Influence of Pelvic Suspension on Beef Meat Quality

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Cover: *M. semimembranosus* from the same carcass; the left muscle is from the pelvic suspended side and the right is from the achilles suspended side.
(photo: Maria Lundesjö Ahnström)

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Abstract

The aim of this thesis was to investigate the effect of pelvic suspension on beef with different background and different genders. The response to pelvic suspension was studied in *longissimus*, *semimembranosus*, *adductor*, *gluteus* and *psoas* muscles from young bulls, bulls, heifers and cows. Prolonged ageing time until 14 days was evaluated for *longissimus* from heifers.

It was concluded that pelvic suspension reduced shear force values for pelvic- compared to achilles-suspended sides in all muscles, except *psoas,* from young bulls and bulls. This decrease in shear force was not found for cows. Heifers responded differently to pelvic suspension depending on breed and showed decreases in shear force for beef- but not for dairy heifers. The variation in shear force, both within sample and between animals, decreased after pelvic- compared to achilles-suspension. Pelvic suspension effectively reduced the need of prolonged ageing time since meat aged 7 days was similar in tenderness to meat from achilles suspended sides aged for 14 days. Sarcomere lengths were elongated in *semimembranosus* for all gender categories after pelvic suspension. Elongation was also found in *longissimus*, *gluteus* and *adductor* for young bulls, bulls and cows. The variation in sarcomere length did not show the same consistent decrease due to pelvic suspension as shear force but was instead increased especially in *longissimus* samples. Purge during ageing and freezing loss decreased in pelvic suspended sides, especially in *longissimus* and *semimembranosus*.

Sensory traits were evaluated for beef heifers and were clearly improved in pelvic suspended samples. Increased tenderness and less visible marbling was found after pelvic suspension. Tenderness also benefitted from four months extended rearing. Pelvic suspension did, however, reduce the need of finishing, since meat from heifers slaughtered directly from pasture was similar in tenderness to meat from heifers after prolonged rearing.

Pelvic suspension is concluded to be a good tool to decrease purge, reduce variation and improve tenderness in beef.

Keywords: Beef, pelvic suspension, tenderness, sarcomere length, shear force, ageing time, young bull, heifer, cow

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Dedication

To the Swedish Beef Industry

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Maria Lundesjö Ahnström, Ann-Charlotte Enfält, Ingemar Hansson and Kerstin Lundström (2006). Pelvic suspension improves quality characteristics in *M. semimembranosus* from Swedish dual purpose young bulls. *Meat Science* 72, 555-559.

II Maria Lundesjö Ahnström, Melvin C. Hunt and Kerstin Lundström. Meat quality in five pelvic suspended muscles from four beef categories (manuscript).

III Maria Lundesjö Ahnström, Anna Hessle, Lisbeth Johansson, Melvin C. Hunt and Kerstin Lundström (2008).Influence of carcass suspension on meat quality of Charolais heifers from two sustainable feeding regimens. *Animal* (accepted).

IV Maria Lundesjö Ahnström, Anna Hessle, Lisbeth Johansson, Melvin C. Hunt and Kerstin Lundström. Influence of slaughter age and carcass suspension on meat quality in Angus heifers (manuscript).

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The greatest virtue of man is perhaps curiosity.
Anatole France

Introduction

Swedish Beef Production

Animal material

Production of beef in Sweden has historically been based on a national cow herd composed predominately of dairy-type cattle. The numbers of dairy cows has, however, decreased about 17% from 1997 to 2006. Since the 1970s the number of suckler herds has increased. To some extent this shift to suckler herds has slowed down the downward trend of beef production (Swedish Board of Agriculture, 2007b).

In 2007, Sweden had about 370 000 dairy cows and 186 000 beef cows. The total number of cattle including heifers, bulls, steers and calves was 1 560 000. The average suckler herd had 15 cows and the average dairy herd had 52 cows. A total of 24 000 beef-producing farms delivered on average 17.6 animals for slaughter during 2007 (SCB, 2008). The number of cattle slaughtered from one farm at a time is generally below five. Therefore, the production background of the 130 000 metric tonnes of beef meat produced in Sweden each year varies considerably. The distribution of gender categories is illustrated in Fig. 1 where it is clear that the major part of the meat is produced from young bulls and cows. The ratio of cattle originating from beef or dairy production also is illustrated; about 70% of the meat originates from animals in the dairy production. It should be noted that the dairy population illustrated in Fig. 1 is pure bred cattle from Swedish Red and Swedish Holstein whereas the beef breeds are represented by Continental beef breeds, British beef breeds, some Swedish traditional breeds and a variety of cross-breeds between these dairy and beef breeds.

While the domestic beef production has decreased, the consumption of beef in Sweden has increased substantially and is now 25.7 kg per person and year. In 1997, 84% of the consumed beef was from Swedish production and in 2007 this number was 58%. Thus the degree of self-sufficiency has decreased. An increased import of beef covers the increased demand (Fig. 2). Fresh beef is mainly imported from Ireland, Germany, Denmark and Brazil. Sweden exports a small quantity of beef mainly to neighbouring countries (Swedish Board of Agriculture, 2007b).

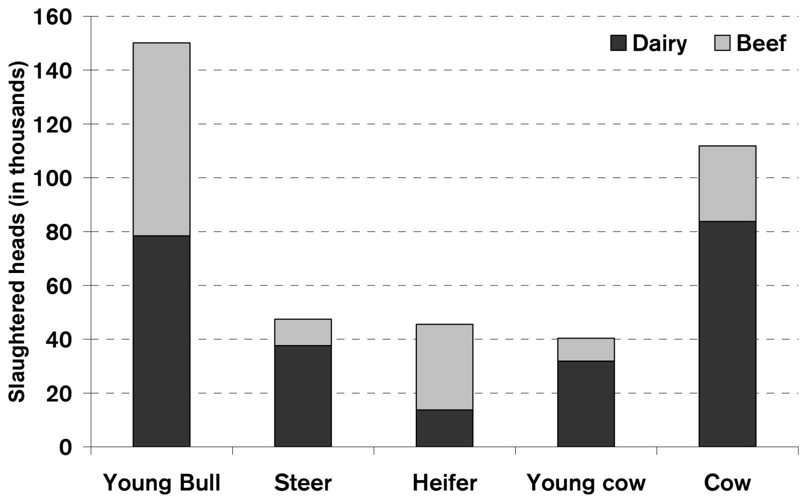

Figure 1. Number of cattle of beef breeds (including cross-breeds) and dairy breeds slaughtered in Sweden during 2007 (data from Taurus, 2008).

Cattle rearing

In Sweden, a majority of all cattle are raised outdoors on pastures during the summer and with winter housing during the cold season. The calves from suckler herds are most often born during the spring and held on pasture together with the cows through summer. When the bull calves are weaned in the autumn, they are housed and intensively fed grass/clover-silage and grain-based concentrates until an average age of 17.3 months and a weight of 343 kg. Bull calves from dairy herds are fed grass/clover-silage and grain-based concentrates in varying proportions. They are generally slaughtered at 18.7 months weighing 310 kg. It is common to raise the male calves as intact bulls. In 2007, only 11% of the cattle slaughtered were steers (42% were bulls). Steers had an average age of 25.8 months and an average weight of 321 kg at slaughter. The heifers raised for slaughter are generally slaughtered at 24.6 months old and a weight near 279 kg (slaughter data from Taurus, 2008). The production of young cows is a minor part of the production scheme in Sweden and is generally the result of culled young cows from both dairy and beef production.

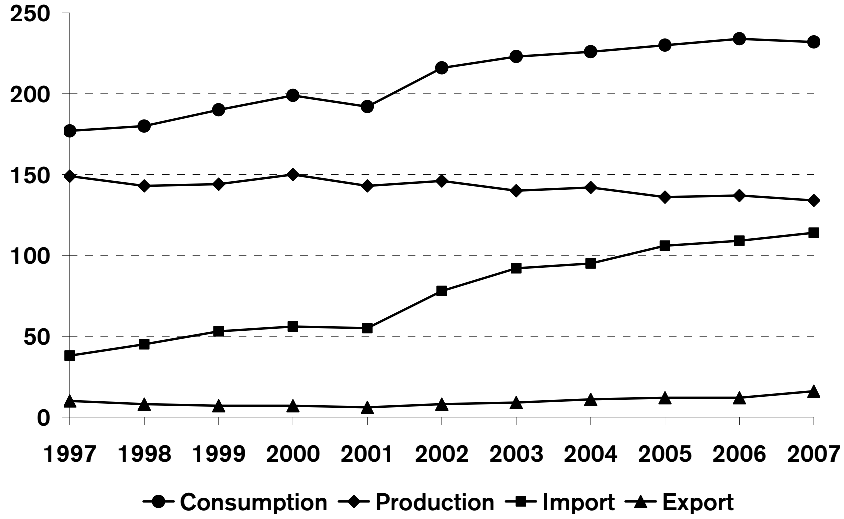

Figure 2. Production, consumption and trade of beef in Sweden 1997-2007, 1000 metric tons. (Data from Swedish Board of Agriculture, 2007b).

Slaughter

In 2007, 420 000 adult cattle were slaughtered and four slaughterhouses accounted for more than half of that slaughter. These plants kill between 700 and 1 500 heads per week. There is, however, a large range of sizes of slaughterhouses, the statistics for 2007 include 47 companies with yearly slaughter ranging from 2 to 78 400 heads of adult cattle. Most of the slaughterhouses in Sweden are integrated and slaughter more than one species; it is therefore possible that they have larger production if including all slaughtered animals at the plant (Swedish Board of Agriculture, 2007a). In 2007, there were 10 slaughterhouses that slaughtered more than 10 000 heads of adult cattle, of which 3 are no longer in operation (August 2008).

Animals are generally stunned by using bullet or captive bolt. The slaughter is performed dry with no rinsing of carcasses. Electrical stimulation is when used done by low voltage either right before evisceration or directly after. All carcasses are graded judging conformation and fatness according to the EUROP schemes modified to the Swedish system in which 15 classes are used (Commission of the European Communities, 2005; Swedish Board of Agriculture, 1998). Pelvic suspension, when used, is performed after grading just before the carcasses enter the cooler, approximately one hour after stunning. Cooling is done using higher air flow during the first cooling cycle to dry the carcass surface and improve hygienic quality. Carcasses are then stored until one or two days after slaughter before cutting. There are of course variations between slaughterhouses in the methods applied.

Electrical stimulation is used in 8 out of 23 slaughterhouses that slaughtered more than 1 000 heads in 2007. The electrical stimulation is in most cases combined with intensive chilling, below 0°C, with high air velocity for approximate one hour followed by storage in 2-4°C until cutting. Many of the slaughterhouses that do not use electrical stimulation use cooling, involving temperatures from 2-5°C with higher air velocity during the first day. No slaughterhouse reported cooler temperatures above 6°C.[1]

Pelvic suspension has, since this doctoral work was initiated, been introduced in parts of the Swedish meat industry and is today used limitedly as a quality assuring method for high quality meat. Approximately 3% of the Swedish slaughter is suspended from the pelvic bone.

