaries from bursting (Grandin, 2001).

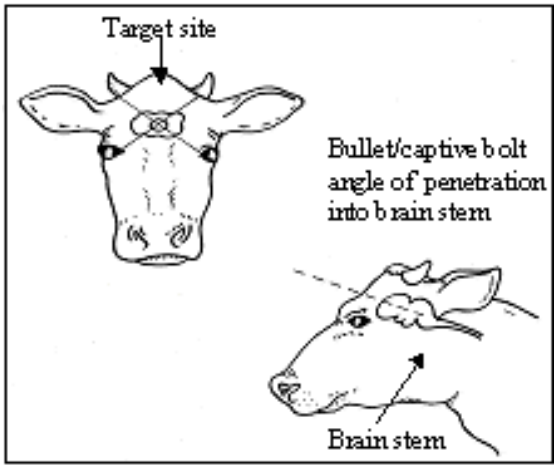


Figure 2.1: Captive-Bolt stunning of cattle (Omafra, 2011).

Sticking of the animal should be done within 15 seconds after stunning (Grandin, 2001). Sticking is executed by cutting the carotid arteries and the jugular vein, and by keeping the animal unconscious but alive, the heart and respiratory function contribute to eliminate blood from the carcases (Pérez-Chabela & Legarreta, 2001).

After bleeding, the carcasses can be **electrical stimulated (ES)** to provoke rigor mortis to occur at an earlier state and thus prevent cold shortening. ES must be done immediately after slaughter to have an effect. When the muscles contract, the stored energy is depleted and pH drops quickly. Low-voltage electrical stimulation is preferred over high-voltage electrical stimulation due to lover installation cost and the safety of the employee (Hollung et al. 2007, Hemmer, 1997).

When the hide is removed and the animal is split and trimmed, the carcass is weighed. The grading is based on fat, colour, gender and age by **EUROP** standard. For muscles fullness the scale ranks from 1-15 where 1 is poor (P-) and 15 excellent (E+). Fat classification ranks from 1-15 where 1 is fat free (1-) and 15 is extremely high in fat (5+). The samples used in this thesis were hot-boned after classification.

Convention suspending of the carcasses is by Achilles tendons, and leaves the most valuable muscles free to contract. The more they contract during rigor mortis, the tougher the meat. To prevent this, a suspending by the pelvic bone can be done. This method is called **Tender-stretch** and reminiscent the live animal posture. It is a cheap and effective method to increase palatability in beef. Two of the main disadvantages are increase labour by re-hanging before chilling and a decrease in chilling capacity (Thompson, 2002).

Hot- boning happens when a muscle is cut of the carcasses before rigor mortis occurs. This requires good hygiene and there is a risk of cold shortening of the muscle when it is cut free from the bones and is no longer stretched by the skeleton. There are two main benefits of hot-boning. Firstly; hot- boned meat gains a good quality on both cuts and production meet. Secondly; there is a financially gain due to lower requirement of both cooling and cold storage without bones. After the muscles are cut free the pieces are vacuumed and slowly cooled so rigor mortis occurs before it reaches a temperature below 10^{o}C. Electrical stimulation of the carcasses used for hot- bone can be done, but is not necessary Hemmer (1997).

Due to rigor mortis contractions in the muscles, the carcass can not be chilled too fast, even after ES. The minimum impact on rigor mortis is at temperature around 15-20^{o}C. To ensure optimal condition of the carcasses, it should be cooled to this temperature and kept there until rigor mortis arises. After rigor mortis, the carcasses can be cooled down to cooling room temperature. This type of cooling method is called **conditioning** Hemmer (1997). This technique can manipulate post mortem glycolysis and prevent cold shortening in the muscle. According to Hollung et al. (2007) this method is not a standard procedure in all slaughterhouses due to increased time at a higher temperature and thus less hygienic.

Cold shortening of the muscle occurs when the temperature drops below 10^{o}C before the muscle enters rigor. Adenosine thriphosphate (ATP) is still present and ultimate pH is not achieved. The muscular contractions are caused by the increased intracellular concentration of calcium ions that generates the cross-binding of actin and myosin. The extent of cold shortening is dependent of ATP concentration and metabolism by- products. If the ATP concentration is high, the contractions increases, while metabolism by- products such as inorganic phosphate and drop in pH can inhibit cold shortening. It can be difficult to cool down deeper muscles in the carcass and the rate of glycolysis will be high and the pH will drop fast. There is also a risk of cold shortening on the surface of the carcasses if exposed to aggressive chilling (Young et al., 2001). Van Moeseke et al. (2001) tested a hypothesis that meat safety would increase by lowering the temperature of the muscle rapidly to create an early activation of calpain due to early supply of free calcium and high pH and thus provide an acceptable tenderness. This method was called **very fast chilling** (VFC). Their results correlated with the already proven cold-shortening theory, and their meat samples were clearly tougher than the ones chilled by conventional method.

2.2 Meat quality

In the United States the latest National Beef Tenderness Survey showed an increase of 18% in consistency of beef tenderness compared to the same survey in 1999, but as Purslow, Archile-Contreras and Cha (2011) emphasis; there is still room for improvement. The length of the sarcomeres, content of connective tissue and proteolysis of myofibrils and other proteins can explain some of the variations in tenderness of meat after post mortem storage (Koohmaraie et al., 2002).

Tenderness is dependant on diet, transportation, organization of the cattle at the slaughterhouse and the slaughter process including cooling and tenderization. The age of the animal will affect how tender the meat is. The older the animal, the tougher the meat in general.

2.2.1 Background toughness

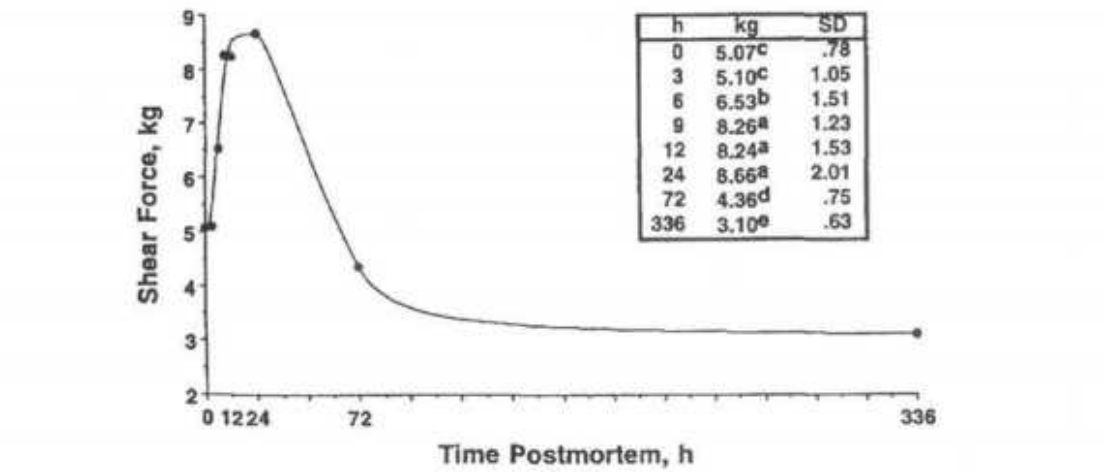
Background toughness does not change over time and are related to the connective tissue (Veiseth & Koohmaraie, 2003). It is the formation of heat stabile cross-bindings of collagen increasing with age that can explain the differences between young and older animals. The diameter of the muscle fibres can also contribute to the variation in tenderness.

2.2.2 Tenderness

A piece of meat can be divided into three categories; tough, medium and tender. This can be done either by sensory analyses or instrumental measurement. Hemmer (1997) writes that sensory analyses must be done by a trained panel that give the same score multiple times. But a study by Wheeler, Shackelford and Koohmaraie (2004) showed that also untrained consumers could accurately and repeatedly detect differences in meat tenderness under controlled conditions. Regardless the sensory analysis will include an overall experience of tenderness such as palpability and juice in addition to fibre strength. The Warner-Bratzler Shear Force (WBSF) measurement will only measure the fibre strength. In spite of this the WBSF measurements correspond well with the sensory evaluation. To measure the toughness in beef by WBSF a V- shaped knife at a set speed is moved through a gap between two fixed plates. The meat is then pushed together and either torn or cut by the knife. The sample is either circular or rectangular cross-section cut lengthwise of the fibres. The shear force is then

recorded and stated as a consistent number. A higher consistency number indicates a tougher beef (Hemmer, 1997).

After rigor mortis and shortening of the muscle, the WBSF measurements are high and indicate tough meat. There is a big difference between the first 24 hours and 72 hours tenderness (Graph 2.1).



h	kg	SD
0	5.07[c]	.78
3	5.10[c]	1.05
6	6.53[b]	1.51
9	8.26[a]	1.23
12	8.24[a]	1.53
24	8.66[a]	2.01
72	4.36[d]	.75
336	3.10[e]	.63

Graph 2.1: Various time measurements of tenderness in ovine longissimus measured by WBSF (Wheeler & Koohmaraie, 1994).

2.2.3 Colour and pH

The colour of the meat is one of the first and vital impressions of the consumer**.** Freshly cut beef have a deep purplish- red colour while faded cured pork tends to have a lighter grey colour. To control the colour of meat there are many factors to take notice of. Fortunately many of these are understood (Boles & Pegg, 2012).

Colour can be measured by a colorimeter. The most common method is called Hunter and the colour is measured in L (lightness), *a (greenness-redness) and *b (blueness- yellowness). L can vary from 0 (black) up to 100 (white) while *a and *b does not have a specific limit. A positive number of *a indicates redness, while negative indicates greenness. For *b it is positive yellowness and negative blue. Greater the values gives more intense colours, Hunterlab (2008).

The colour in meat is dependant on the water- soluble protein **myoglobin**. This protein stores oxygen for aerobic metabolism in the muscle and consists of a protein portion and a nonprotein porphyrin ring with a central **iron** atom. The colour of the meat is dependent on

the oxidation state of the iron and the compound attached to the iron portion of the molecule. The compounds can be oxygen, water or nitric oxide. The demand of oxygen varies from muscle to muscle as a result of their activity, thus the myoglobin and colour concentration also varies from muscle to muscle. If the muscles have a high concentration of myoglobin the colour of the muscle intensifies. The level of myoglobin is increased by ageing and exercise. (Boles & Pegg, 2012)

Freshly cut meat has a deep purplish- red colour. The oxygen present in air connects with the surface and binds to the iron. As a result the meat changes colour to bright cherry red while the oxygen gets oxygenated, and the pigment is called **oxymyoglobin**. This colour is the one the consumers associate with freshness. If myoglobin and oxymyoglobin losses an electron the pigment turns into a brown colour and yields **metmyoglobin**. The colour of meat can change between myoglobin, oxymyoglobin and metmyoglobin according to the storage conditions. When meat is cooked the brown pigment "denatured metmyoglobin" is formed and is not normally able to change again. This change is shown in figure 2.2.

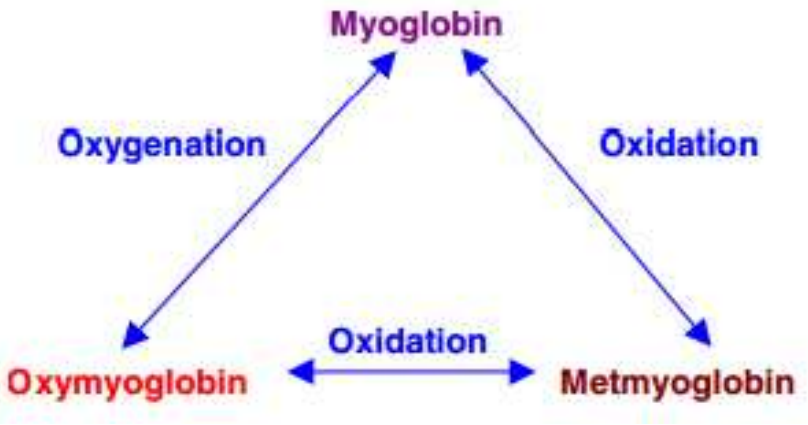


Figure 2.2: The correlation between the pigments in meat. When myoglobin oxidizes it turns into oxymyoglobin with a bright red colour. Both myoglobin and oxymyoglobin can oxidize to metmyoglobin (Boles & Pegg, 2012).