Most beef sold in Sweden is cut from the carcass two days after slaughter and the cuts are then aged in vacuum. There is a recommendation from the Swedish Meat Industry Association that meat should not be sold to the consumer until 7 days after slaughter. During the last five years a transformation has occurred in the way meat is sold to Swedish consumers. Meat is most commonly aged in vacuum and was earlier cut and prepared for consumer packages in the shops. Now, processing, including slicing, cubing and grinding, have been centralised and the preparation for consumer packages are done centrally. Modified atmosphere packaging (MAP) with high oxygen has become the most common delivery system but vacuum packages and meat on trays wrapped in plastic film are also found in the shops.

[1] Information gathered by the author through phone calls to the 23 slaughterhouses that slaughtered more than 1,000 adult cattle in 2007 and was still operating in August 2008.

Meat quality

Consumer concerns about meat quality are focused on one major factor: meat tenderness (Miller *et al.*, 2001). The inconsistency in tenderness and prevalence of tough meat are the major issues for the beef meat industry world-wide. This has lead to more than 50 years of meat quality research with the goal of improving meat tenderness. Numerous methods are currently used to improve tenderness including electrical stimulation, pelvic suspension of carcasses and prolonged ageing times. To understand more about how these techniques influence meat quality it is important to know what meat is and how muscle is converted to meat.

Meat structure

Muscle tissue consists of about 75% water and 20% protein. The remaining 5% is mainly fat together with small amounts of carbohydrates (glycogen) and minerals. The muscle proteins have wide ranging physiological functions. The contractile (myofibrillar) proteins, mainly actin and myosin, make contraction of the muscle possible and the structural proteins, collagen and elastin, provides support forming connective tissue. Enzymes, hormones and antibodies are other important proteins as are haemoglobin and myoglobin, which are responsible for the oxygen transport in the blood and muscle. The myofibrillar proteins represent ~60% of the muscle proteins whereas sarcoplasmic proteins represent ~30% and about 10% are structural proteins (Lawrie & Ledward, 2006).

The contractile and structural proteins and their contribution to meat properties will be further discussed below together with the importance of intramuscular fat.

Muscle

Skeletal muscle is the most important component of meat and it is therefore important to know the detailed structure of muscle to be able to understand how meat quality can be affected.

The structure and function of mammalian skeletal muscles have been described by many authors (for example Sjaastad *et al.*, 2003). The main functions of muscles in the living animal are to generate contraction forces that can be transmitted through the skeletal limbs and result in movement. The long myofibres that are organised in bundles in the muscle structure contain myofibrils with the contractile proteins actin (thin filament) and myosin (thick filament) that perform most of the work during contraction

(Harper, 1999). Actin and myosin are in the myofibrils organised in repeating structures called sarcomeres. Sarcomeres give the muscle fibres apparent striations visible in microscope (Fig. 3). The length of one sarcomere is defined as the distance between the Z lines and varies with the state of contraction of the muscle (Huxley & Hanson, 1954). Within the sarcomere, structure is held in place by a cytoskeleton composed of a number of other proteins, titin, nebulin troponin, desmin and filamin, recently reviewed by Lonergan & Lonergan, (2008). The fluid (sarcoplasm) surrounding the myofilaments contain the water-soluble sarcoplasmic proteins such as proteolytic and glycolytic enzymes, the heme pigments and other cellular elements (Lawrie & Ledward, 2006).

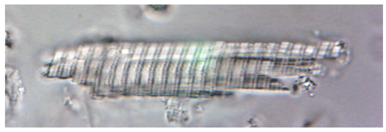

Figure 3. Part of a myofibre under the microscope with the sarcomeres visible as striations along the fibre. Picture taken during sarcomere length analysis.

Muscles can only contract, not stretch themselves, and therefore they need to be arranged in pairs or opponent groups around the skeleton. These opposing pairs balance contraction and relaxation making movement possible (Sjaastad *et al.*, 2003).

The contraction of muscles has been widely investigated and the sliding filament theory was proposed in 1954 by Huxley and Hanson. The myofilaments slide over one another and create overlaps of different degree, hence producing different amounts of contractile force. The maximum force is obtained when all binding-sites of the thick filaments are aligned to the thin filaments (Fig. 4). The degree of overlap can be detected by the change in sarcomere length.

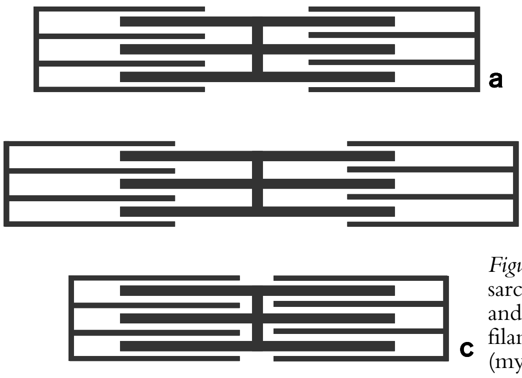

Figure 4. Schematic drawing of one sarcomere in relaxed (a), stretched (b) and shortened (c) state. The thin filaments (actin) and thick filaments (myosin) show different degree of overlap due to state of contraction.

Muscles in living animals contract as a response to nervous stimulation. Such stimulation leads to a large release of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum into the sarcoplasm. The increase in Ca^{2+} around the proteins of the sarcomere units removes the normal inhibition of attraction between actin and myosin and a contraction can occur. In a resting muscle ATP (adenosine triphosphate) helps to keep the muscle in a relaxed state because it prevents the temporary formation of actomyosin. When ATP is hydrolysed to ADP (adenosine diphosphate) contraction can occur. Energy in the form of ATP is therefore needed in the contraction process as well as the relaxation phase. ATP is mainly formed through glycolysis from glycogen in the muscles or from free fatty acids or glucose in the blood. ATP is used both as energy supply for the contractile elements and to fuel the calcium pump of the sarcoplasmic reticulum. When Ca^{2+} concentration is decreased the inhibition between actin and myosin returns, the contraction ceases and the filaments return to a relaxed state (Sjaastad *et al.*, 2003).

Connective tissue

The structural proteins include collagen and elastin that form connective tissue. Collagen is the main component of connective tissue and is present in several forms. The collagen fibres are made up of long rod-like molecules formed by polypeptide chains twisted together to form a coiled triple helix. Cross-links formed between these rod-like molecules give collagen its strength. The cross-linking makes the collagen more stable and resistant to tensile stress. When animals get older the cross-links become more stable and the diameter of the collagen fibrils increase. The connective tissue has different properties and names depending on where in the muscle it is located. The whole muscle is surrounded by and contained within the epimysium and the connective tissue surrounding the muscle fibre bundles is called perimysium. A fine network of endomysium encloses the individual muscle fibres. Collagen fibres are straight and non-branching. They are also inextensible in contrast to elastin fibres which are branched and elastic (Lawrie & Ledward, 2006).

The epimysium is the thickest and most rigid connective tissue sheet associated with muscle but it does not contribute much to the perceived toughness of meat since it is generally located on the outside of the muscle and cut away before eating. Instead over 90% of the intramuscular connective tissue of meat is located in the perimysium (McCormick, 1994).

Connective tissue is believed to represent the background toughness, more or less insensitive to ageing, in beef (Bailey & Light, 1989). The collagen and elastin content varies much between muscles within a carcass and is largely responsible for the different tenderness properties of muscles (Jeremiah *et al.*, 2003; Prost *et al.*, 1975). The amount of connective tissue is also related to the gender and age of the animal with higher content in males and older individuals (Prost *et al.*, 1975; Valin, 1995) .

Intramuscular fat

Fat occurs in the carcass as subcutaneous, intermuscular and intramuscular. Intramuscular fat consist of fat cells situated in the perimysium and the endomysium, surrounding the myofibrils and muscle fibre bundles (Wood, 1990). The content of intramuscular fat (marbling) is of main importance for cuts presented as slices or steaks. It contributes primarily to the experiences of flavour and juiciness and, to a lesser extent, the tenderness of meat (Webb & O'Neill, 2008). Intramuscular fat is known to develop late in the maturation of cattle and the content of fat will increase with higher age (Pethick *et al.*, 2004). In some countries, e.g. Australia, marbling is rated in the classification system (Meat Standards Australia, 2008). In the European countries consumers generally select against meat with much marbling (Ngapo & Dransfield, 2006).

Conversion of muscle to meat

When the animal is slaughtered the conversion of muscle to meat starts. After slaughter the carcasses are generally cooled for a day or more before the muscles are cut from the carcass and then kept for further tenderization or ageing before it is presented to the consumer. Throughout this time different biochemical changes occur in the muscles.

Muscles continue to metabolise after death and the first major difference after slaughter is the absence of blood flow and therefore lack of oxygenation of muscles and the blockage of removal of waste products. This process was thoroughly described in the late 1940s by Bate-Smith (1948). The lack of oxygen in the muscle leads to a shift from aerobic production of energy to reactions that are anaerobic. The breakdown of glycogen to produce ATP via anaerobic chemical pathways now generates lactic acid, which accumulates in the muscle. The lactic environment makes muscle pH fall from a neutral pH of about 7 to an ultimate pH of around 5.5 to 5.7. Glycolysis is eventually stopped by depletion of glycogen storages or when the enzyme systems associated with glycolysis are deactivated due to the low pH.

When glycolysis is halted no more ATP can be produced and since the muscles still metabolise the ATP will eventually be depleted. *Rigor mortis* occurs when the level of ATP is too low to maintain relaxation. At *rigor* the actin and myosin filaments attach irreversibly to each other and form actomyosin. The cross-bridges formed are permanent. Each muscle fibre goes into *rigor* individually when ATP is locally depleted and the individual *rigor* contractions gradually increase overall muscle stiffness (Honikel *et al.*, 1983). This increase in muscle stiffness is generally significant when pH is about 6 (Thompson *et al.*, 2006). The *rigor* shortening is restricted by the skeleton and tendons, muscles can contract until restraint limits further action.

Beef carcasses cool slowly due to their large bulk. Effective cooling systems have been introduced to enhance the microbiological status of the carcasses and make a high throughput possible (Lawrie & Ledward, 2006). The temperature of the muscle when it enters *rigor* has been shown to significantly affect the muscle shortening. Locker and Hagyard (1963) showed that minimal shortening occurred when muscles were held at 15°C and maximal shortening about 2 to 4°C. This cold-induced toughening called cold shortening occurs because the calcium-pump in the sarcoplasmic reticulum does not work well under cold conditions. The calcium ions activate the binding between actin and myosin and contraction can occur. This is mainly a problem for muscles close to the carcass surface and especially in carcasses without a protective fat cover on the back muscles. Cold shortened meat does not tenderize much during ageing. It has been suggested that cold shortening can be prevented if the muscle temperature does not fall below 10°C until muscle pH reaches 6 (Honikel *et al.*, 1983). Simmons *et al.* (2008) have recently re-evaluated the conditions needed to produce cold shortening. They showed that to avoid cold-induced toughening muscle pH should be below 6.2 when the sample temperature was reduced to 0°C. Very extreme cooling conditions would have to be applied to the carcasses to achieve cold-induced toughening by this new criterion.

Electrical stimulation

Electrical stimulation is done to accelerate *rigor mortis* and cause a more rapid pH decline in the muscles. This is performed by passing an electrical current through the carcass stimulating the muscles to contract and utilize glycogen and ATP (Hwang *et al.*, 2003; O'Neill *et al.*, 2004; Strydom *et al.*, 2005). Carcasses can be chilled faster since the muscle is less responsive to the cold temperatures thus resulting is less cold-induced shortening and toughening. Electrical stimulation without more rapid chilling can lead to less desirable colour and colour stability of muscles that chill more slowly e.g. muscles in the round (Simmons *et al.*, 2008).

Electrical stimulation can, besides from preventing cold shortening, also influence the ageing process positively through a more rapid onset of *rigor* and hence earlier activation of proteolysis (Dransfield, 1994). The improvement in tenderness achieved by electrical stimulation is similar to the effect of pelvic suspension (Taylor, 1996).

Ageing

Several authors have reported the improving effect of ageing on meat tenderness. The response of ageing, however, depends on multiple factors related to the animal such as species, age, sex and diet and several factors related to the meat such as location of cut, degree of muscle shortening, the temperature during ageing and length of the ageing period. The pH decrease and the temperature during chilling also substantially affect the ultimate tenderness. Jeremiah and Martin (1978) stated that the combined effects of the temperature 2°C and the ageing time 6 days were successful in producing acceptable tenderness in meat from young cattle of all sexes, sizes and degrees of fatness.

Tenderisation during ageing depends predominantly on the activity of the proteolytic enzymes present in muscles. In the living tissue proteolytic enzymes break down and recycle proteins continuously. During *post mortem* proteolysis in meat the calpains are the most important enzymes; they occur in several forms but *m*-calpain and μ-calpain are so far the best categorised (Goll *et al.*, 2003). These enzymes require calcium to be active and the names refer to the levels of calcium needed for activation. Calpains do not degrade the major myofibrillar proteins actin and myosin, but considerably affect other myofibrillar proteins associated in the sarcomere (Goll *et al.*, 2003). The protein inhibitor of calpains, calpastatin, prevents calpains from binding to membranes and the subsequent protein degradation. Calpains eventually degrade themselves and their inhibitor calpastatin, the rate at which this occurs is dependent on the rate of glycolysis and tenderisation in meat (Goll *et al.*, 1995). Proteolysis is more complex than one enzyme and the degradation of one or two substrate proteins. It is more likely the combined action of multiple proteases and their target myofibrillar proteins are probably related to the *post mortem* changes in muscle during ageing (Lonergan & Lonergan, 2008).

Pelvic suspension

In 1960 an article from New Zealand was published where the author suggests that it is possible to stretch the *longissimus* muscle by altering the carcass suspension (Locker, 1960). That statement has led to a huge body of research. Pelvic suspension is a method where the carcass is hung from the pelvic bone (verses the achilles tendon), which leads to a carcass position more typical of the animals' natural state (Fig. 5). The weight and the tension of the hind limb when it undertakes a more relaxed position leads to straightening of the vertebral column and prevention of muscle shortening due to skeletal restraint in both the back and the leg (Hostetler *et al.*, 1970).

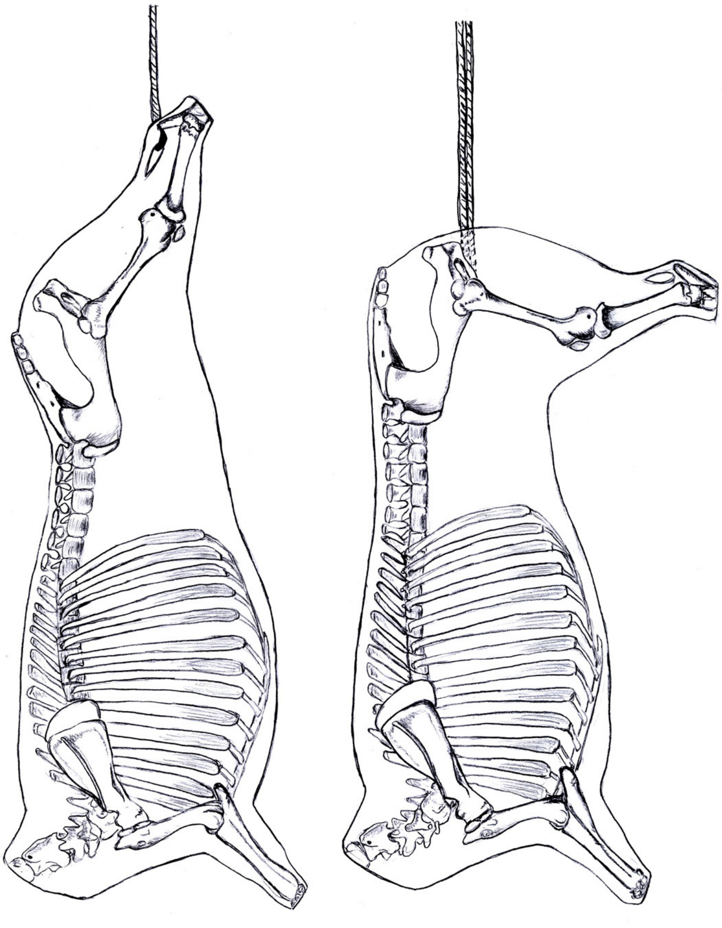

Figure 5. Schematic drawing of suspension methods. Carcass shape is affected by suspension from the achilles tendon (left) and the *obturator foramen* of the pelvic bone (right).

Different names for this method have been suggested including hip suspension, aitch-bone hanging and The Texas A&M Research Center have presented the name Tenderstretch based on work done of Hostetler *et al.* (1970; 1972; 1975). When Tenderstretch is applied to a carcass an S-shaped hook is inserted in the *obturator foramen* of the pelvic bone or in the pelvic ligament. In Sweden the method is called "pelvic suspension" and a slight modification is made using a rope to re-hang the carcasses instead of a hook (Fig. 6). The rope is attached around the *cranial* branch of *pubis*. This modification was introduced since the *caudal* branch of *pub*is between the *obturator foramen* and the *pubic symphysis,* where the carcass is split, is considered to be too weak. Attaching the rope on the wrong site could lead to breakage of the bone and a risk that carcasses fall off the rail.

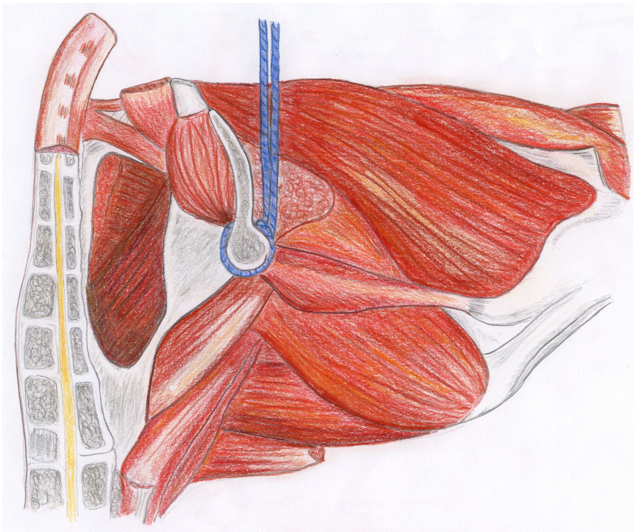

Figure 6. Illustration of the attachment of a rope to the pelvic bone for pelvic suspension.

There are also other methods which aim to prevent muscle shortening mainly in the back muscles. They involve the insertion of specific cuts that sever the skeleton in the back to physically stretch the muscles. Scientists at Virginia Polytechnic Institute and State University introduced the TenderCut method in 1994 (Wang *et al.*, 1994). This method involves making cuts in the skeleton at specific locations, which leads to a stretching effect although the carcass is still suspended by the achilles tendon. Cuts can be strategically located but one cut is often placed between the 12^{th} and 13^{th} thoracic vertebrae, near the normal position for splitting the carcasses into quarters in the USA (Fig. 7). Muscles adjacent to the *longissimus* such as the *multifidus dorsi* are also severed. The second cut severs the *ischium* of the pelvic bone and the junction between the 4th and 5th *sacral vertebrae* with adjacent connective tissue. Gaps of significant size should appear in the cutting areas to ensure sufficient stretching effects (Wang *et al.*, 1994). Modified versions of the TenderCut method have been presented by Canadian and Norwegian researchers (Aalhus *et al.*, 1999; Sørheim *et al.*, 2001).

Stretching, or the prevention of shortening, has a large contribution to the influence of the myofibrillar structure to tenderness (Hostetler *et al.*, 1970; 1972; Marsh & Leet, 1966). Herring *et al.* (1965) found muscle fibre diameter to be highly variable in achilles-suspended sides; with pelvic suspension this variation decreased. Most of the muscles in the loin and the round gained smaller muscle fibre diameters after pelvic suspension. The stretched muscles, with longer sarcomeres and smaller fibre diameter, were also lower in shear force compared to achilles samples (Herring *et al.*, 1965). Correlations between muscle fibre size and tenderness were also found by Crouse *et al* (1991).

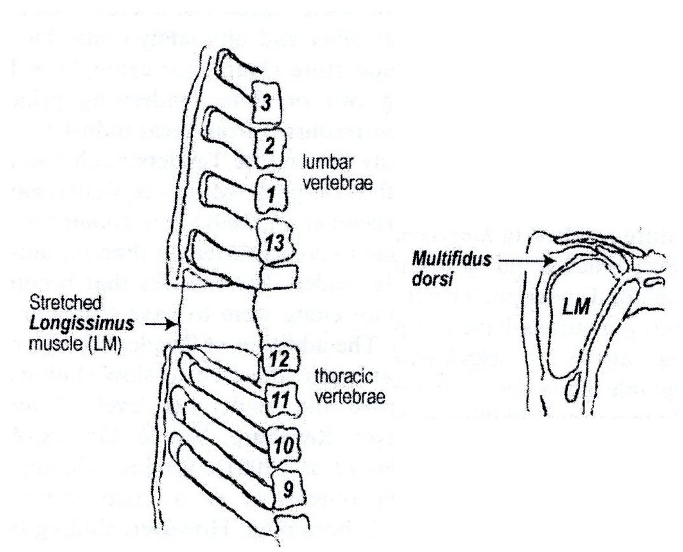

Figure 7. Schematic drawing of the TenderCut system with a cut in the 12th/13th vertebrae region of a beef carcass side. A cross section of the loin (LM) is shown to the right (Claus, 1997).

Objectives

Inconsistency in tenderness is a major concern for consumers of beef meat. Swedish beef production is decreasing but the demand for Swedish meat with consistent high quality is still prevalent.