The pH will also have an effect on the colour. The pH drops from 7.0-7.2 down to pH 5.5-5.7 24 hours post mortem. If the pH drops rapidly the meat appear to be very pale and soft (PSE), hence a lower pH will result in paler meat colour. If the pH decrease is slow, the meat will be dark, firm and dry (DFD). If the pH is higher than 5.7 the colour becomes darker. In figure 2.3 the effect of ultimate pH on colour is shown (Boles & Pegg, 2012).

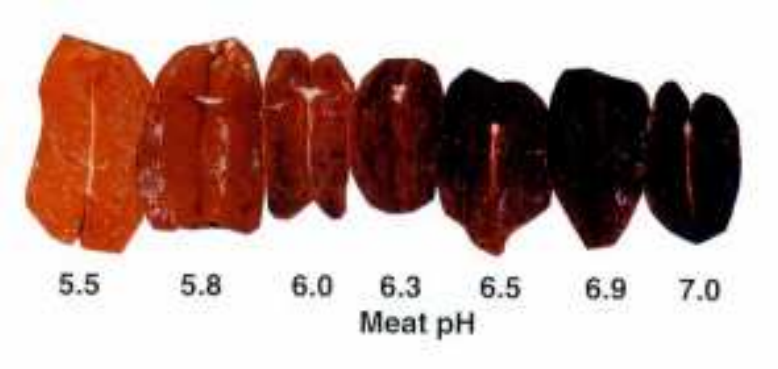


Figure 2.3: Ultimate effect of pH on colour in meat. (Boles & Pegg, 2012).

2.3.3 Intramuscular fat

Intramuscular fat (IMF) content in muscles differs between species, breeds, sex, age, feed and muscle type. The content can also be regulated by adjusting the diet. Lipids are primarily synthesized in the liver and transported to the muscle following the blood stream. By decreasing the fat intake, the IMF content can be reduced. IMF is linked to influence the taste, juiciness, flavour and tenderness positively and in some countries the visible IMF is desired while in others it is not. In the United States IMF is a part of the grading system, while it is not in EUROP. Saturated fat is linked to obesity, type-2 diabetes, cancer and other diseases and is likely to be one of the reasons why visible IMF is not desired. IMF consists of phospholipids (cell membranes), triglycerides (energy reserves) and cholesterol. Animals with high muscularity and high glycolytic activity tend to have a reduced content of IMF (Hocquette et al., 2009).

2.3 Muscle structure

The main constituent in meat is about 75% water, 19-25% protein, 1-2% minerals and glycogen. Their composition varies from species, muscles and cut (Horquette et al., 2009).

Muscles are responsible for locomotion of the skeleton, posture control and have specialized functions as respiration, swallowing and peristalsis. They are divided into two main groups; striated and nonstriated. The **nonstriated** muscles have similar function as the striated ones but have different structure. They are found in the linings of the gastrointestinal tract and the circulatory system.

Striated muscles are either cardiac or skeletal muscles. The **cardiac** muscle distributes and collects the blood throughout the body. They are highly aerobic and require higher

concentration of oxygen than skeletal muscles. The **skeletal** muscles are associated with the skeleton and lay either next to a bone or attached to various bones. Skeletal muscles can contract and relax and are responsible for locomotion, posture control and protecting vital organs. Depending on their function and location the muscles varies in size, shape, colour and concentration of tendons. Skeletal muscles contain muscle fibres, a complete vascular supply, a great amount of supportive connective tissue and nerves who controls the muscle fibres. They also serve as a storage depot for lipids and contain large quantities of extracellular fluid Hemmer (1997).

The muscle fibres can be divided into red and white because of their colour. **Red muscle fibres** contracts slower and usually rely on oxidative metabolism for generation of ATP. **White muscle fibres** have larger glycolytic enzyme content, they contract faster and their energy metabolism depends on glycolysis. The red and white classifications of fibres are inadequate and another system based on myosin isoforms is developed. The **red fibres** are called **type I**, while **white fibres** are divided into **type IIA, type IIB** and **type IIX**. This classification is also incomplete because of the muscle fibres are mixed even at fibre bundle level Greaser (2001).

2.3.1 Proteins in muscles

Proteins are the building blocks of nature. They have evolved to perform specific functions and are dependent on their three- dimensional structure. Proteins are linear chains of amino acids bonded by polypeptide bonds (Branden & Tooze, 1999). There are 20 different amino acids that can be linked together in different quantities and combined countless times. The digestive system hydrolyzes the protein into amino acids and rebuild protein specific to the organism (Cheftel, Duq & Lorient, 2001).

The molecular mass of proteins may vary from 5000 to many million daltons. They are complex macromolecules and consist of carbon, hydrogen, oxygen, nitrogen and often sulphur. The amino acids are linked together by peptide bonds which creates polypeptide chains. The polypeptide chains can consist of several hundred linked amino acids (Cheftel, Cuq & Lorient, 1985). All proteins have a central carbon atom (Cα) attached to a hydrogen atom, an amino group (NH_2), a carboxyl group (COOH) and a side chain (R) (Fig 2.4). The amino acids are linked together with peptide bonds created between a carboxyl group and an amino group of another amino acid, and can consist of several hundred amino acids linked together (Cheftel, Cuq & Lorient, 1985, Branden & Tooze, 1999).

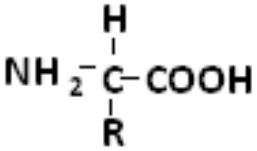


Figure 2.4: Basic chemical structure of amino acids.

There are three main proteins in muscle (Koohmaraie et al., 2002, Hemmer, 1997):

- Myofibrillar (salt- soluble)
- Connective tissue (acid- soluble)
- Sarcoplasmic (water-soluble)

The **myofibrillar proteins** are responsible for contraction of the muscle. The main proteins are myosin, actin, titin, tropomyosin, toponin, desmin and actinin Hemmer (1997). A muscle consists of multiple muscle bundles that are consisting of multiple muscle cells or fibres. The fibres consist of myofibrils that again are divided into thinner and thicker filaments made out of the proteins actin and myosin.

After birth the number of muscle cells is relatively constant but increases in size due to larger cell length and diameter. By using a microscope on muscle tissue a number of bands occur. Dark bands are called **A**-bands, while the light bands are called **I**-bands. The **Z-line** is a thin line that cuts the I-band. The area between the Z-lines is called sarcomeres and is the smallest functional unit of the myofibril. The muscle cells have various degree of shortening which affects the length of the sarcomeres. The A-bands have all the same length, while I- bands decrease in length in myofibrils with shorter sarcomeres. The **H-zone** has a lower intensity and is found in the middle of myofibrils with long sarcomeres, while the **M-line** is in the middle of the sarcomere binding the thick filaments together.

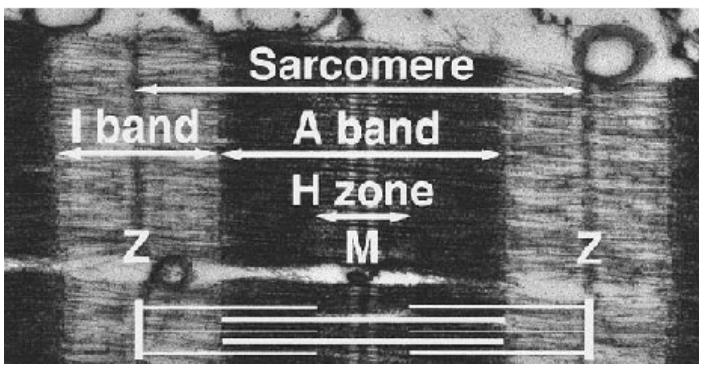


Figure 2.5: Longitudinal view of a muscle in electron microscope (Greaser, 2001).

An **electron microscope** can be used to understand what the filaments responsible for these patterns is composed of. In the A- bands a thick filament can be found that interacts with thinner filaments that are attached to the Z- lines. During muscle contractions these filaments slide over another. The thicker filament consists mainly of the protein **myosin** while the thinner consists mainly of the proteins **actin**, troponin and tropomyosin. Titin is elastic and it is believed to be of importance for myofibrils and to protect against overstretching. It is a large protein that extends from the Z-line. Nebulin is assumed to control the length of the thin filaments, and are attached to the Z-line. Titin and nebulin are sometime called **cytoskeletal** proteins, Greaser (2001).

Bone, cartilage and tendons consist of **connective tissue**. Their task is to transfer muscle power to the skeleton, to form skin and to protect the inner organs. For the living animal the connective tissue has to be strong and rigid but is perceived as toughness in meat. The connective tissue protein collagen has the highest influence on tenderness followed by elastin, Hemmer (1997).

Age plays an important role according to connective tissue. Calves have a great amount of connective tissue but dissolves to gelatine by heating, Hemmer (1997). In young animals the collagen is less cross-linked and the rigid organized helical structure “melts” when heated above 60^{o}C and forms gelatine. When the animal gets older, the cross-links to collagen increases and get more heat stabile (Young & Gregory, 2001; Purslow, 2005; Hemmer, 1997). Intramuscular connective tissue (IMCT) determinds the “**background toughness**” and is difficult to change (Koohmaraie et al., 2002). IMCT consist mostly of the two proteins collagen and elastin and are sounded by a proteoglycan (PG) matrix. The collagen content in beef varies from 1% up to 15%, and elastin 0.6 up to 3.7%. (Purslow, 2005)

The **Intramuscular connective tissue (IMCT)** structures (epimysium, perimysium and endomysium) have different structure and composition. They play a central role in growth, transmission of signals to muscle cells and they distribute forces between the fibres in the muscle. The amount, composition and morphology are dependant on the muscles and their function Purslow (2002). As shown in figure 2.4 the muscle is surrounded by a connective tissue layer called **epimysium**. The epimysium consists mostly of collagen and is attached to the bones along with the tendons. It consist of two layers of collagen fibres that intersect and when the muscle relaxes, the collagen fibres are arranged at an approximately 55^{o} angel to the long axis of the muscle fibres. Most of the muscles are arranged like this but some can be

arranged parallel to the long axis which creates a denser surface layer with a surface tendon function. The **perimysium** divided the muscle into fascicles or muscle fibre bundles. They are a continuous network of connective tissue and the fascicles ranks from tendon to tendon. Inside the perimysium each muscle fibre bundle or fascicle is covered by a thin layer called **endomysium**. (Purslow, 2010)