The objective of this thesis was to gain further knowledge on the effect of pelvic suspension on several muscles in different gender categories. Pelvic suspension is known to improve tenderness and reduce variation in tenderness but have mostly been studied on grain-fed steers. Since Swedish production is focused on forage-feeding on pasture as well as indoors and involves a large production of young bulls it was of considerable interest to make studies with the following objectives:

Investigation of the effect of pelvic suspension on males and females of Swedish Red cattle (I and II). The objective of these studies was to investigate whether pelvic suspension in comparison with normal hanging of the carcass by the achilles tendon would improve tenderness and reduce variation in *longissimus, semimembranosus, adductor, gluteus* and *psoas* from dual purpose young bulls, bulls, heifers and cows.

Evaluation of pelvic suspension as a tool to reduce the need for extended rearing (III and IV). The major aim of this work was to evaluate the combined effects of finishing grazing heifers on a forage-based diet and the benefits of using pelvic suspension as an effective tenderness intervention post slaughter.

Evaluation of the need of ageing on pelvic suspended meat (III and IV).
The main purpose was to examine the effects of prolonged ageing on meat from achilles- and pelvic-suspended carcasses.

Investigation of the relationships between instrumental and sensory tenderness measurements in meat from pelvic suspended carcasses (IV). The aim was to investigate the relationships between different instrumental measurements and sensory tenderness in meat from animals differing in within- and between-animal variation.

Methods

Animal material and muscle sampling

Slaughter has been performed at two different commercial slaughterhouses, A and B. In Papers I and II carcasses were randomly selected directly from the slaughter line in slaughterhouse A. In Papers III and IV, the animals were of known background from other studies as described below and slaughtered at slaughterhouse B.

Paper I

Thirty-four Swedish Red young bulls were randomly selected on the slaughter line in slaughterhouse A and slaughtered as described below. *M. semimembranosus* muscles were excised and put in vacuum bags for ageing for 5 days in 4°C. After the first ageing period muscles were unpacked and prepared for further analysis. *Semimembranosus* was split into a dorsal and ventral section. A 10-cm piece of the dorsal section towards the middle of the muscle were vacuum packed and aged for another 7 days in 4°C, total ageing time 14 days.

Paper II

Carcasses from Swedish Red cattle with unknown nutritional background were selected on the slaughter line in slaughterhouse A and slaughtered as described below.

Whole muscles, *M. longissimus dorsi*, *M. semimembranosus*, *M. adductor*, *M. psoas major* and *M. gluteus medius*, were uniformly removed at cutting. The thick epimysial layer was removed from the LD and all muscles were trimmed of fat and thick connective tissue. Cuts were vacuum packaged and aged at 4°C until day 7 post slaughter. Muscles were unpacked and sampling for Warner-Bratzler analysis was performed, the samples were then frozen giving a total ageing time of 7 days.

Paper III

The animals were a sub-sample from a larger study concerning the effect of feed intensity and slaughter age on production traits (Hessle *et al.*, 2007). The subgroup was composed of 35 heifers with at least 75% Charolais breeding. During the grazing period, all heifers were kept together on semi-natural grasslands described by *Hessle et al.* (2007) without supplemental feeding. Two-thirds of the heifers (n = 22) were slaughtered directly from pasture at 18 months of age, whereas one-third (n = 13) was finished indoors and slaughtered at 22 months of age. During the indoor finishing period, the heifers were fed ad libitum intake of silage combined with 2 kg of mixed grain (65% oat and 35% barley) per animal per day.

The animals were slaughtered at a commercial plant, slaughterhouse B as described below. At cutting, 2 days after slaughter, *longissimus*, and *semimembranosus et adductor* were vacuum packed and aged in 4°C until day 7. The *semimembranosus* and *adductor* muscles were separated in their natural seam. *Semimembranosus* were split into a dorsal and a ventral section and a 10-cm piece of the dorsal part were vacuum packed and aged for another 7 days in 4°C, total aging time 14 days. *Adductor* muscles from the achilles- and pelvic-suspended sides were cut into comparable shaped cuts, vacuum packed and aged for another 7 days at 4°C, total aging time 14 days. *Longissimus* muscles were cut and samples for Warner-Bratzler analysis were frozen or additionally aged for 7 days to produce aging differences and hence total ageing times of 7 and 14 days. Sensory samples were aged in total for 14 days.

Paper IV

A subgroup of heifers with at least 75% Angus breeding was selected from a larger study about the effect of feed intensity and slaughter age on production traits (Hessle *et al.*, 2007). Half of the heifers (n = 20) were slaughtered directly from pasture at 18 months of age. A second group (n = 20) was kept indoors over winter and slaughtered at 22 months of age. During the grazing period, all heifers were kept together on semi-natural grasslands as described by Hessle *et al. (2007)*, without supplemental feeding. During the indoor period, the heifers were fed grass silage.

The animals were slaughtered at a commercial plant, slaughterhouse B as described below. At cutting, 2 days after slaughter, *longissimus* muscles were vacuum packed and then aged at 4°C until day 7. *Longissimus* was cut and samples for Warner-Bratzler analysis were frozen or additionally aged for 7 days to produce aging differences and hence total ageing times of 7 and 14 days. Sensory samples were aged for 14 days in total.

Slaughter procedure

Carcasses were de-hided, eviscerated and then electrically stimulated (low voltage, 80V, 30 s) 30 min after exsanguination. The left side from each carcass was hung by the *obturator foramen* of the pelvic bone while the right side was suspended by its achilles tendon. In slaughterhouse A (I and II) carcasses were chilled intensively for the first hour; 20 min at -2°C, 10 min at -4°C and 20 min at -2°C. Then carcasses were stored at 2-4°C for 2 days. In slaughterhouse B (III and IV) carcasses were chilled at 2-4°C for 2 days. The pH was measured in the centre of the *longissimus* muscle at the 11th rib using a probe electrode (Knick SE104, Germany) attached to a pH meter (WTW pH 340, Germany). No carcasses with a pH above 5.8 at 48 h *post mortem* were selected. During commercial cutting, the fore- and hindquarters were separated between ribs 10 and 11. Cutting was done anatomically, presenting the large muscles from the round as individual cuts. Muscles are in this thesis named by their Latin names.

Specific Methodology

Weight, length and width

Muscles were weighed at the slaughterhouse before packing (I and II) or after 7 days of aging (III and IV). The length and width were recorded with ruler (I and II).

pH measurement

Final pH was measured at designated times of ageing using a probe electrode (Knick SE104, Germany) attached to a pH meter (WTW pH 340, Germany).

Sarcomere length

Samples for sarcomere length measurements were removed from the central part of the muscles after 7 days of ageing (I and II), placed in glutaraldehyde fixative and prepared according to Cross, West, & Dutson (1980). The samples (app. 50 mg) were put into the first fixative (0.1M KCl, 0.039M boric acid, and 5mM EDTA in 2.5% glutaraldehyde) for 2 h, and then transferred to the storage solution (0.025M KCl, 0.039M boric acid, 5mM EDTA in 2.5% glutaraldehyde). The samples were stored in +4°C until analysis. For analysis, about 25 mg of the sample was homogenised at low speed in 2 ml 0.25M sucrose solution with an Ultra-Turrax T25 homogeniser S25N-10G. A drop of homogenate was transferred to a slide and covered with a cover slip. The slide was then examined with a Nikon Microphot FXA microscope with a flat Apo 60X lens. The lengths of 10 sarcomeres were measured in 10 myofibrils from each sample (Fig. 3).

Intramuscular fat (IMF)

Papers III and IV; Samples (1.5 cm thick and frozen at day 7) were partially thawed, chopped into small pieces, and mixed. Duplicate sub-samples were analysed for IMF by the SBR method (NMKL, 1989) after hydrolysis with HCl diethyl ether and petroleum ether for extraction. Values for the duplicates were averaged.

Paper II; The IMF content was chemically determined and analysed using petroleum ether for extraction (Soxtec system H^+ equipment, Tecator AB, Höganäs, Sweden).

Colour measurements

On day 7, a freshly cut sample was bloomed for 1 h in room temperature, (approximately 20°C) and evaluated using a Minolta Chroma Meter CR300 (Osaka, Japan) with an 8-mm aperture and a 0° viewing angle with the specular component included (III and IV). A white tile was used for standardization (L*=97.47, a*=-0.20, b*=1.79) according to the manufacturer's recommendations. CIE L* (lightness), a* (redness), and b* (yellowness) values (Illuminant D-65) were obtained from three scans from each steak and averaged for statistical analysis.

Water holding capacity

In Paper I and II muscles were weighed after cutting before vacuum packaging. The purge was accumulated during 5 days in the vacuum bag and after ageing the muscles were unpacked, lightly blotted to remove excess moisture, and re-weighed so that percentage purge could be calculated.

Frozen samples were thawed overnight at 4°C in their original vacuum package and then, while still sealed in the bag, placed in a water bath (20°C) for 1 h to standardize the sample temperature before evaluating the resulting juice loss. The samples were unpacked, blotted dry and weighed before re-vacuum packaged and cooked in the vacuum bag in a water bath for 2 h at

70°C. The weights of the meat samples were recorded before freezing, after thawing and after cooking to calculate the freezing and cooking losses.

In Papers III and IV the samples aged 14 days were after additional aging frozen without re-packing, thus freezing losses after 14 days of aging is the combined losses from purge during ageing and thawing. In Paper I no freezing losses were determined since Warner-Bratzler samples were cut from slightly frozen samples.

Instrumental tenderness

Instrumental tenderness was measured on all muscles using a Stable Micro System Texture Analyser HD 100 (Godalming, UK). Samples were cooked as described above. The cooked meat samples were stored at 4°C until evaluated the next day. From each sample, 30-40 mm × 10 mm × 10 mm strips (a shear area of 100 mm^2) were removed with the fibre direction parallel to the longitudinal dimension of the strip.

Warner-Bratzler Shear force

The Warner-Bratzler shear force method described by Honikel (1998) with modifications as indicated below was used for shear force determinations. Peak force (N) and total energy (Nmm) were recorded in Papers I, II, III and IV (table 1). In Paper IV shear firmness (N/mm) was measured as described by Larmond and Petrasovits (1972). The Warner-Bratzler shear force was analysed using a blade with a rectangular hole of 11 mm × 15 mm. The blade was 1 mm thick and had a cross-head speed of 0.83 mm/s when cutting through the strips.

Texture profile analysis by Compression

In Paper IV the compression force method described by Honikel (1998) was used with modifications as indicated below. Hardness (N), compression area 1 and 2 (Nmm), springiness (mm), cohesiveness and gumminess were recorded. In Paper IV the Warner-Bratzler analysis and compression assessments were made 15 mm from each end, which made it possible to use both methods on every strip. Compression measurements were conducted perpendicular to the fibre direction with a squared flat-ended plunger (10 × 10 mm) driven at 0.83 mm/s twice vertically 80% of the way through the strip. The strips were placed in a metal cell fitted with two lateral walls along the long dimension of the strips (10 mm wide × 20 mm high × 50 mm long) allowing lateral strain on both sides and movement possible only in the longitudinal direction along the fibre axis.