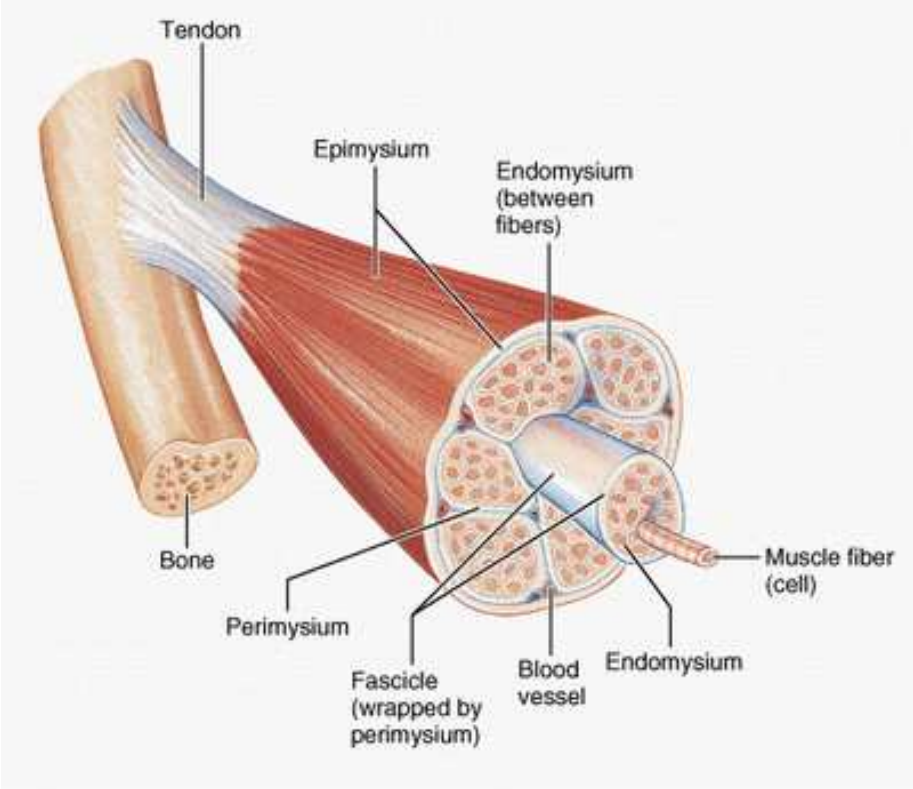


Figure 2.4: Composition of skeletal muscle connective tissue (Medicalook, 2012)

If the muscle is short in length the collagen fibres in the endomysium can adjust the diameter of the fibres while a long muscle can extend in the longitudinal direction. The endomysium can distribute forces laterally and thus grow and repair sarcomeres without loosing their contractile function. The perimysium consists of two structures. The small fascicles or muscle bundles are called primary perimysium, while larger is called secondary perimysium and is usually thicker than the primary perimysium. The thickness, size and shape differ between the primary and secondary perimysium. (Purslow, 2010)

Sarcoplasmic proteins are not structural proteins and thus not directly affecting meat tenderness (Koohmaraie et al., 2002).

2.3.2 Ante mortem muscle

When the animal is alive the locomotion is provoked by a **nerve impulse** from the spinal cord passes to a motor end plate in the muscle cell. A monitor unit is a nerve fibre that connects with hundreds of muscle fibres. A small amount of acetylcholine gets released from the end plate of the nerve fibre. The **acetylcholine** diffuses to the surface of the muscle cell and binds it to the receptors in the muscle cell membrane. This results in a local **depolarization** of the cell membrane that influences the polarization of special perpendicular invaginations (**T-tubes**). T- tubes penetrate the centre of the muscle cell and are attached by the protein receptor ryanoide to the sarcoplasmic reticulum. When a nerve signal from the spinal cord reaches the muscle, the depolarization passes by the receptors and t- tube and ends up at the **ryanoide receptor**. They respond by releasing calcium ions into the **cell cytosol**. The released calcium ions diffuse to the myofibrils where it binds to tropoins and causes it to change shape and leads tropomyosin to move deeper into actin. This causes the heads of the myosin on the surface of actin to be exposed and the myosin binds and either pulls or pushes **actin** and the **myosin** heads releases and can be re- attached to another actin. The polarity of the cell membrane is restored by the **sodium- potassium pump** in the outer cell membrane. Both the calcium and the sodium-potassium pump require ATP to function (Greaser, 2001).

2.3.2 Rigor mortis

When the carcass has bled and the oxygen supply to the muscle stops, the **glycogen** storage in the muscle is used to create energy. This accumulates lactic acid and the pH drops from about pH 6.7 down to 5.7 (Wheeler & Koohmaraie, 1994; Greaser 2001). When the glycogen storage is depleted, rigor mortis occurs. So far the process is somewhat similar in all animals (Veiseth & Koohmaraie, 2003). If the animal is ES the glycogen storages are depleted earlier and the carcass goes into rigor at an earlier stage than without ES.

When **rigor mortis** occurs, the muscles become **rigid** and **inextensible** because of irreversible cross-bridges between actin and myosin, the two main proteins responsible for contraction. The myosin heads attaches to actin and precludes the free-sliding of the filaments and the muscle goes into rigor mortis. The higher the temperature in the muscle is the greater contraction. This is called heat shortening but occurs at all temperatures as long as the muscle is warm and can be called rigor shortening (Zamora, Chaïb & Dransfield, 1998). In a live muscle the calcium ions leaks from sarcoplasmic reticulum when the muscle contracts and is

pumped back when it releases. This **calcium pump** is an ATP dependant enzyme system and works faster at higher temperatures, while the leakage is relatively temperature insensitive. If the temperature is too low, the calcium pump becomes less efficient and the concentration of intracellular calcium ions increase and the muscle contracts. (Young et al., 2001). Thaw-, cold- and rigor shortening of the muscle will increase the toughness of meat even after ageing. There are to main factors that occur at different stages of tenderization, the myofibrillar contraction dependent on the calcium concentration and the proteolytic ageing (Zamora, Chaïb & Dransfield, 1998).

When the ATP concentration declines to low levels, myosin and actin binds irreversible and the muscle is contracted. Rigor mortis can occur for 8-15 hours. It starts usually around pH 6.0 and is maximal at ultimate pH. Due to type of fibre, glycogen content and other factors, the individual fibres enters rigor at different time (Young et al., 2001). Stress can affect the animal by draining the glycogen storages which leads to an abnormal decline in pH were the ultimate pH is higher than normal and the meat will be classified as **DFD** (dark, firm and dry).

After rigor mortis the sarcomeres length stays unchanged and is not affected by the proteolysis. The contraction during rigor mortis determines the length. If the contraction is great it will result in tough beef (Wheeler & Koohmaraie, 1999).

2.3.3 Tenderization influencing factors

Enduring toughness in meat can be because of connective tissue and myofibrillar toughness. By cooking less tender meat over a longer period of time, the connective tissue can be defeated while myofibrillar toughness can not. (Young & Gregory, 2001) Meat ageing is mostly the weakening of the myofibrillar structure by the endogenous proteolytic calcium dependant enzymes calpains and the lysosomal-located cathepsins (Young et al., 2001). In addition, a study by Nishimura, Hattori & Takahashi (1994) showed that there were little change in the intramuscular connective tissue (IMCT) after 10 days post mortem but a clear difference after 14 days post mortem. When the animal is alive, the calpains is one of the proteolytic systems involved in protein turnover in cells (Young & Gregory, 2001).

Enzyme catalysts were formulated first by Linus Pauling in 1946. Some proteinases can be found as a domain in large multifunctional proteins while others are independent smaller peptide chains. The activity of **proteinases** is dependent of the availability of **inhibitors** that

can block them. Based on their functional group of the active site the proteinases are divided into proteinase families (Branden & Tooze, 1999). Most of the proteases are destructive and their catalytic activity is activated by proteolysis (Creighton, 1997). Calpains primary function is to degrade excess or broken proteins or to activate zymogens. Compared to MMPs and their remodelling of connective tissue, the calpains are less vital (Cha & Purslow, 2009). For several MMPs to become active, both calcium and zinc is required (Barrett, 1977).

The **protease** activity is different in various carcasses and so is the tenderness (Veiseth & Koohmaraie, 2003). Calpain proteases are a large family and consist of 3 isoforms µ- calpain, m- calpain and p94 (calpain 3) in skeletal muscle. They need Ca^{2+} to be activated. Calpastatin inactivates the calpains by wrapping the inhibitory domain around the calpains (Kemp et al., 2009). The findings of Morton et al. (1999) supports the theory that µ-calpain and calpastatin plays a great role in tenderisation of meat. There was a difference between lamb and beef. The activity of the two enzymes declined over 12h after slaughter in beef, while 24h in lamb. Underwood, Means & Du (2008) showed that caspase 3 did not effect tenderization of beef. Caspase 3 was not activated, the activity decreased during post mortem and is not associated with WBSF.

Two of the **calpain family** is the protease enzymes **µ-** and **m- calpain**, while calpastatin inhibits and regulates their activity. The calpains are optimally active around neutral pH but is also active at lower pH. **Cathepsins** are enclosed within lysosomes and have to be released from this organelle to be activated. The protease family of cathepsins have a lower pH optimum. When the concentration of calcium ions increases in the post mortem muscle, the µ-calpain is activated and thus **proteolysis** can begin. If the temperature is high the calpains are activated early and the proteolysis occurs rapidly but short due to inactivation because of calpain autolysis. This will provide tougher meat than by more slowly pH decline combined with lower temperature. Then the proteolysis last over a longer period but the reactions is slower. During and immediately after rigor the µ- calpain is the main enzyme responsible for tenderization of meat. After 3- 4 days it is believed that the cathepsins play an important role. (Young et al., 2001; Young & Gregory, 2001)

It exist little information on **matrix metalloproteinase (MMPs**) and their role in tenderisation of meat. In general, all the MMPs are **zinc** dependant enzymes responsible for degradation of extracellular matrix (EMC). The MMPs are primary enzymes responsible for turnover and remodelling of connective tissue such as intramuscular connective tissue

(IMCT) (Snoek- van Beurden & Von den Hoff, 2005). They are also linked to several deceases such as arthritis, cancer and osteoporosis (Woessner, 1991)

Inside the cell, MMPs and collagen are respectively expressed as **latent** (pro-enzyme) and pro- collagen. MMPs are activated by proteolysis extracellularly while collagen is modified and aggregates into fibrous collagen. The amount of active MMP is dependant on the amount of their inhibitor TIMP present. It is the activity of MMP enzymes and the synthesis of new matrix components that will affect the turnover and remodelling of IMCT. (Purslow, Archile-Contreras & Cha 2011)

MMPs can be divided into 5 groups (Mannello & Medda, 2011):

1) Matrilysis (MMP-7 and -26)
2) Collagenases (MMP-1 and -13)
3) Gelatinases (MMP-2 and -9)
4) Stromelysin (MMP-3 and -7)
5) Membrane-type (MT-MMP).

Cha and Purslow (2010) observed that **collagenases** and **gelatineases** are mainly active at the borderline of each muscle cell, most importantly at the **borderline** between muscle fascicles around or at the endomysial and perimysial IMCT.

MMPs are either secreted into extracellular space or bound in the membrane (Cha & Purslow, 2009). Post mortem, the MMPs are involved in proteolysis where they are secreted in a latent form and activated by plasmin or membrane- type MMP (MT-MMP) (Balcerzak et al., 2001) by breaking up the **cysteine switch** (Cys-Zn^{2+}) interaction and remove propeptide (Geesink & Veiseth, 2009).