Table 1. *Variables for shear, compression and sensory analysis*

Shear variables	
Peak Force (N)	Maximum peak force. Force needed to shear through a standardised meat sample
Total Energy (Nmm)	The area under the curve
Shear Firmness (N/mm)	The slope of a line drawn from the origin of the curve to its peak
Compression variables	
Hardness (N)	Peak of first compression curve
Comp. area 1 and 2 (Nmm)	The area under the first and second compression curves
Springiness (mm)	Width of the second curve
Cohesiveness	The ratio of the area under the second curve to the area under the first curve
Gumminess	Hardness × cohesiveness
Sensory Variables	
Tenderness	Overall tenderness judged after chewing 8 times
Bite resistance	Force needed to bite through a slice of meat
Threadiness	A perceived sensation of a threadlike net on the tongue after chewing 8 times
Juiciness	Juiciness perceived after chewing 4-5 times
Marbling	Judged visually as amount of marbling using pictures as reference
Fatty taste	Fatty taste and degree of a fat film in the mouth after chewing 8 times
Acidity	Acidulous taste after chewing 5 times
Meat flavour	Overall meat taste after chewing 8 times

Sensory analysis

The sensory samples were thawed overnight at 4°C. They were wrapped in aluminium foil and cooked in a conventional oven at 125°C to an internal end-point temperature of 70°C. A descriptive test (conventional profiling; (ISO, 1985) was performed by a 7-member panel. The panelists were experienced at assessing beef and were trained in accordance with ISO 8586-1 (1993) before profiling. The samples were tempered to room temperature and served in coded petri dishes in replicates as rectangular, 3.8-mm-thick slices. The crust and outer layer of connective tissue were removed before presentation to the panel. The sensory attributes (Table 1) of acidity, fatty taste, meat taste, juiciness, tenderness, bite resistance, visible marbling (III and IV) and threadiness (IV) were judged on an intensity scale from 0 to 100. The assessments were performed in duplicate in a room with separate booths for each judge and a normal white light according to ISO 8589 (1988). The PSA program (PSA system/3 2.09 1994) was used for data collection. Unsalted wafers and water were available for use between samples.

Statistical methods

Statistical evaluation was performed using the Procedure Mixed in SAS (Version 8.02 (I) and 9.1 (II, III and IV) SAS Institute Inc., Cary, NC, USA). The within-animal variation was calculated as the coefficient of variation (CV; in %) from mean and S.D. for the measured strips or sarcomeres from each sample. The between-animal variation was calculated as the CV from mean and S.D. for each sub-group. Differences between subgroup least-squares means were evaluated using the 'pdiff' option (II and III) or 'estimate' option (IV) when at least one of the main effects were significant ($P<0.05$).

Paper I

The effects of suspension method, as fix factor, and carcass, as random factor, were included in the statistical model.

Paper II

The statistical analyses were made separately for each muscle. The model contained the fixed effects of sex and suspension method and the interaction between these, and the random effect of animal.

Paper III

For the carcass and fat traits, slaughter age (18 and 22 months) was included in the model as a fixed effect. For the meat quality traits, suspension method and slaughter age and their interaction were included as fixed effects with animal as a random effect. Differences due to ageing were evaluated with the same model but with ageing time added (7 or 14 days). For the sensory evaluation, the random effect of panel member also was included.

Paper IV

For the carcass and fat traits, slaughter age (18 and 22 months) was included in the model as a fixed effect. For the meat quality traits, suspension method and slaughter age were included as fixed effects with animal as a random effect. As only achilles suspension was used in animals slaughtered at 22 months, no interaction effect between suspension method and slaughter age could be included. For the sensory evaluation, the random effect of panel member also was included. Differences due to ageing (7 vs. 14 days) were evaluated within suspension method/slaughter age subgroup with ageing time as fixed and animal as random effect.

Summary of presented papers

Paper I

To study whether pelvic suspension would improve tenderness in comparison with normal hanging from the achilles tendon, carcasses from 34 Swedish Red young bulls were randomly selected at the slaughter line. The left sides of the carcasses were re-hanged by the pelvic bone before cooling whereas the right sides remained hanging from the achilles tendon. Quality characteristics and length and weight were studied for *M. semimembranosus*. Tenderness was measured by the Warner-Bratzler shear force method. Purge and cooking loss were determined and sarcomere length was measured. Pelvic suspension considerably affected the shape of *M. semimembranosus;* the length increased by 38% in comparison with achilles suspension. The elongation of the muscle was parallel with an increase in sarcomere length from 1.6 to 2.9 μm. The water-holding capacity was improved because both purge and cooking loss were lower for pelvic suspended carcasses. Tenderness was improved and the coefficient of variation between animals for Warner-Bratzler shear force was reduced from 26% for achilles tendon suspension to 12% for pelvic suspension. Likewise, the within-animal variation for shear force decreased significantly from 13.3% to 10.0%.

Therefore, pelvic suspension seems to be a reliable method to reduce texture variability in *M. semimembranosus* from young bulls.

Paper II

The effects of pelvic and achilles suspension methods were evaluated in four gender categories (young bulls, bulls, heifers and cows) and five muscles [*M. longissimus dorsi* (LD), *M. semimembranosus* (SM), *M. adductor* (AD), *M. psoas* *major* (PM) and *M. gluteus medius* (GM)]. The animals (Swedish Red breed) were slaughtered and electrically stimulated and one side of each carcass was re-suspended from the pelvic bone. Carcasses were assessed according to the EUROP carcass classification system. Meat quality was evaluated by sarcomere length, muscle length and width, intramuscular fat, drip loss, colour, and shear force measurements.

Pelvic suspension reduced the possibility for the muscles to contract due to the more natural position of the muscles in relation to the skeleton restraint, which resulted in longer sarcomeres and more tender meat. The longest sarcomeres were found in the SM, but elongation also occurred in LD, GM and AD muscles, except in heifers. The elongation of sarcomeres was accompanied by extended muscle length for the LD, SM, AD and GM muscles from all genders. However, the changes in sarcomere length did not always produce proportional changes in tenderness. Peak force was reduced in LD of young bulls (P=0.008) and bulls (P=0.005), but for heifers and cows shear force was not affected by suspension method. Similar results were found for GM muscles. SM decreased in shear force after pelvic suspension for young bulls (P<0.001), bulls (P<0.001) and heifers (P=0.006) and for the AD the only decrease was found in bulls (P=0.034). Water-holding capacity significantly increased due to pelvic suspension. Although differences were not always significant, purge in the vacuum bags during ageing was lower for all pelvic-suspended muscles except the PM. The purge for LD, SM and AD muscles was reduced significantly ranging from 0.3 to 2.7 units. The increased yields found in the LD from both males and females may be of considerable importance to the industry.

Paper III

This study investigated the effects of pelvic suspension on *M. longissimus dorsi*, *M. semimembranosus* and *M. adductor* from 35 heifers with at least 75% Charolais breeding. Two-thirds of the heifers were slaughtered directly from pasture at 18 months of age and one-third was finished indoors and slaughtered at 22 months. After slaughter and electrical stimulation one side of each carcass was re-suspended from the pelvic bone. In addition, the *longissimus* muscles were aged 7 or 14 days before meat quality was evaluated by drip loss, colour, shear force and sensory analysis. Heifers (22 months old) were heavier, more muscular and fatter (P<0.05) compared with heifers slaughtered at 18 months of age. IMF also increased with slaughter age (P<0.003). Muscle pH and freezing losses were lower in pelvic-suspended sides for all three muscles. Pelvic suspension reduced *longissimus* shear force values and total energy (P<0.05) in heifers slaughtered at 18 months.

Semimembranosus showed an even stronger response to pelvic suspension with significantly lower peak force and total energy values. The finishing time of 4 months did not affect *longissimus* shear force values. When evaluating the effects of ageing, achilles suspended samples had lower shear force values after 14 vs. 7 days of ageing. The pelvic suspended samples aged 7 days were, however, just as tender as those aged 14 days. Sensory analysis of *longissimus* samples aged 14 days showed that samples from pelvic suspended sides had higher tenderness, lower bite resistance, more meaty taste and less visible marbling compared with samples from achilles-suspended carcasses.

Pelvic suspension was a very effective tenderization intervention because it improved tenderness of almost all treatment combinations and it reduced significantly the additional aging period required to make cuts more acceptably tender.

Paper IV

This study investigated the effects of pelvic suspension on *M. longissimus dorsi* from 40 heifers with at least 75% Aberdeen Angus breeding. Twenty heifers were slaughtered directly from pasture at 18 months of age and 13 was finished indoors and slaughtered at 22 months. After slaughter and electrical stimulation one side of each carcass from the 18 months-old heifers was re-suspended from the pelvic bone. In addition, the *longissimus* muscles were aged 7 or 14 days before meat quality was evaluated by drip loss, colour, shear force, compression and sensory analysis. Heifers, 22 months old, were heavier and fatter but not more muscular compared with heifers slaughtered at 18 months of age. IMF also increased with slaughter age. Muscle pH and freezing losses were lower in pelvic suspended sides from 18-month-old heifers. Pelvic suspension reduced both between- and within-animal variation for peak force, total energy and compression peak in heifers slaughtered at 18 months. Pelvic suspension also decreased peak force, total energy and compression variables for *longissimus* samples aged both 7 and 14 days from heifers slaughtered at 18 months. The finishing time of 4 months was effective and decreased peak force and total energy to levels comparative to pelvic suspended sides at 18 months. When evaluating the effects of ageing, achilles suspended samples had lower shear force values after 14 vs. 7 days of ageing. Peak force and total energy from the pelvic suspended sides aged for 7 days were not different from those aged 14 days but shear firmness and all compression variables except cohesiveness were reduced after ageing. The importance of aging was more obvious for samples from the achilles-suspended sides from 18-month-old heifers than from the 22-

month-old animals, where peak force was similar after 7 and 14 days of aging. However, total energy and all compression parameters except gumminess showed effect of ageing also in the 22-month-old samples. Sensory analysis of *longissimus* samples showed that samples from pelvic suspended sides had greater tenderness, lower bite resistance and less threadiness, higher juiciness and meat flavour and less visible marbling than meat from achilles-suspended sides at 18 months. Prolonging the feeding period from 18 to 22 months increased tenderness and decreased bite resistance and threadiness in the achilles-suspended sides to values similar to pelvic suspended sides. Visible marbling and fatty taste were higher at 22 months of slaughter. The correlations between the different instrumental measurements and sensory tenderness were considerably higher for carcasses suspended by the achilles tendon compared with those hung by the pelvic bone. More correlations between sensory evaluated tenderness and shear variables were significant after 7 days of ageing than after 14 days.

Clearly, meat from pelvic suspended carcasses will be equal to or more tender than meat hung by the Achilles tendon and aged for at least 7 more days.