Extracellularly, MMPs are activated by proteolysis, and their activity depends on how much of their inhibitor (TIMP) is present. It is the activity of MMP enzymes and the synthesis of new matrix components that will affect the turnover and remodelling of IMCT (Purslow, Archile- Contreras & Cha, 2011). It is their interaction with proteins, proteoglycan core proteins and/or their glycosaminoglycan chain (among other molecules) that determine the activation, activity and localization of MMPs. (Mannello & Medda, 2011) To regulate the activity of MMPs there are the inhibitors called TIMP. TIMP-1 can form noncovalent bindings with the active MMP, while TIMP-2 binds to MMPs inactive form and stabilities it

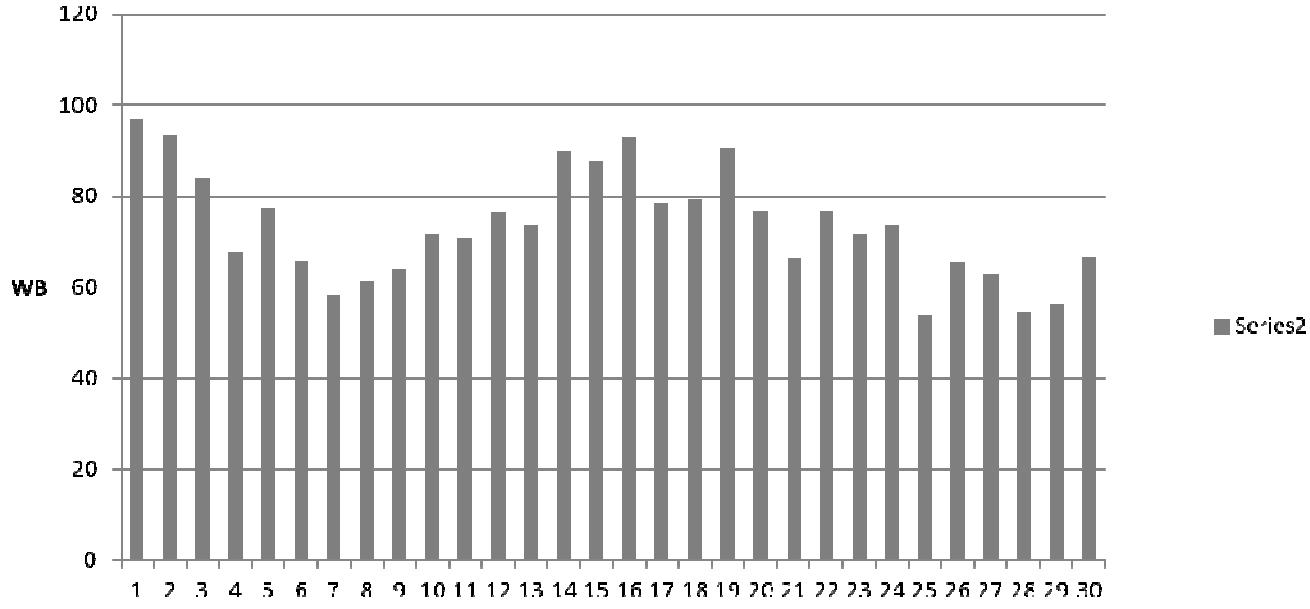
by inhibiting activation of proteinases. (Geesink & Veiseth, 2009) Elastin is little or not digested by the MMP family members (Woessner, 1991).

3 Materials and methods

3.1 Animals

The project “Mer og Bedre Biff” (More and better meat) has collected data from a total of 550 NRF (Norwegian Red) bulls, sons of 25 elite sires and thus 25 families. About 20 were exposed for severe stress at the beginning of the project and consequently withdrawn. The slaughter period started autumn 2008 and was finished during winter 2012. All the animals were slaughtered at Nortura Rudshøgda. The preliminary heritability (h^2) of Warner-Bratzler was calculated to be about 0.35 and means that there is a significant variation between the tenderness of the families.

The bulls were slaughtered in 30 batches with the average of approximately 20 bulls each day. The average WBSF measurements of the various batches is presented in Fig. 3.1



Graph 3.1: Variation of WBSF- measurements between the 30 slaughter batches.

For this thesis the selection of animals were 111 sons of 4 tender sires and 4 tough sires with 14 sons each. They were chosen on the basis of Warner-Bratzler shear force measurements (WBSF) (graph 3.2) and divided into tender/tough groups with high/low standard deviation:

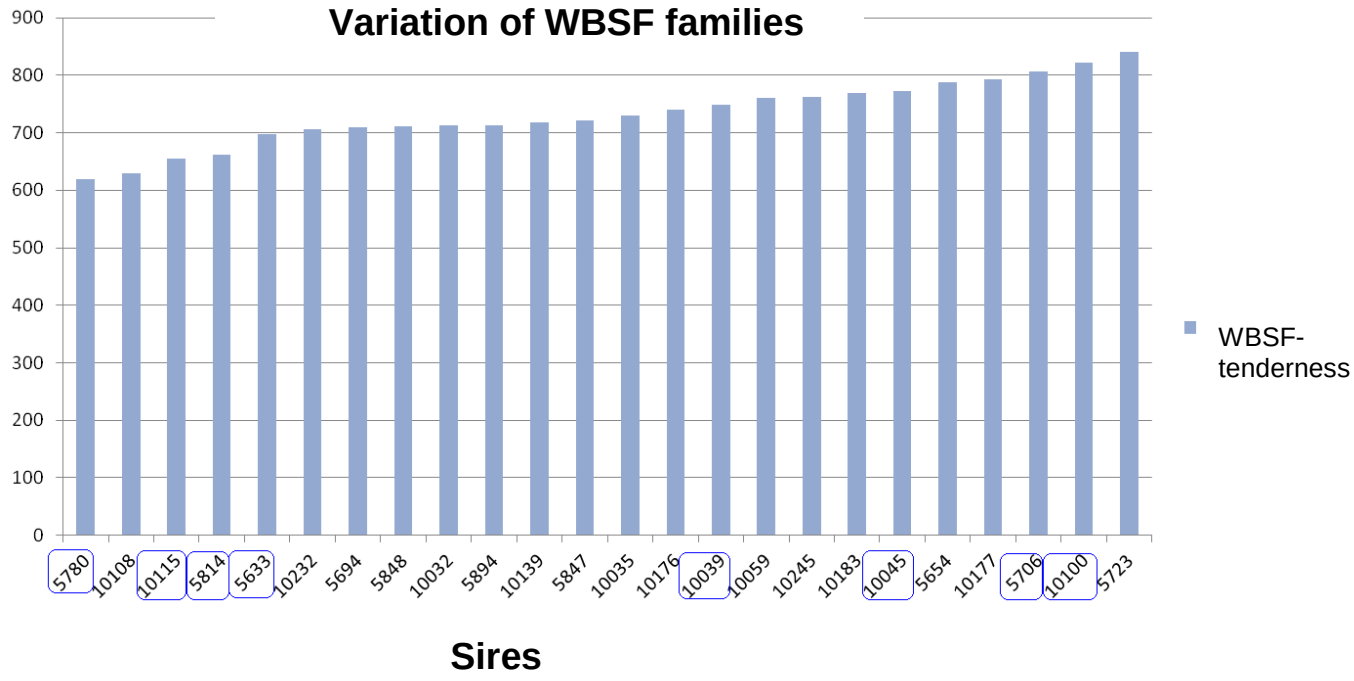
1-1 Tender low standard deviation

1-2 Tender high standard deviation

2-1 Tough low standard deviation

2-2 Tough high standard deviation

Some of the extreme sires did not have enough sons to be presented in each group and thus the “next best” were chosen. From the 550 bulls the tender bulls came from the sires 5780, 10115, 5814 and 5633. The sires hereditary the tougher meat was 10100, 5706, 10045 and 10039. The selected sires are marked with a blue circle in graph 3.2.



Graph 3.2: Average of WBSF of the sons of their respectively sire. One sire was excluded due to too few sons.

The bulls in this study came from 6 farmers, located in the South-East of Norway. As shown in table 3.1, all of the farmers were represented in each group.

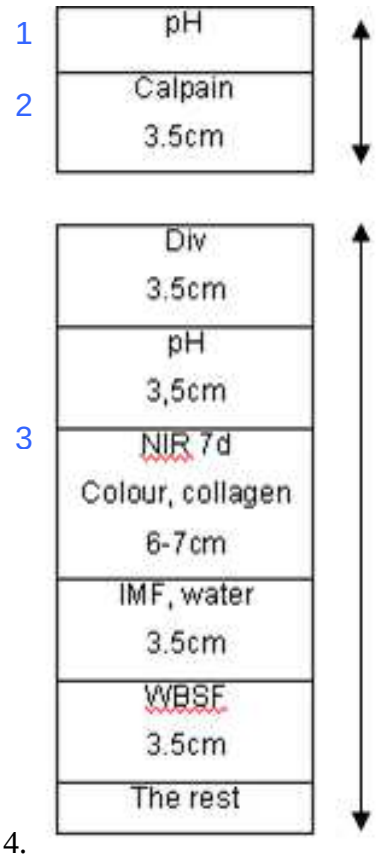
Table 3.1: Distribution of bulls in relation to the 6 farmers divided into 4 different groups and the average of WBSF measurements in the slaughter batches associated with each group.

Farmer no.	Tender low std	Tender high std	Tough low std	Tough high std
1	3	6	3	6
2	7	6	6	5
3	8	4	9	2
4	1	3	2	6
5	3	2	3	5
6	6	6	5	4
Average WBSF in slaughter batches	71,0N	71,4N	76,4N	77,8N

For the MMP-2 activity analysis 109 samples were included. 1 sample was not to be located in the freezer, while the other one gave various results even after repeated attempts. The colour measurements after 1 week also included 109. The calpain activity was only measured on 87 samples. These analyses are not finished for the project in total, causing a lack in data ready for this thesis. For the rest of the traits, data on all of the 111 samples were completed (Table 4.3 and 4.4).

3.2 Slaughter parameters

The animals was transported to the slaughter house and stayed over night. They were all stunned by a captive-bolt gun and killed by sticking of the carotid arteries and jugular vein and electrical stimulated at 90V after 20 minutes. After removal of bones, hide, head, intentional the carcass was cut in two pieces. The slaughter weight was measured and the carcass was classified by EUROP standard. For muscles fullness the scale ranks from 1-15 where 1 is poor (P-) and 15 excellent (E+). Fat classification ranks from 1-15 where 1 is fat free (1-) and 15 is extremely high in fat (5+). After classification the muscle samples for this thesis was cut free. The samples was 40cm *m. Longissimus dorsi* cut free 5-10cm prior of the 11th vertebral and back on the right side of the carcass. The muscle was then cut into 3 pieces:



1. 6.5cm was put into water bath at 11°C and used to measure pH and temperature at 1h, 6h and 10h after slaughter.
2. 3.5cm was cut into smaller pieces and frozen directly in liquid nitrogen, transported back to Nofima on dry ice and stored at -80°C. These samples was used for both calpain and matrix metalloproteinase measurements.
3. 21cm piece was vacuum-packed and put into water bath at 11^{o}C for 10 hours and subsequently stored at 4°C before it was transported to Nofima in Ås, and cut into smaller pieces (figure 3.1). pH was measured 48h. After 7days NIR, colour, collagen, intra muscular fat (IMF), water and WBSF measurements.

Figure 3.1: How the muscle was cut.

The left half of the animal was kept overnight at 4^{o}C in the hanging hall. 24h post mortem, NIR, Minolta (colour) and pH was measured.

3.3 Meat parameters

To measure pH a glass- stick probe (InLab427 Combination pH Puncture Electrode, Mettler, Toledo Intl. Inc., Greifensee, Switzerland) connected to a pH meter (Portamess 752 Calimatic, Knick, Berlin, Germany) was inserted into the muscle and the pH was measured 1, 6, 10 and 48 hour post mortem.

During conditioning the temperature were monitored by loggers (EBI-125 A, Temperature Data Logger, Ebro Electronic, Ingolstadt, Germany). They were placed in the water baths and coolers throughout the storage period. Several temperature loggers were inserted into LM to give estimates of the temperature drop in the muscles. It was not observed any abnormal temperature decline during the experiment.

After 7 days the WBSF measurements of the sons were conducted. To measure the tenderness of the samples a Warner- Bratzler shear force (WBSF) instrument with a V- shaped blade attached to an Instron Materials Testing Maschine (Model 4202, Instron Engineering Corporation, High Wycombe, UK) was used. A 3.5cm thick slice of each sample (location in the muscle, see Fig. 3.1) was vacuum-packed in polyethylene bags and heated in a water bath at 70,5^{o}C for 50 min and chilled in ice water for 50 min. The core temperature was 70,5^{o}C in the samples, based on simultaneous test performed at Nofima laboratory. The repetitive parallels for WBSF were cut as shown in figure 3.2a. A rectangular piece of 1 x 1 x 3 cm was cut along the fibre direction and 10 parallels were sheared perpendicular to the fibre direction (figure 3.2b). The average maximum force for the 10 parallels, measured as N/cm^{2}, was used in the data analysis.

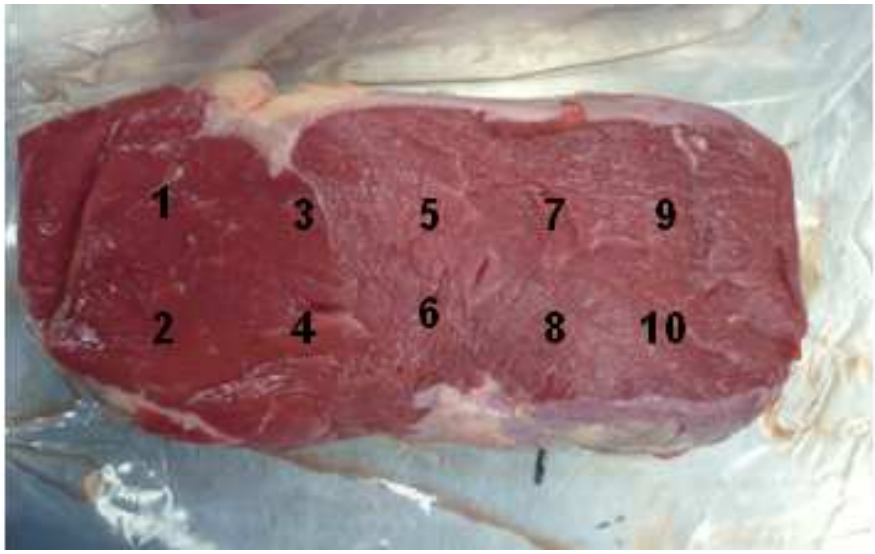


Figure 3.2a: One sample cut into WBSF pieces and their location on beef.

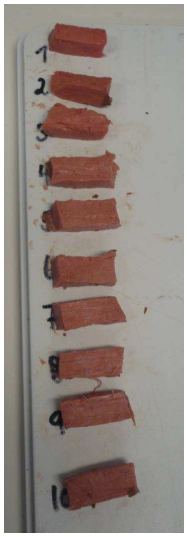


Figure 3.2b: One sample cut into paral

The colour was measured with MINOLTA CR-200 by Hunter L*a*b- system. The measurements took place at the cross-section of the back muscle on the left side of the carcass in the hanging hall at Nortura Rudshøgda one day after slaughter and at the Nofima Ås 7 days post mortem on the piece cut of the right side of the carcass. The slice was 3.5cm broad (location in the muscle, see Fig. 3.1) and the first measurement was in the centre of the muscle and the second closest to the later part of the muscle (figure 3.3). Two parallel measurements for each position were measured and their average calculated.

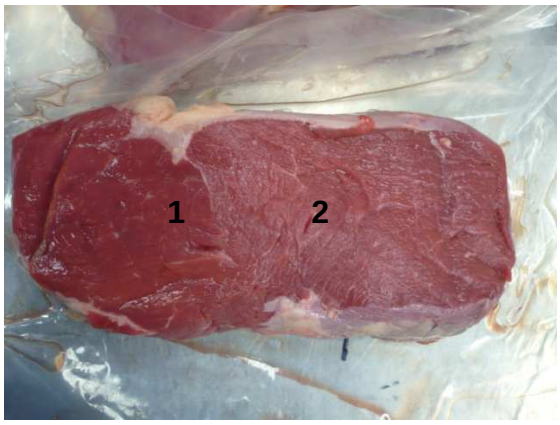


Figure 3.3: Position of the colour measurements-.

A third slice (Fig. 3.1) was handled the same way for analysis of fat % (intramuscular fat) and water %. To analyze fat % Soxtec (EU DIR 98/64 m) was used, and to analyse water % (NMKL 23 1991). Iron was detected by NMKL No 139 1991 and Calcium by NMKL 161 mod;ICP-AES.

The hydroxyproline analysis was also done by Eurofins based on the method SS-EN ISO 13903:2005. The sample was oxidized with performic acid for 16 hours. The oxidation was stopped by adding sodium bisulfite and hydrolyzed in 6M hydrochloride acid on sand for 23 hours. After pH adjustment and filtering the sample was loaded over to the amino acid analyzer. Biochrom was used during cation exchange chromatography. The sample was injected into an exchange column by an auto-injector and different buffers with different pH and ionic strength were pumped through the column and the various amino acids were separated. The temperature of the column changed between 50-90^{o}C. When the amino acid reached its isoelectrical point was eluted from the column and ninhydrin was added to create colour products detectable by photometer. The absorption of light was then measured at 570nm and 440nm and the concentrations of amino acids were plotted as a series of peaks. The peak retention time identifies the amino acid. The evaluating program EZchrome Elite was used to evaluate (pers.comm. Eurofins, 2008).

3.3 Protease analyzes

3.3.1 Calpain activity

Approximately 1 hour after slaughter the samples for determination of µ- and m- calpain activity by casein zymography (Raser et al., 1995) was snap- frozen in liquid nitrogen. All of the samples were measured in duplicate, and each zymogram contained a duplicate reference sample. The regions of transparent gel were measured (Quantity One, Version 4.5.0, Bio- Rad Laboratories Inc., Hercules, CA) and calpain activity was expressed as the density of the bands from the sample within each zymogram. At present, these analyses are not finished for the project in total, causing a lack in data ready for this thesis (calpain data for only 87 out of 111 animals in this study). The calpains for this subsample of animals in the study were analysed together with the rest (total 550 samples) divided into three periods, each consisting of 9 days with 12 samples analysed per day, referred to as “perday” in results.

3.3.2 Matrix metalloproteinase activity

To prepare enzyme extractions for matrix metalloproteinase analysis, the samples were taken out of the -80^{o}C freezer and kept on liquid nitrogen. The samples were numbered according to sampling dates, and about 300mg was measured into a Precellys tube. 1000µl extraction buffer (50 mM Tris- HCl, pH 8,3, 10mM EDTA) was added to 300mg muscle. The amount of extraction buffer was adjusted to get the accurate concentration of buffer to muscle weight.

The tubes were then put into a Precellys machine set at 6,000 rpm for 2 x 20sec with a 5 sec pause in between. After the samples were shaken they were transferred into a centrifuge set at 13,000 rpm at 4^{o}C for 30 min. The samples were then divided into three phases; the fat phase on top, water phase in the middle and the solid phase at the bottom. 300µl of the water phase was transferred into an eppendorf tube on ice before it was divided into 3 marked eppendorf tubes and kept at -80^{o}C. One of the samples was chosen as a standard, and this sample was included on all gels and used for correcting possible batch effects related to the zymography analysis.

The bulls were divided into 14 groups, referred to as ”agroup” in results. Each sire was represented with one son in each group. The extracts were thawed in a refrigerator for 1 hour, while the sample buffer (62,5mM Tris-HCl, pH 6,8, 10% glycerol, 2% SDS and 0,0025% Bromophenol blue) was allowed to reach room temperature. After 1 hour the extract and the sample buffer was mixed at the ration 1:2. (10µl extract to 20µl sample buffer). The samples were then applied to 10% Zymogram (gelatine) gels. For each sample, 20µl was applied into two wells, resulting in four different samples in addition to the standard sample on each gel. After assembling the gels the electrophoresis buffer (25mM Tris- HCl, pH 8,3, 192mM glycine and 0,1% SDS) was added. The electrophoresis was run at 125V for about 100 minutes with the expected voltage of 30-40 mA/gel; 8-12 mA/gel at the end. After electrophoresis the gels were removed from their plastic holders, marked in the top corner to keep track of orientation, placed into individually containers and added 50ml/gel of renaturation solution (2,5% Triton X-100) 2 x 15 minutes at room temperature. The gels were then incubated in 50 ml/gel incubation solution (50mM Tris- HCl, pH 8, 5mM $CaCl_2$) for 15 minutes before the gels were incubated in 100 ml/gel incubation buffer at 37^{o}C for 20 hours. After 20 hours 50 ml/gel of staining solution (0,1% Coomassie brilliant blue R-250, 50% methanol, 7% acetic acid) was added and the gels were incubated at room temperature. After 1 hour the gels were rinsed with distilled water and incubated in 100 ml/gel destaining solution (20% methanol, 7% acetic acid) for 2 hours. After destaining the gels were rinsed and kept in distilled water before scanning with Epson Scan, Tif format with the settings 240dpi and Grayscale 16. An example is shown in figure 3.4.

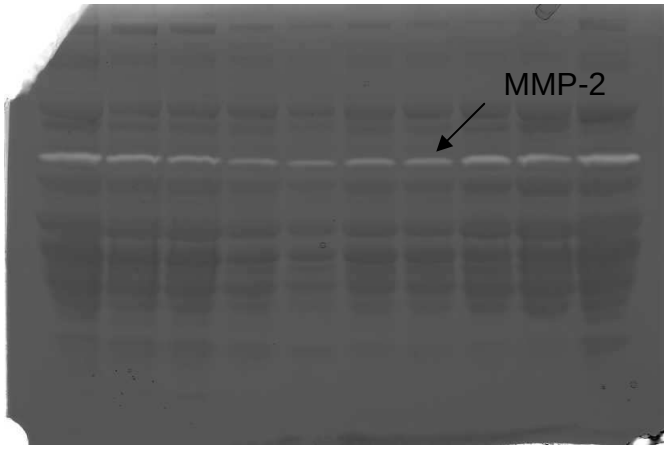


Figure 3.4: Example of the transparent MMP-2 bands (shown by the arrow). The standard samples was injected at the end on both sides with 4 samples with duplicates in the middle. This gel was group 1 and gel 2 (the four last of group 1).

To evaluate the gels the quantification program "ImageQuant TL, version 7.0 (GE Healtcare) was used. The transparent regions of the gel were measured and MMP2 activity was expressed as the density of the bands from the sample relative to the density of the standard sample within each zymogram. To remove background, the rolling ball function of the software was used.

3.3 Statistical Analysis

The data were analysed using various procedures in the statistical package SAS (SAS Inst., Inc., Cary, NC). Initially, the data set was examined for overall sampling and balance within the various possible fixed effects, and which fixed effects to include in the models. These effects were farm, slaughter batch and age of slaughter.

All farms were represented and acceptably distributed in each sire-STD group (11, 12, 21 and 22). Sire-STD group 11 and 12 was dominated with animals from slaughter batch 1-11 and 24-30, while sire-STD groups 21 and 22 was dominated by batch 10-30 (figure 3.1). However, there were no indications that these differences should have affected the data by special batch effects related to this skewed sampling. This because the mean WBSF values for all bulls slaughtered in the corresponding batches as the subsample of 111 bulls in groups 11 to 22 was quite similar (max. 7 N difference; Table 3.1).