Discussion

Pelvic suspension influences muscles in the carcass differently. Many of the muscles in the round and the back are affected by a change in the position of the hind leg. Normal hanging from the achilles tendon pulls back the hind leg, which affects the muscles on the leg. In relation to the femur, the posterior muscles are allowed to shorten and the anterior muscles are stretched. The vertebral column is curved and pushed together. In pelvic suspension the hind legs hang vertically from the carcass reversing the effects on the muscles involved and the vertebral column is straightened and slightly separated as described by Hostetler *et al.* (1970). This reduces the possibility for the muscles to contract, which results in altered shape of the muscles compared to achilles suspension. The effect of pelvic suspension on different variables is substantial in the papers included in this thesis. The main reason for this is believed to be the elongation of sarcomeres since similar responses can be seen for different stretching techniques (Sørheim & Hildrum, 2002).

Muscle characteristics

The pelvic suspension considerably affected the shape of muscles and especially *semimembranosus* which became more flat and elongated due to stretching (I and II). Muscle length was extended also in *longissimus*, *adductor* and *gluteus* from all gender categories (II). Aberle & Judge (1979) found similar effects of pelvic suspension on the shape of muscles both in the loin and the round. The yield was affected in *longissimus* where muscles from pelvic-suspended sides showed higher weights than achilles-suspended counterparts (II). Pelvic suspended *longissimus* was 9% (400 g) and 7% (270 g) heavier for males and females respectively. This is probably due to difficulties to separate the fore- and hindquarter similar for both suspension methods. Nevertheless it may be of considerable importance since this part of the loin is one of the most valuable cuts on the carcass. The predominant way to sell the loin in Sweden is as consumer-ready slices.

Sarcomere length

Sarcomere length is determined by the state in which the muscle enters *rigor* (Huxley & Hanson, 1954; Marsh & Carse, 1974). If the muscle is exposed to a stretching technique such as pelvic suspension before *rigor* has occurred, muscle shortening will be prevented and stretched muscles will show longer sarcomere lengths. A strong relationship between sarcomere length and muscle toughness when sarcomeres are shorter than 2 μm was proved as early as in 1967, but this relationship disappears with increasing sarcomere lengths (Herring *et al.*, 1967). Eisenhut *et al.* (1965) investigated how the angle at which the muscle fibres are attached to the spine changes depending on stretching of the loin. They discovered that when *longissimus* was stretched, fibre angles, both from the *transverse processes* and the *spinous process*, decreased and longer sarcomeres were produced. The fibre angles were more similar along the *longissimus* when the muscle was stretched.

The results from Paper II where five muscles from young bulls, bulls, heifers and cows have been achilles- and pelvic-suspended clearly demonstrates that response to pelvic suspension differs between gender categories and muscles (Table 2). In the literature much of the work on pelvic suspension has been done on steers and sarcomere elongation, similar to our study, has been reported for several muscles (Aberle & Judge, 1979; Barnier & Smulders, 1994; Bouton *et al.*, 1973; Derbyshire *et al.*, 2007). In Paper II, a large, significant effect of pelvic suspension on sarcomere elongation was found in all muscles from male animals and cows except the *psoas,* whereas differences were much smaller and insignificant in heifers. This is contradictory to results by Joseph and Connolly (1977) who found effect on sarcomere length of pelvic suspension in several muscles from heifers.

Sarcomere lengths from achilles-suspended sides are similar across gender categories within muscles and also across muscles (II). *Longissimus*, *semimembranosus*, *adductor* and *gluteus* all have sarcomere lengths at 2 μm or slightly shorter when from achilles-suspended sides. *Psoas* have sarcomeres of about 3 μm, all results are in agreement with Bouton *et al.* (1973) and Wahlgren *et al.* (2002) who reported similar values. In our study, pelvic suspension made sarcomere lengths more inconsistent, with larger variations both within and between animals compared to achilles suspension (Table 2). The effect in stretched muscles varies from a decrease in sarcomere length of

0.2 μm for heifer *adductor* to an elongation of 1.3 μm for bull *semimembranosus*. In *psoas*, where fibres are allowed to shorten in pelvic-suspended sides the differences vary between gender categories and are shortened by a range from 1μm in cows to 0.2 μm in heifers. Herring *et al.* (1965) found that variation in sarcomere length decreased when muscles were stretched. However their study was conducted with carcass sides assigned for stretching lying on a table with the limbs oriented and fixed perpendicular to the long axis of the sides. In our study the hind leg of the pelvic-suspended side hung freely and was therefore not restricted in any way. Muscles may therefore be differently stretched depending on the weight of the hind limb. This may be one of the reasons for the differences in effects of pelvic suspension between genders.

The interaction between sarcomere length and the extent of post mortem proteolysis is still unclear. An increased overlap between thin and thick filaments in the sarcomere may reduce the availability of proteolytically susceptible sites (Wheeler & Koohmaraie, 1994). The same authors later concluded that the effect on sarcomere length on tenderization occurred because of the lower initial tenderness and not depending on proteolysis (Wheeler & Koohmaraie, 1999). A recent study by Weaver *et al.* (2008) discovered that earlier *post mortem* samples with longer sarcomeres showed lower amounts of the protein troponin T and higher amounts of protein degradation products compared to samples with shorter sarcomeres. This implies a more tender sample when sarcomeres are longer. They suggest that the lower proteolytic activity in shorter sarcomeres may be due to the steric hindrance from the dense matrix in the shortened sarcomere. Migration of calpains could be hindered and therefore the proteolysis will slow down but not be entirely stopped. These suggestions need to be further investigated to fully understand the combined mechanism of sarcomere elongation and proteolytic activity and its effect on muscle tenderness.

Table 2. *Overview of sarcomere length means, range and variation in pelvic- and achilles-suspended sides from four gender categories (Paper II)*

Muscle	Gender	Susp.	Mean	S.D.	Min.	Max.	CV B[1]	CV W[2]
Longissimus	Young bull	AS	1.8	0.06	1.8	1.9	3.3	5.5
	Young bull	**PS**	**2.0**	**0.16**	**1.9**	**2.3**	**8.0**	**6.9**
	Bull	AS	1.8	0.04	1.7	1.8	2.2	5.1
	Bull	**PS**	**2.0**	**0.19**	**1.8**	**2.3**	**9.5**	**5.2**
	Heifer	AS	1.7	0.04	1.6	1.7	2.4	7.1
	Heifer	**PS**	**1.8**	**0.11**	**1.7**	**1.9**	**6.1**	**9.3**
	Cow	AS	1.7	0.08	1.6	1.9	4.7	5.8
	Cow	**PS**	**2.0**	**0.18**	**1.9**	**2.4**	**9.0**	**4.8**
Semimemb.	Young bull	AS	1.7	0.07	1.6	1.8	4.1	13.4
	Young bull	**PS**	**2.7**	**0.17**	**2.4**	**2.9**	**6.3**	**7.3**
	Bull	AS	1.8	0.25	1.3	2.1	13.9	11.2
	Bull	**PS**	**2.7**	**0.21**	**2.4**	**3.0**	**7.8**	**9.4**
	Heifer	AS	1.7	0.06	1.6	1.8	3.5	10.4
	Heifer	**PS**	**2.0**	**0.32**	**1.7**	**2.6**	**16.0**	**5.4**
	Cow	AS	1.7	0.15	1.5	2.0	8.8	8.2
	Cow	**PS**	**2.7**	**0.15**	**2.6**	**3.0**	**5.6**	**7.7**
Adductor	Young bull	AS	2.1	0.57	1.7	3.3	27.1	7.7
	Young bull	**PS**	**2.9**	**0.61**	**1.7**	**3.6**	**21.0**	**6.5**
	Bull	AS	1.8	0.18	1.5	2.0	10.0	8.0
	Bull	**PS**	**3.1**	**0.29**	**2.5**	**3.3**	**9.4**	**5.8**
	Heifer	AS	2.4	0.73	1.8	3.3	30.4	10.0
	Heifer	**PS**	**2.2**	**0.64**	**1.7**	**3.5**	**29.1**	**8.9**
	Cow	AS	1.9	0.04	1.9	2.0	2.1	7.4
	Cow	**PS**	**2.9**	**0.13**	**2.7**	**3.0**	**4.5**	**7.2**
Gluteus	Young bull	AS	1.7	0.05	1.7	1.8	2.9	5.6
	Young bull	**PS**	**2.6**	**0.14**	**2.4**	**2.8**	**5.4**	**6.4**
	Bull	AS	1.9	0.27	1.6	2.4	14.2	7.0
	Bull	**PS**	**2.3**	**0.31**	**1.8**	**2.6**	**13.5**	**4.7**
	Heifer	AS	1.7	0.08	1.6	1.8	4.7	7.9
	Heifer	**PS**	**1.9**	**0.36**	**1.6**	**2.6**	**18.9**	8.3
	Cow	AS	1.8	0.06	1.7	1.9	3.3	**8.0**
	Cow	**PS**	**2.6**	**0.09**	**2.4**	**2.7**	**3.5**	5.5
Psoas	Young bull	AS	3.0	0.26	2.6	3.3	8.7	14.6
	Young bull	**PS**	**2.6**	**0.40**	**2.1**	**3.1**	**15.4**	**8.4**
	Bull	AS	3.2	0.36	2.6	3.5	11.3	4.4
	Bull	**PS**	**2.7**	**0.50**	**2.2**	**3.5**	**18.5**	**9.2**
	Heifer	AS	2.9	0.73	1.8	3.5	25.2	5.0
	Heifer	**PS**	**3.1**	**0.52**	**2.0**	**3.4**	**16.8**	**5.7**
	Cow	AS	3.2	0.14	2.9	3.3	4.4	4.5
	Cow	**PS**	**2.2**	**0.14**	**2.1**	**2.5**	**6.4**	**6.7**

[1] Coefficient of variation between animals.

[2] Coefficient of variation within animal.

Water holding capacity and pH

Within the muscle, most of the water is held within the myofibrils, between myofibrils, between muscle cells and muscle bundles. (Huff-Lonergan & Lonergan, 2005). The state of contraction can therefore easily affect the amount of water held in the muscles (Offer & Knight, 1988a). Purge is also related to the level of intramuscular fat in the sample where a higher fat content lead to less water to drip (Cheng & Sun, 2008; Jeremiah *et al.*, 2003).

Pelvic suspension decreased losses during ageing (I and II) for all muscles except *psoas* from all gender categories. The purge was, depending on gender category, decreased by 0.3 - 2.7 units, in *semimembranosus* (I and II), *longissimus* and *adductor* (II). Lower purge due to stretching has been demonstrated by several authors (Claus *et al.*, 1997; Joseph & Connolly, 1977; Wahlgren *et al.*, 2002), indicating that a more stretched sarcomere would have less overlap between thick and thin filaments and hence larger interfilament space to hold water.

Freezing losses or thaw losses are of less importance in Sweden since nearly all Swedish meat is sold fresh on the market. Freezing has been shown to both increase losses and decrease tenderness (Lagerstedt *et al., (2008).* In our studies, samples were, however, frozen due to logistic reasons. All samples from the studies included in this thesis were treated similarly, in relation to freezing, before analysis.