In the GENO breeding programme, the sires are excluded from matings after a certain time period and replaced by new elite sires. During the 4-year project period, this practice led to a confounding between sires and batches, although some overlap occurred. However, based on

the same argumentation as above, this was not considered to affect the results (no indications that special batch effect trends occurred over the actual time period).

The initial analyses showed that there were no significant effects of farm, slaughter batch or age at slaughter on any of the traits MMP2, hydroxyproline, μ- or m-calpain. However, as age of slaughter was significantly different between the standard deviation groups and groups 12 and 21 (Table 4.2), this variable was included in the final statistical models for all traits (except pH-traits) to adjust for possible effects of this difference.

One bull had extremely high ultimate pH (pH_{48} of 6.87). The possible influence of this high pH level was investigated initially and found to be of no importance for all traits except for the colour measurements, where this bull were omitted from the analyses.

The data was finally analysed by the statistical ANOVA model which included the overall mean μ, the fixed effect FGi of sire group (i=1,2), the fixed effect STDj of the standard deviation group (j=1,2), the interaction between sire and STD group (FSTD)ij (i=1,2;j=1,2) the regression b1(X1ijk) of age at slaughter on Yijkl and the random error eijkl associated with the l'th observation.

Additionally, for MMP-2 the statistical model included the fixed effect of AGROUP and for calpains the fixed effect of PERDAY, as these had highly significant effects ($p<0.0005$ and $p<0.01$ respectively) on the enzyme data.

The full genetics of the trait MMP-2 is still not described in the literature, thus, it was of interest to analyse the data in order to explore if some genetic variation among the 8 families were present in the data. This model contained the fixed effect of AGROUP, the regression b1X1 of age at slaughter, the random effect of sire and the random error effect.

4 Results

4.1 General overview

The studied characteristics is presented in table 4.1

Table 4.1: *Number of samples (n), mean, standard deviation, minimum and maximum values.*

Characteristics	n	Mean	STD	Minimum	Maximum
Live animals					
Weight gain (g/day)	111	539	59	361	689
Age at slaughter (days)	111	579	72	440	777
Carcasse quality					
Slaugther weigth (kg)	111	310	35,7	216	404
EUROP class (1-15)	111	5,22	0,71	3	7
EUROP fat class (1-15)	111	6,06	1,12	3	9
Meat quality					
WBSF, 7 d (N)	111	72,1	21,15	36,4	156,8
std	111	9,54	5,79	25	412
range	111	2,94	1,76	8	136
Water (%)	111	74,5	0,79	71,3	76,3
IMF (%)	111	1,42	0,72	0,2	4,6
Iron (mg/100g)	111	1,6	0,34	0,83	2,4
Calcium (mg/100g)	111	3,26	0,39	2	5,3
µ-Calpain activity 1 h (units/g)	87	0,99	0,22	0,49	1,59
m- Calpain activity 1 h (units/g)	87	1,06	0,39	0,25	2,2
MMP-2 activity 1 h (units/g)	109	0,67	0,23	0,15	1,17
Hydroxyproline (g/kg)	111	0,80	0,22	0,40	1,60
pH					
1 h	110	6,56	0,29	5,82	7,47
6 h	111	5,85	0,26	5,38	6,98
10 h	110	5,63	0,21	5,3	7,07
24 h	106	5,59	0,2	5,29	7,06
48 h	111	5,54	0,17	5,09	6,87
Colour					
L	92	36,5	2,63	29,98	44,9
*a	92	22,3	3,01	15,28	30,18
*b	92	10,9	2,9	3,63	15,78
1 week L	109	42	3,46	28,62	50,75
1 week *a	109	15,4	1,99	10,39	19,6
1 week *b	109	5,6	2,35	-3,7	9,45

Overall the sons grew on average 539 g/day and their age ranked from 440 to 770 days. General EUROP classification was low. There were great differences between the WBSF-measurements, standard deviation and range, IMF, MMP2 and hydroxyproline content. The

pH after 48 hours was normal, except for the bull that had extremely high ultimate pH (pH_{48} of 6.87). The colour of the surface of *m. Longissimus dorsi* 24h post mortem on the carcass was darker (L-value) and with higher intensity (*a and *b values) than when measured on the bloomed (1h) surface of the conditioned filet (from the right side of the carcass) 7 days post mortem.

4.2 Analysis of variance

Analysis of variance is shown in table 4.2.

Table 4.2: Table of ANOVA with sums of square for sire-group, std-group, sires X std group, agroup, perday, slaughter age, residual and R^2

	Sire groups	Std groups	Sire and Std groups	Agroup	Perday	Slaughter age	Residual	R^2
Age at slaughter (days)	7167	**15934***	998				5132,67	0,042
EUROP class (1-15)	0,0102	0,5506	0,9787			**2,3478****	0,48	0,082
EUROP fat class (1-15)	1,1408	0,0159	0,0338			**4,124***	1,26	0,037
WBSF, 7 d (N)	**3028278*****	**279660*****	**47270***				14525,54	0,684
std	**35828*****	**124509*****	**12037****				1817,09	0,472
range	**3350*****	**10717*****	**1080****				175,88	0,448
IMF (%)	**235,9****	0,1654	114,93			11,06	49,86	0,068
Water (%)	**292,3****	5,77	16,09			2,02	62,07	0,047
Iron (mg/100g)	286,18	1453	117,08			**31522*****	872,63	0,289
Calcium (mg/100g)	251,41	253,98	1657,51			3388,08	1476,46	0,046
MMP-2 activity 1 h (units/g)	**0,279****	0,027	**0,159****	**1,787*****		0,03	0,04	0,387
Hydroxyproline (g/kg)	227,45	125,45	2,54			0,98	496,49	0,007
µ-Calpain activity 1 h (units/g)	0,027	0,015	0,048		**2,026****	0,008	0,04	0,495
m- Calpain activity 1 h (units/g)	0,037	0,031	0,068		4,419	0,004	0,14	0,366
Colour								
L	2,719	4,185	0,557			**40,49****	6,71	0,078
*a	11,628	6,275	0,544			**76,44****	8,42	0,116
*b	0,93	0,717	1,692			**42,387****	8,27	0,059
1 week L	18,265	0,397	6,383			**57,985****	10,01	0,074
1 week *a	4,562	**25,075****	**9,492***			**13,303****	3,29	0,153
1 week *b	**30,181****	1,107	0,06			0,077	4,57	0,066

* p≤ 0.10
** p≤ 0.05
*** p≤ 0.001

R^2 express the amount of variation in the actual trait explained by the statistical model, which in this study varied between 0.007% and up to 68.4%. The ANOVA-table shows that the sire

groups had an effect on WBSF, IMF, water, MMP-2 activity and *b after 1 week which indicate that genetic variation was present for these traits. The standard deviation had an effect on slaughter age, WBSF and *a after 1 week. Standard deviation and sire effected WBSF, MMP-2 activity and *a after 1 week. The agroup affected the MMP-2 activity, and perday the µ-calpain activity. Slaughter weight had an effect on EUROP class and fat class, iron and all of the colour parameters except *b 1 week.

4.3 LSMEANS

The 4.3 table shows the significant differences between the sire groups, standard deviation groups and the four (sire + standard derivation) groups 1-1, 1-2, 2-1 and 2-2 and their measurements.

Table 4.3: Shows differences between the groups

		Sire groups		Standard deviation group		Groups (Sire + Std)			
Source	n	Tender (1)	Tough (2)	Low (1)	High (2)	1-1	1-2	2-1	2-2
WBSF (N)	111	**55,5^{a}**	**88,5^{b***}**	**67,0^{a}**	**77,0^{b***}**	**52,5^{a}**	**58,4^{b*}**	**81,4^{c***}**	**95,6^{d***}**
Standard deviation	111	**7,8^{a}**	**11,3^{b*}**	**6,2^{a}**	**12,9^{b***}**	5,4^{a}	**10,1^{b***}**	7,0^{a}	**15,7^{c***}**
Range	111	**24^{a}**	**35^{b***}**	**20^{a}**	**39^{b***}**	17^{a}	**31^{b***}**	22^{a}	**48^{c***}**
Slaughter age (days)	111	571	587	**591^{a}**	**567^{b*}**	580	**562^{a}**	**602^{b**}**	572
EUROP class (1-15)	111	5,2	5,2	5,3	5,2	5,2	5,3	**5,4^{a}**	**5,1^{b*}**
EUROP Fat (1-15)	111	6,0	6,2	6,1	6,1	5,9	6,0	6,2	6,2

LSMEANS with different letters were significantly different at level:
* p≤ 0.10
** p≤ 0.05
*** p≤ 0.001

For WBSF-measurements and range, the sires are significant difference (p≤ 0,001) and for standard deviation (p≤ 0,10). The two standard groups were significantly different at WBSF, standard deviation and range (p≤ 0,001), and slaughter day (p≤ 0,10).

The four groups were significantly different (p≤ 0,001) at WBSF, except 1-1 and 1-2 (p≤ 0,10). At standard deviation and range, both group 1-2 and 2-2 stands out. Group 2-2 is significantly different (p≤ 0,001) from the others. The slaughter age was significantly different (p≤ 0,10) between the standard deviation groups. Group 2-1 and 2-2 had a significant difference (p≤ 0,10) in values for EUROP class. No significant differences in EUROP fat class values between the groups. In addition to the parameters above, the colour

of the carcasses was measured and there were no significant differences between the groups. The average, range, minimum and maximum can be found in table 4.1.

Table 4.4 shows the average values and significant differences between the sire-STD groups of different parameters.

Table 4.4: LSMEANS* for different parameters divided into the various groups.

		Sire groups		Standard deviation		Groups (Sire + Std)			
Source	n	Tender (1)	Tough (2)	Low (1)	High (2)	1 1	1 2	2 1	2 2
MMP-2 1h (unit/g)	109	**0,72^{a}**	**0,62^{b***}**	0,69	0,65	**0,78^{a}**	**0,66^{b*}**	**0,59^{b***}**	**0,64^{b**}**
Hydroxyproline (g/kg)	111	0,78	0,81	0,81	0,79	0,79	0,77	0,83	0,80
µ-calpain 1h (unit/g)	87	1,02	0,98	0,98	1,01	1,03	1,00	0,93	1,02
m-calpain 1h (unit/g)	87	1,06	1,10	1,06	1,10	1,07	1,04	1,05	1,16
IMF (%)	111	**1,3^{a**}**	**1,6^{b**}**	1,4	1,4	**1,2^{a}**	1,4^{ab}	**1,7^{b***}**	1,5^{ab}
Water (%)	111	**74,63^{a}**	**74,30^{b**}**	74,48	74,44	**74,69^{a}**	74,56^{ab}	**74,28^{b*}**	**74,31^{b*}**
Iron (mg/100g)	111	1,61	1,64	1,66	1,59	1,66	1,57	1,67	1,62
Calcium (mg/100g)	111	3,28	3,25	3,31	3,21	**3,37^{a}**	**3,19^{b*}**	3,26^{ab}	3,24^{ab}
Colour 1 week									
L	109	41,65	42,49	42,01	42,13	41,83	41,47	42,18	42,79
*a	109	15,62	15,20	**15,90^{a}**	**14,92^{b***}**	**15,81^{a***}**	**15,43^{a**}**	**15,99^{a***}**	**14,41^{b}**
*b	109	**5,11^{a}**	**6,19^{b**}**	5,75	5,55	**5,19^{ab}**	**5,04^{a**}**	**6,32^{c**}**	**6,06^{bc}**

LSMEANS with different letters were significantly different at level:
* p≤0.10.
** p≤ 0.05
*** p≤ 0.01

The MMP-2 activity was clearly significantly different between the sire groups (p≤ 0,001), and especially group 1-1 stands out. The greatest different was between 1-1 and 2-1 (p≤ 0,001). There were significantly lower content of IMF in the tender sire group (p≤ 0,05) than in tough, and also between 1-1 and 2-1 (p≤ 0,001). The water content was higher in the tender sire group (p≤ 0,05), and was significantly higher in 1-1 than 2-1 and 2-2 (p≤ 0,10). Calcium content was higher (p≤ 0,10) in 1-1 than 1-2. The *a colour after one week was significantly higher (p≤ 0,001) in the groups with low standard deviation, 1-1 and 2-1, and significantly higher in (p≤ 0,05) in 1-2. *b had a significantly higher value in the tough sire group (p≤

0,05). There was no significant difference between the samples in content of hydroxyproline, µ- calpain, m- calpain, iron and colour L after one week.

Table 4.5 shows the average pH values and significant differences between the sire-STD groups.

Table 4.5: LSMEANS* of pH

		Sire groups		Standard deviation		Groups (Sire + Std)			
Source	n	Tender (1)	Tough (2)	Low (1)	High (2)	1 1	1 2	2 1	2 2
pH 1h	110	6,54	6,56	6,58	6,51	6,59	6,48	6,57	6,55
pH 6 h	111	**5,88^{a}**	**5,80^{b*}**	5,82	5,86	**5,89^{a**}**	**5,86^{a*}**	**5,76^{b}**	5,85^{ab}
pH 10 h	110	5,64	5,59	5,61	5,62	**5,64^{a*}**	5,63^{ab}	**5,57^{b}**	5,61^{ab}
pH 24 h	106	5,57	5,59	5,56	5,60	5,57^{ab}	5,57^{ab}	**5,55^{a*}**	**5,62^{b}**
pH 48 h	111	5,53	5,52	5,52	5,53	5,53	5,53	5,51	5,52
pH 1-6 h	110	**0,67^{a}**	**0,76^{b**}**	**0,76^{a}**	**0,66^{b**}**	**0,70^{a*}**	**0,63^{a***}**	**0,81^{b}**	**0,70^{a*}**
pH 1-10 h	109	0,91	0,97	0,98	0,91	0,95^{ab}	**0,87^{a*}**	**1,00^{b}**	0,94^{ab}
pH 6-10 h	110	0,24	0,21	0,22	0,24	0,25	0,24	0,19	0,24
pH 1-24 h	105	0,97	0,97	**1,02^{a}**	**0,92^{b*}**	1,02	0,92	1,02	0,92
pH 1-48 h	110	1,01	1,04	1,06	0,99	1,06	0,96	1,07	1,02
pH 6-48 h	111	0,35	0,29	0,30	0,33	0,36	0,34	0,25	0,32
pH 10-48 h	110	0,10	0,07	0,09	0,09	0,11	0,09	0,07	0,08

LSMEANS with different letters were significantly different at level:
* p≤0.10.
** p≤ 0.05
*** p≤ 0.01

The tender sire group had a significantly higher (p≤ 0,1) pH at 6h than the tough sire group. 2-1 was significantly lower than 1-1 (p≤ 0,05) and 1-2 (p≤ 0,10). The pH was higher in 1-1 than 2-1 (p≤ 0,10) at pH 10h. After 24h the 2-2 had highest pH and was significantly higher than 2-1. The pH decrease between 1-6h was significantly higher (p≤ 0,05) in the tough sire group and low standard deviation group than tender sire group and high standard deviation. Between the four groups, the pH decline was greatest in 2-1 and the largest difference was to 1-2 (p≤ 0,001) in addition to 1-1 and 2-2 (p≤ 0,05). There were significant difference between pH decline between 1-10h in 2-1 (p≤ 0,10) and 1-2. Between 1-24h the pH in the low standard deviation group was (p≤ 0,10) lower than the high standard deviation group.

Using the PROC CORR procedure the correlation between the relevant parameters was calculated. Increasing WBSF gave increase of standard deviation for WBSF by 0.61 ($P<0.0001$), decrease of MMP-2 activity by -0.19 ($P<0.05$). An increase of µ- calpain gave and increase of m- calpain 0.62 ($P<0.0001$) (data not shown in table).

The degree of heritability was calculated to be $h^2 = 0.16$ for MMP-2 activity (data not shown in table).

5 Discussion

The studied characteristics in this thesis was more or less in average compared to the 550 bulls slaughtered in the project. MMP-2 activity and hydroxyproline analysis was mostly done for this thesis.

The **sire groups** had a great effect on WBSF, std, range, IMF content, water content and MMP-2 activity, while the **standard deviation** group was effected by slaughter age, WBSF, std, range and *a after one week (Table 4.2 and 4.3). Silva, Patarata & Martins (1999) found that tenderness increased in beef from 1 to 6 days post mortem with no further increase in 13 days. This relates with the 7 day matured samples used for WBSF-measurements. The samples in this thesis were chosen due to their WBSF-measurements and the standard deviation, hence the great differences in WBSF-measured values, standard deviation and range. The IMF content was lower in the tender sires than the tough. This does not correlate with literature (Hocquette et al., 2009). Due to lower IMF content of tender sires higher water content follows. The MMP-2 activity was also affected by sires and the degree of heritability (h^2) was calculated to be 0.16 for MMP-2. The low standard deviation group was slaughtered at higher age, and especially there was a difference between 1-2 and 2-2.

The **slaughter age** affected both of the EUROP classifications, iron content and most of the colour measurements. To be classified as “young bull” the slaughter age has to be between 301-730 days (Animalia, 2011). The 111 bulls in this study ranked between 440 to 770 days and thus some was classified as “bulls”, but the majority was “young bulls”. Their average slaughter weight (310kg) was higher than normal (295kg), but their average EUROP class (O) correspond well with the norm for young bulls (O to O+), and so did their average EUROP fat class (2+) where the norm is 2+ Røe (2009). When cattle get older, there is a larger accumulation of iron in the muscles, and thus will the iron affect the colour of the meat.

The **MMP-2 activity** was significantly higher in the tender sire group and significantly highest in 1-1 than the others. Pambuka et al. (2006) found an increase of MMP activity in ostrich meat after 21-days ageing. This indicates that the activation of MMP happens post mortem and hence plays a part in post mortem tenderisation. Due to relatively stable content of hydroxyproline, µ- and m-calpain activity there seem to be only MMP-2 that affect tenderness in these samples. The MMP-2 method was greatly affected by analyse group (agroup table 4.2), but due to randomisation of the bulls and the presence of each sire in each

group the results are valid. The hydroxyproline analysis only measured the total content of collagen and did not distinguish between degraded and not degraded collagen, but it seems that the bulls in group 1-1 had a higher MMP-2 activity and consistently more tender meat. The correlation analysis indicates that the MMP-2 activity followed WBSF and increased when the WBSF decreased. Other factors that may affect the tenderness is IMF% and water%. In theory the tenderness is positively influenced by greater values of IMF% (Hocquette et al., 2009) but in these samples the tough sire group had a significantly higher content, and hence lower water content.

In contrary to other work (Veiseth & Koohmaraie, 2000; Uytterhaegen et al., 1994ab; Steen et al., 1996), it seems that the **calpains** did not affect the tenderness in this study. The calpain analysis was only finished for 87 of the samples and may have affected the variation in activity. The content was approximately at the same levels in all of the samples, only 2-1 stands out slightly ($p \leq 0{,}18$) and may be one of the difference between 2-1 and 2-2. The correlation analysis indicates that the µ-calpain activity followed m- calpain activity and increased when m-calpain increased. Thelack of difference of the calpain activity the toughness in the meat can be due to myofibrillar toughness.

The detected **calcium** content was significantly higher in 1-1 than 1-2. Christensen et al. (2003) concluded that the calcium content did not affect the weakening of the myofibrillar components but the initial stiffness of the muscle fibres. Since the difference in calcium content was significant between the two tender groups, the calcium content may have affected the 1-1 to become tender throughout the piece of meat.

The meat **colour** 24h post mortem was measured to be darker (L-value) and with higher intensity (*a and *b values) than when measured on the bloomed (1h) surface of the conditioned filet 7 days post mortem. The colour depends on multiple factors such as breed, gender, age, weight, fat content and diet. Grass feed animals tend to have more yellow meat (Dunne et al., 2008), and an increase of age in cattle increases the myoglobin content in the muscles, hence the iron content and colour (lighter L-values). After one week the *a parameter was more intense in the low standard deviation group. The a* one week post mortem was significantly lower in 2-2 than the rest. The *b one week post mortem was lower in the tender sire group. In the (sire + std) groups there were significantly differences between most of the groups. This may be due to different absorbance of light waves in the surface of the muscle structure. The colour measuring can be affected by the fibre type. Red fibres have

a larger content of myoglobin that will affect the L-value to decrease. Red fibres consist of type IA fibres, while white consist of type IIB. Typical for type IA fibres is higher content of myoglobin, hence more intense *a and *b and lower L-value. The IMF% and iron content are also higher than for type IIB. Type IIB have lower content of myoglobin, hence less intense *a and *b with a greater L-value. The IMF% and iron content is lower than in type IA. These fibre differences may be part of the reason why there is differences between the samples.

One of the bulls had an abnormal high **pH** both at 1h (7.47) and ultimate pH 48h (6.87). This is most likely due to pre-slaughter stress that depletes the glycogen storage and results in DFD-meat. The rest of the animals had a normal pH drop from about 6.56 down to 5.54. Some of the factors that influence the drop in pH pre-slaughter is hereditary factors, feeding and ante mortem stress (Hollung et al., 2007). There was a significant difference in the pH drop 1-6h between the sire groups, where the tough with low standard deviation dropped more rapidly. In theory a rapid pH drop induces an earlier start of proteolysis and hence a more tender meat. The post mortem glycolysis is affected by pre-slaughter stress and feeding among others (Hollung et al., 2007). A rapid glycolysis gave Hwang & Thompson (2001) a lower shear force value after both 1 day and 14 days. Their samples with a higher shear force were associated with later activation of calpains. In contrast to this in this study it seems that slower pH decline results in more tender beef.

6 Conclusions

The 111 animals analyzed in this thesis were selected on the basis of the WBSF-measurements and high and low standard deviations. Not surprisingly the four groups were significantly different at WBSF, standard deviation and range.

There are a few parameters in this study that are very interesting in terms of affecting tenderness in beef. Those are the hydroxyproline content, IMF content, the activity of the calpains and MMP-2. When the levels of these are approximately the same in all of the samples, there must be something else affecting the tenderness such as MMP-2. There is little research available on the relation between MMP-2 activity and tenderness in beef, but due to little effect of other tenderisation parameters in this study there is likely to be a connection. In this master thesis the selection of animals is low and gives only an indication of the effect of MMP-2. It would therefore be necessary to do more experiments to drawn final conclusions. The μ-calpain had a tendency of lower activity in 2-1, but this was not significant and may be affected by the still not finished calpain analysis.

One parameter not been taken into account is the MMP-9 bands. During the MMP-2 analysis there were some other bands detectable in the gels, MMP-9 bands. For further study it would be interesting to figure out if there is a parallel towards tenderness for MMP-9.

References

Animalia (2011) *Storfe: Nye klassifiseringsregler*. Available at: http://www.animalia.no/Artikler/2011/Storfe-Nye-klassifiseringsregler/ (Assesed: 12.05.2012)

Archile- Contreras, A.C., Mandell, I.B. & Purslow, P.P. (2010) Phenotypic differences in matrix metalloproteinase 2 activity between fibroblasts from 3 bovine muscles. American Society of Animal Science. *Journal of Animal Science*. P. 4006-4014,

Balcerzak, D., Querengesser, L., Dixon, W. T. & Baracos, V. E. (2001) Coordinate expression of matrix-degrading proteinases and their activators and inhibitors in bovine skeletal muscle. *Journal of Animal Science*, 79:94-107.