Freezing losses were lower for pelvic-suspended sides, except for *psoas* samples. *Longissimus* showed lower freezing losses for pelvic-suspended sides after ageing for both 7 and 14 days (III and IV). However, for samples aged 14 days these results cannot be entirely separated from the purge during ageing since samples were not re-packed before freezing. In Paper II no differences were found for either *longissimus* or *gluteus* but a large difference were found in *semimembranosus,* where all gender categories had lower freezing losses in pelvic-suspended sides. Mitchell *et al.* (1991) found higher sensory scores for fresh compared to thawed samples for both *longissimus* and *semimembranosus,* but no differences were found for shear force values depending on freezing or thawing methods.

Cooking loss differences due to pelvic suspension were small and when significant, inconsistent between studies. Pelvic suspension decreased cooking loss in *semimembranosus* from young bulls (I) but increased cooking loss in *longissimus* from cows (II) and heifers aged 7 days (IV) whereas no effect were found for heifers in Paper II and III. The large variations in sarcomere length may influence the effect on losses of cooking. Cooking losses related to pelvic suspension have been shown to decrease (Barnier &

Smulders, 1994), increase (Eikelenboom *et al.*, 1998) or be unaffected (Derbyshire *et al.*, 2007; Joseph & Connolly, 1977) in comparison with achilles-suspended samples. Cooking losses can be highly affected by the cooking method applied, i.e. dry cooking on a grill or moist cooking with meat in a bag immersed in water (Cheng & Sun, 2008). Different end-point temperatures are also seen in the literature varying from 50°C to 90°C with higher temperatures leading to higher losses (Offer & Knight, 1988b).

Total loss, i.e. purge, freezing loss and cooking loss together, was lower for young bulls and cows in both *semimembranosus* and *adductor* in Paper II. For the other muscles, *longissimus*, *psoas* and *gluteus*, no differences in total loss were found between suspension methods. The consistently lower purge for pelvic-suspended muscles are nonetheless of economic significance for the industry.

Colour

Colour was not similarly affected by pelvic suspension in Paper III and IV. In Paper III pelvic-suspended samples were darker compared to achilles-suspended samples whereas in Paper IV the contrary was found. In Paper IV pelvic-suspended samples were slightly more yellow compared to achilles-suspended samples; a^* and b^* values were otherwise not affected by pelvic suspension. A higher slaughter age gave more red and more yellow colour (III and IV) in agreement with Vestergaard *et al.* (2000a). The effect of pelvic suspension on meat colour was minimal and is not believed to be of any practical importance.

Instrumental tenderness

Shear force

Warner-Bratzler shear force is an objective measurement of meat tenderness introduced as early as 80 years ago (Warner, 1928). According to Culioli (1995) it is the most widely used method for instrumental tenderness determinations. A higher value demonstrates a less tender sample. Much information can be obtained from the curves received in the analysis but the most common way to report results is by peak force (N) which is the value at the highest peak of the curve (Fig. 8). This reflects the strength needed to shear through the sample. Another value that can be obtained is the total energy (Nmm) registered as the area under the curve, reflecting more of the

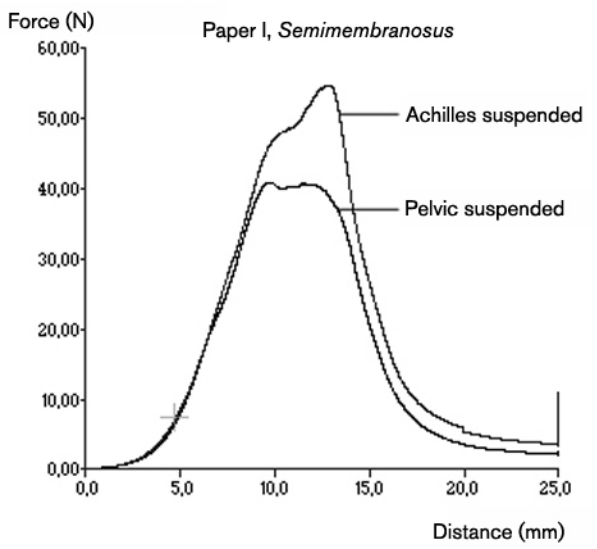

Figure 8. Warner-Bratzler shear force curves (average values, I) from achilles- and pelvic-suspended *semimembranosus* muscles.

total work done chewing a sample. Hence, samples can produce curves similar in peak force but still show differences in total energy or vice versa.

Pelvic suspension has been reported to decrease shear force compared to achilles suspension (Bouton *et al.*, 1973; Joseph & Connolly, 1977; Mooney *et al.*, 1999; Wahlgren *et al.*, 2002) and this was confirmed in our studies (Fig. 9 and 10). The highest peak force values were found in achilles-suspended *semimembranosus* and *longissimus* muscles from male animals (I and II). This was also generally where the largest improvements due to pelvic suspension were found. Decreases in average peak force for males were 21% (I, 14 d) and 26% (II, 7 d) for *semimembranosus* and 20% (II, 7 d) for *longissimus*. The corresponding decreases for females were 24% (III, 14 d) and 11% (II, 7 d) for *semimembranosus* and 36% (IV, 7 d), 20% (IV, 14 d), 10% (insignificant, II, 7 d), 7% (insignificant, III, 7 d) and no difference (III, 14 d) for *longissimus*. The largest effect of 36% was surprisingly found in heifers. *Gluteus* muscle showed no decrease in peak force due to pelvic suspension in samples from female animals, but peak force was reduced for both young bulls and bulls. For the *adductor* samples peak force was reduced only in bulls, (II) whereas there was a reverse effect in cows with higher peak force for pelvic-suspended sides. This was also found for heifers in Paper III where there was a tendency towards higher peak force values for pelvic-suspended *adductor* samples. The only muscle that was unaffected by pelvic suspension in relation to peak force was the *psoas*.

The differences in total energy depending on pelvic suspension most often showed patterns similar to differences in peak force (Fig 9). In Paper II (Fig. 10) the relative difference between suspension methods was larger for peak force values than for total energy values in male animals. In females the two measurements responded similarly to suspension methods. However, when evaluated statistically the response for the two measurements were identical in all muscles and genders (II). It should be noted, that total energy seems to be able to detect ageing effects in tender meat better than peak force. In Paper III and IV ageing significantly decreased total energy in meat for heifers slaughtered at 22 months of age. No corresponding difference was found for peak force or in pelvic-suspended sides.

The patterns of peak force and total energy are very similar, despite this, more clear differences between ageing times and suspension methods were found in total energy. Higher precision may be obtained when using the information from the entire curve. In addition to traditional evaluation of shear force measurements as maximum peak force and total work, also the entire Warner-Bratzler shear force curve with its individual data points have been evaluated using multivariate data analysis (Hildrum & Narum, 2006; Rosenvold *et al.*, 2002). The results were only marginally improved by the use of the entire curve, but Hildrum & Narum (2006) concluded that the use of multivariate techniques was useful to improve the quality control of the data.

The differences observed between the achilles-suspended muscles were not surprising since the inherent tenderness have been shown to differ between muscles (Jeremiah *et al.*, 2003; Von Seggern *et al.*, 2005). More interesting were the uneven response to pelvic suspension that lead to peak force and total energy values on a consistent low level for all muscles and genders included in our four studies. Barnier & Smulders (1994) found similar elongations in sarcomere lengths to our studies but they found no coherent effect in shear force. The *semimembranosus* and *gluteus* muscles even showed increased shear force in pelvic-suspended samples. *Longissimus* samples did, however, show decreased peak forces (Barnier & Smulders, 1994). Most authors report positive effects on pelvic-suspended samples from the muscles included in our studies, especially for the *longissimus* and *semimembranosus* (Bouton *et al.*, 1973; Joseph & Connolly, 1977; Lively *et al.*, 2006; Mooney *et al.*, 1999; Wahlgren *et al.*, 2002)

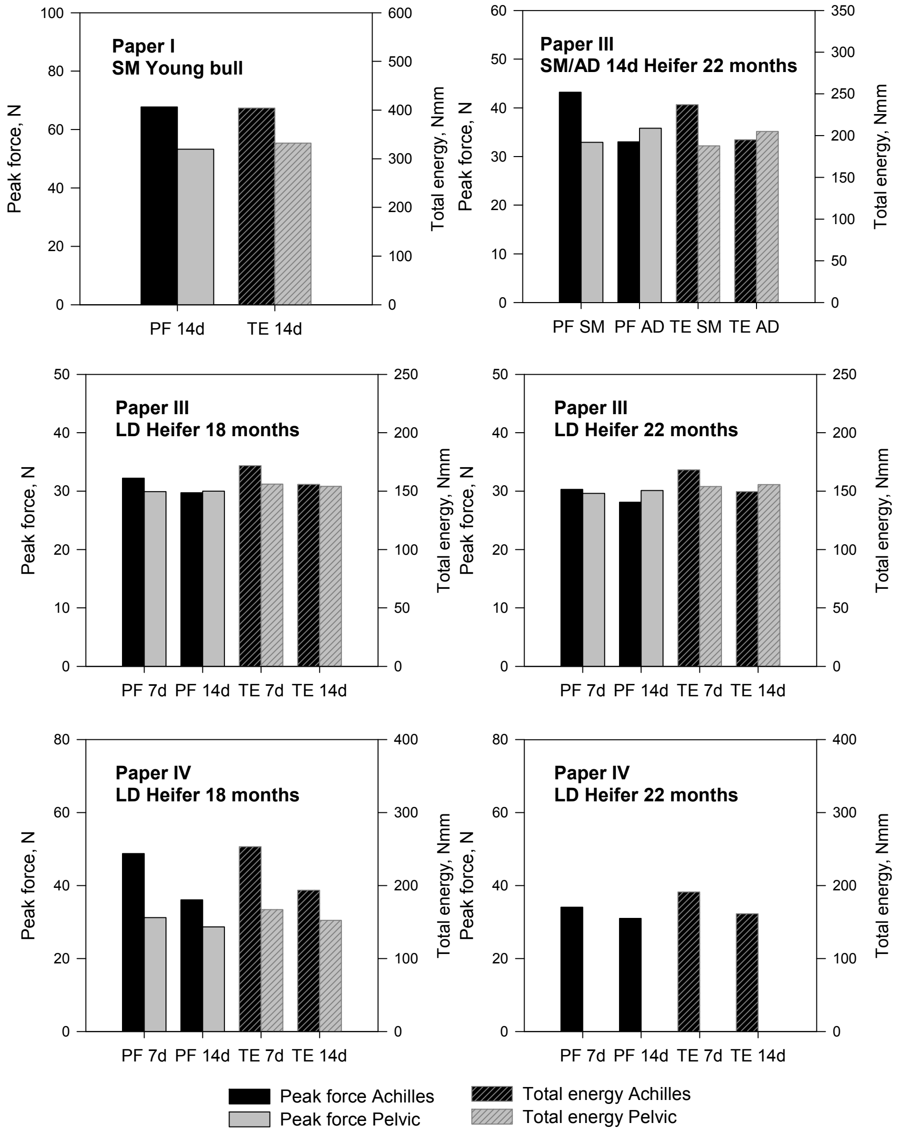

Figure 9. Peak force (PF) and Total energy (TE) values for *longissimus* (LD, III and IV), *semimembranosus* (SM, I and III) and *adductor* (AD, III) samples analysed after 7 and/or 14 days of ageing. Note the difference in peak force scale between papers.