Barrett, A. J. (1977) *Proteinases in mammalian cells and tissues.* North- Holland publishing company. Volume 2

Boles, J.A. and Pegg, R. (2012) *Meat Color*. Available at: http://www.safespectrum.com/pdfs/meatcolor.pdf (Assesed 25.04.2012)

Brande, C. & Tooze, J. (1999) *Introduction to Protein Structure*. Second Edition. Garland Publishing, Inc.

Cha, M. C. & Purslow, P.P (2009) Matrix metalloproteinases are less essential for the in-situ gelatinolytic activity in heart muscle than in skeletal muscle. j.cbpa.2010.04.014

Cheftel, J.C., Duq, J. L. & Lorient, D. (1985) *Chapter* 5 Amino acids, peptides and Proteins. In *Food Chemistry*, Fennema, O.R. (red.). Second edition. Published by Marcel Dekker, Inc. ISBN: 0-8247-7271-7

Christensen, M., Young, R.D., Lawson, M.A., Larsen, L.M. & Purslow, P.P. (2003) Effect of added μ-calpain and post mortem storage on the mechanical properties of bovine single muscle fibres extended to fracture. *Meat Science*, 66: 105-112

Creighton, T.E. (1997) *Proteins Structures and Molecular Properties.* Secon edition. W.H. Freeman and company, New York

Dunne, P.G., Monahan, F.J., O'Mara, F.P. & Moloney, A.P. (2008) Colour of bovine subcutaneous adipose tissue: A review of contributory factors, associations with carcass and meat quality and its potential utility in authentication of dietary history. *Meat Science* 81 (2009) 28–45

Gerelt, B., Rusman, H., Nishiumi, T. & Suzuki, A. (2004) Changes in calpain and calpastatin activities of osmotically dehydrated bovine muscle during storage after treatment with calcium. *Meat Science* 70 (2005) 55-61

Geno (2009) *Avlsmålet for NRF*. Available at: http://www.geno.no/no/Forsiden/NRF/Avlsmal/Avlsmalet-for-NRF/ (Assesed: 25.April 2012)

Geesink, G. H. & Veiseth, E. (2009) Muscle enzymes: proteinases. In: *Nollet*, L.M.L, Toldra, F. (Eds.), Hand Book of Muscle Food Analysis. CRC Press, Boca Raton

Grandin, T. (2001) Chapter 10 Antemortem Handling and Welfare. In *Meat Science and Application,* Hui, E.H. et. al (red.). A. Marcel Dekker, Inc. ISBN: 0-8247-0548-3

Greaser, M. L. (2001) Chapter 2 Postmortem Muscle Chemistry. In *Meat Science and Application,* Hui, E.H. et. al (red.). A. Marcel Dekker, Inc. ISBN: 0-8247-0548-3

Hemmer, E. (1997) *Kjøtt- teknologi*. Tapir forlag, Trondheim. ISBN: 82-519-1278-4

Hollung, K., Veiseth, E., Frøystein, T., Aas, L., Langsrud, Ø. & Hildrum, K. I. (2007) Variation in the response to manipulation of *post mortem* glycolysis in beef muscles by low-voltage electrical stimulation and conditioning temperature. *Meat Science* 77 (2007) 372-383

Hocquette, J.F., Gondret, F., Médale, F., Jurie, C. & Pethick, D.W. (2009) Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. The Animal Consortium 2009. *Animal* (2010), 4:2, pp303-319

Hunterlab (2008) *Hunter L, a, b Color Scale*. Vol. 8,No 9 Availabel at: http://www.hunterlab.com/appnotes/an08_96a.pdf (Assesed: 10.05.2012)

Hwang I.H. & Thompson, J.M. (2001) The interaction between pH and temperature decline early postmortem on the calpain system and objective tenderness in electrically stimulated beef *longissimus dorci* muscle. *Meat Science* 58 (2001) 167-174

Kemp, C. M., Sensky, P. L., Bardsley, R. G., Buttery, P. J. & Parr, T. (2009) Tenderness- An enzymatic view. *j.meatsci*.2009.06.008 page 248- 255.

Kim, Y.S. (2001) Chapter 24 Meat Production. In *Meat Science and Application,* Hui, E.H. et. al (red.). A. Marcel Dekker, Inc. ISBN: 0-8247-0548-3

Koohmaraie, M. & Geesink, G.H. (2006) Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. *Meat Science* 74 (2006) 34-43

Koohmaraie, M., Kent, M.P., Shackelford, S.D., Veiseth, E. & Wheeler, T. (2002) Meat tenderness and muscle growth: is there any relationship? *Meat Science* 62 (2002) 345-352

Lovdata (2005) Forskrift om endring i forskrift om transport av levende dyr. [online] http://www.lovdata.no/ltavd1/filer/sf-20041007-1555.html [10.05.2012]

Mannello, F. & Medda, V. (2011) Nuclear localization of Matrix metalloproteinases. *j.proghi*.2011.12.002

Medicalook(2012) *Skeletal muscle fiber.* Available at: http://www.medicalook.com/human_anatomy/organs/Skeletal_muscle_fiber.html (Assesed 11.04 2012)

Morton, J. D., Bickerstaffe, R., Kent, M. P., Dransfield, E. & Keeley, G.M. (1999) Calpain-calpastatin and toughness in *M. longissimus* from electrically stimulated lamb and beef carcasses. *Meat science* 52 (1999) 71-79

Nishimura, T., Hattori, A. & Takahashi, K. (1994) Structural Weakening of Intramuscular Connetive Tissue during Conditioning of Beef. *Meat Science* 39 (1995) 127 133

Nyhus, G. (1990) *Norge rundt med NRF*. Landbruksforlaget, Oslo ISBN:82-529-1419-5

Omafra (2011) *On- Farm Euthanasia of Cattle and Calves*. Available at: http://www.omafra.gov.on.ca/english/livestock/animalcare/facts/info_euthanasia_cc.htm (Assesed: 02.05.2012)

Pambuka, S.E, Adebiyi, A.P., Muramoto, K. & Naudé, R.J. (2006) Purification and partial characterisation of a matrix metalloproteinase from ostrich skeletal muscle, and its activity during meat maturation. *Meat Science* 76 (2007) 481–488

Pérez-Chabela, M.D.L & Legarreta, I.G. (2001) Chapter 11 Slaughtering and Processing Equipment. In *Meat Science and Application*, Hui, E.H. et. al (red.). A. Marcel Dekker, Inc. ISBN: 0-8247-0548-3

Purslow, P. P. (2002) The structure and functional significance of variations in the connective tissue within muscle. *Comparative Biochemistry and Physiology Part A* 133 (2002) 947-966

Purslow, P. P. (2005) Intramuscular connective tissue and its role in meat quality. *Meat Science* 70 (2005) 4-35-447

Purslow, P. P. (2010) Muscle fascia and force transmission. *Journal of Bodywork & Movement Therapies* (2010) 14, 411e417

Purslow, P. P., Archile- Contreras, A.C. & Cha, M. C. (2011) Meat Science and muscle biology symposium: Manipulating meat tenderness by increasein the turnover of intramuscular connective tissue. *J ANIM SCI* 2012, 90:950-959

Ree, M. S., Wheeler, T. L., Shackelford, S. D. & Koohmaraie, M. (2004) Variation in plalability and biochemical traits within and among eleven beef muscles. *J ANIM SCI* 2004, 82:534-550

Røe, M. (2009) *Ung okse i 2008: Nedgang i tilførsel*. Available at: http://www.animalia.no/Artikler/2008/Storfe-i-2007-Hoyere-slaktevekter-og-hoyere-klasser/ (Assessed: 12.05.2012)

Silva, J.A., Patarata, L. & Martins, C. (1999) Influence of ultimate pH on bovine meat tenderness during ageing. *Meat Science* 52 (1999) 453-459

Snoek-van Beurden, P.A.M. & Von den Hoff, J.W. (2005) Zymographic techniques for the analysis of matrix metalloproteinases and their inhibitors. *BioTechniques* 38:73-83

Solem, T. (2007) *Norsk, rød og internasjonal.* Available at: http://www.forskning.no/artikler/2007/desember/1196939694.42/print (Assesed: 25.04 2012)

Steen, D., Claeys, E., Uytterhaegen, L., De Smet, S. & Demeyer, D. (1996) Early Post- Mortem Conditions and the Calpain/Calpastatin System in Relation to Tenderness of Double- muscled Beef. *Elsevier Science, Ltd.* 0309-1740

Schwartzkopf-Genswein, K.S., Faucitano, L., Dadgar, S., Shand, P., González, L.A. &Crowe, T.G. (2012) Road transportation of cattle, swine and poultry in North America and ist impact on animal welfare, carcass and meat quality: a review. *J.meatsci.*2012.04.010

Thompson, J. (2002) Managing meat tenderness. *Meat Science* 62 (2002) 295-308

Underwood, K. R., Means, W. J. & Du, M. (2008) Capase 3 is not likely involved in the post mortem tenderixation of beef muscle. *J ANIM SCI* 2008: 86:960-966

Uytterhaegen, L., Claeys, E. & Demeyer, D. (1994a) Effects of exogenous protease effector on beef tenderness development and myofibrillar degradation and solubility. *J ANIM SCI* 1994, 72:1209-1223

Van Moeseke, W., De Smet, S., Clayes, E. & Demeyer, D. (2001) Very fast chilling of beef: effects on meat quality. *Meat Science* 59 (2001) 31-37

Veiseth, E. & Koohamarie, M. (2000) Effect of extraction on estimating calpain and calpastatin activity in post mortem ovine muscle. *Meat Science* 57 (2001) 325-329

Veiseth, E., Shackelford, S.D., Wheeler, T.L. & Koohmaraie, M. (2003) Indicators of Tenderization are detectable by 12 h post mortem in Ovine Longissimus. *J ANIM SCI* 2004: 82(5):1428-36

Villarroel, M., María, G.A., Sañudo, C., Olleta, J.L. & Gebresenbet, G. (2002) Effect of transport time on sensorial aspects of beef quality. *Meat Science* 63 (2003) 353-357

Wheeler, T.L. & Koohmaraie, M. (1994) Prerigor and postrigor changes in tenderness of ovine longissimus muscle. *J.Anim Sci,* 72 (5): 1232-1238

Wheeler, T.L., Shackelford, S.D & Koohmaraie, M. (2004) The accuracy and repeatability of untrained laboratory consumer panellists in detecting differences in beef longissimus tenderness. *J ANIM SCI* 2004, 82:557-562

Woessner, J.F.Jr.(1991) Matrix metalloproteinases and their inhibitors in connective tissue remodelling, *FASEB J.*, 2145,

Young, O.A et al. (2001) Chapter 13 Carcass Processing: Quality Controls. In *Meat Science and Application,* Hui, E.H. et. al (red.). A. Marcel Dekker, Inc. ISBN: 0-8247-0548-3

Young, O.A. & Gregory, N.G. (2001) Chapter 12 Carcass Processing: Factors Affecting Quality. In *Meat Science and Application,* Hui, E.H. et. al (red.). A. Marcel Dekker, Inc. ISBN: 0-8247-0548-3

Zamora, F., Chaïb, F. & Dransfield, E. (1998) Calpains and Calpastatin from Cold-shortened Bovine *M. longissimus lumborum*. *Meat Science,* Vol. 49, No. 1, 127-133,1998