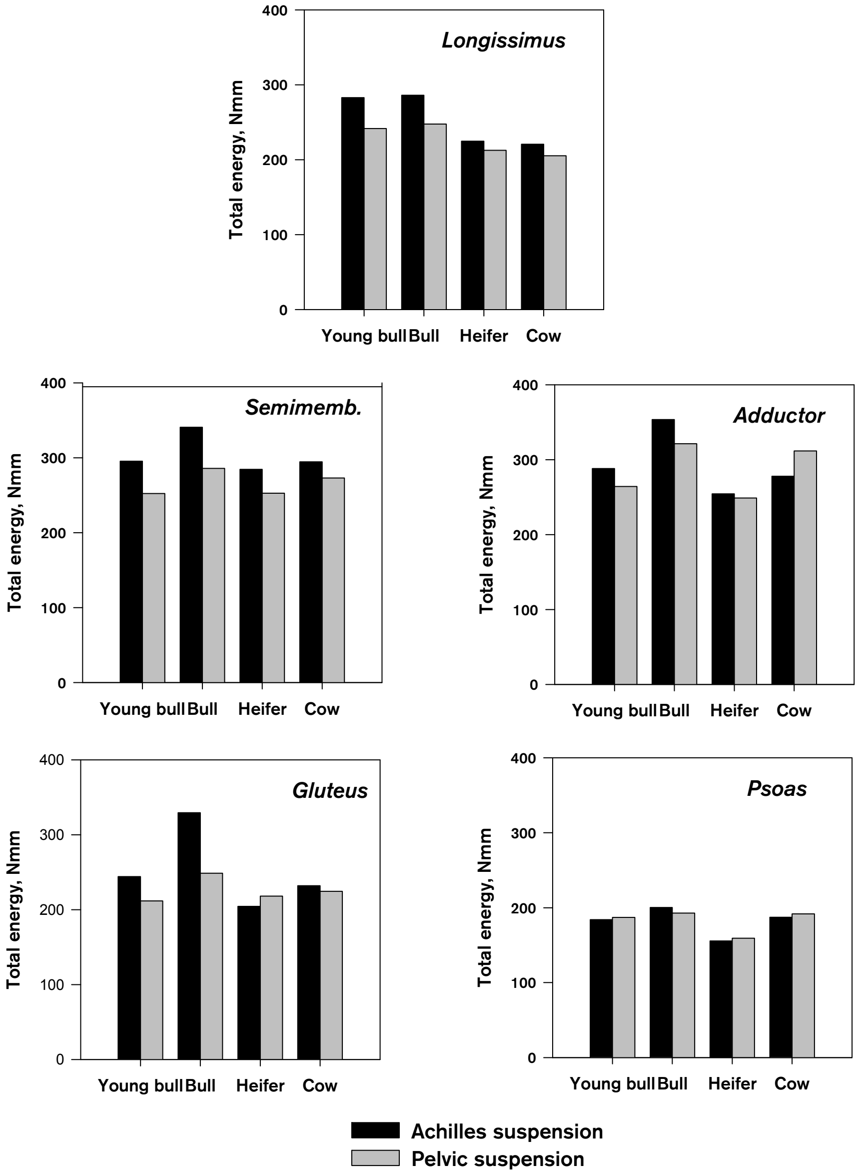

Figure 10. Total energy (Nmm) values for *longissimus*, *semimembranosus*, *adductor*, *gluteus* and *psoas* from young bull, bulls, heifers and cows. for pelvic- and achilles-suspended (II).

Variation in Warner-Bratzler shear force was clearly decreased in pelvic-suspended samples compared to achilles-suspended samples (Table 3). Between animal-variation and within-animal variation decreased in samples aged for both 7 and 14 days. This confirms the study made by Wahlgren *et al.* (2002) where pelvic suspension reduced variation in shear force and was therefore suggested as a good tool to use for tenderness assurance of meat originating from different slaughterhouses.

Prolonged ageing is an effective tool to increase tenderness, especially until 14 days of ageing, after which only smaller improvements can be detected (Campo *et al.*, 1999; Gruber *et al.*, 2006; Smith *et al.*, 1978). The studied ageing times in Paper III and IV are relatively short but often used by the industry. Pelvic suspension has early been shown to reduce the need for ageing (Bouton *et al.*, 1973) and this was clearly demonstrated in our studies. When evaluating the effects of 7 vs. 14 days of ageing (III and IV) for the *longissimus,* samples from achilles-suspended sides aged 14 days were more tender with reduced peak force and total energy compared to those aged 7 days. However, the pelvic-suspended sides aged 7 days were just as tender as those aged 14 days. The within-animal coefficients of variation for both peak force and total energy decreased with pelvic suspension at both 7 and 14 days of ageing for *longissimus* samples, except from Charolais heifers slaughtered at 22 months and aged 14 days (III). Ageing until 14 days also reduced the differences between finishing times.

Finishing feeding is another tool used to increase tenderness. It has previously been shown that shear force can be lowered by finishing feeding or by intensive feeding compared with more extensive feeding (Bowling *et al.*, 1977; Bowling *et al.*, 1978; Dubeski *et al.*, 1997; Hoving-Bolink *et al.*, 1999; Schroeder *et al.*, 1980) However, there are also studies where no differences in shear force from finishing feeding have been found (Bruce *et al.*, 2004; Harrison *et al.*, 1978).

The feeding period was extended for four months in studies III and IV. This gave no further increase in tenderness for *longissimus* samples from heifers in study III. In study IV, heifers slaughtered at 22 months had lower peak force values than achilles-suspended samples from 18 months but no difference was found between pelvic-suspended heifers 18 months and achilles-suspended heifers at 22 months. Pelvic suspension thus reduced the need for further finishing.

The need for further improvement can however be discussed since the samples from our studies can be considered tender based on values from the shear force analysis. A peak force value below 50 N has been suggested by Maher *et al.* (2005) to indicate tender meat. Miller *et al.* (2001) reported a

Table 3. *Overview of peak force means, range and variation in pelvic- and achilles-suspended sides*

Paper	Gender	Muscle	Age.	Susp.	Mean	S.D.	Min.	Max.	CV B[1]	CV W[2]
Paper I	Young bull	SM	14d	AS	67.7	17.4	44.5	123.8	25.7	13.3
	Young bull	**SM**	**14d**	**PS**	**53.3**	**5.7**	**42.6**	**67.1**	**10.7**	**10.0**
Paper II	Young bull	SM	7d	AS	58.2	8.9	44.2	66.3	15.3	13.9
	Young bull	**SM**	**7d**	**PS**	**44.5**	**2.3**	**42.8**	**49.1**	**5.2**	**12.0**
	Bull	SM	7d	AS	67.7	14.1	49.7	87.8	20.8	13.3
	Bull	**SM**	**7d**	**PS**	**47.9**	**7.7**	**35.8**	**56.8**	**16.1**	**11.5**
	Heifer	SM	7d	AS	54.6	8.2	43.2	63.5	15.0	10.0
	Heifer	**SM**	**7d**	**PS**	**45.0**	**7.8**	**34.7**	**53.5**	**17.4**	**11.6**
	Cow	SM	7d	AS	47.3	5.4	41.4	57.1	11.4	9.5
	Cow	**SM**	**7d**	**PS**	**45.4**	**2.5**	**43.0**	**49.6**	**5.5**	**10.8**
Paper II	Young bull	LD	7d	AS	55.4	12.3	42.4	72.0	22.2	17.9
	Young bull	**LD**	**7d**	**PS**	**45.1**	**3.7**	**40.2**	**51.1**	**8.3**	**12.4**
	Bull	LD	7d	AS	55.8	20.5	34.9	80.9	36.7	13.1
	Bull	**LD**	**7d**	**PS**	**43.9**	**9.0**	**36.1**	**56.5**	**20.4**	**9.3**
	Heifer	LD	7d	AS	40.2	11.4	26.9	61.4	28.4	16.5
	Heifer	**LD**	**7d**	**PS**	**37.4**	**9.6**	**26.7**	**56.8**	**25.8**	**18.7**
	Cow	LD	7d	AS	40.4	9.3	29.1	51.7	23.0	13.0
	Cow	**LD**	**7d**	**PS**	**35.0**	**4.0**	**28.9**	**39.4**	**11.5**	**10.4**
Paper III	Heifer-18	LD	7d	AS	32.2	6.6	23.3	45.7	20.5	15.9
	Heifer-18	**LD**	**7d**	**PS**	**29.9**	**3.4**	**24.1**	**38.9**	**11.3**	**10.0**
	Heifer-18	LD	14d	AS	29.7	4.9	21.7	38.6	16.5	13.9
	Heifer-18	**LD**	**14d**	**PS**	**30.0**	**4.1**	**21.5**	**37.4**	**13.7**	**10.5**
	Heifer-22	LD	7d	AS	30.3	5.0	23.1	42.2	16.5	13.2
	Heifer-22	**LD**	**7d**	**PS**	**29.6**	**3.4**	**24.1**	**36.4**	**11.5**	**9.8**
	Heifer-22	LD	14d	AS	28.1	5.8	23.2	45.4	20.6	10.5
	Heifer-22	**LD**	**14d**	**PS**	**30.1**	**3.3**	**25.2**	**37.0**	**11.0**	**9.7**
	Heifer-22	SM	14d	AS	43.2	5.9	32.1	51.5	13.7	11.2
	Heifer-22	**SM**	**14d**	**PS**	**33.0**	**5.9**	**25.5**	**46.4**	**17.9**	**11.4**
Paper IV	Heifer-18	LD	7d	AS	48.8	18.0	29.6	91.2	36.9	28.0
	Heifer-18	**LD**	**7d**	**PS**	**31.2**	**3.7**	**25.1**	**39.9**	**11.9**	**14.5**
	Heifer-18	LD	14d	AS	36.1	11.2	24.1	70.1	31.0	21.2
	Heifer-18	**LD**	**14d**	**PS**	**28.7**	**4.8**	**22.2**	**45.5**	**16.7**	**13.2**
	Heifer-22	LD	7d	AS	34.1	10.3	20.3	63.2	30.2	21.2
	Heifer-22	LD	14d	AS	31.0	14.6	19.3	75.2	47.1	18.9

[1] Coefficient of variation between animals.
[2] Coefficient of variation within animal.

100% consumer acceptability for samples below 30 N. Most samples in Paper III and IV were below the 50 N limit also with achilles suspension and several of both the achilles- and pelvic-suspended samples reported shear forces of about 30 N.

Compression

Texture profile analysis is believed to be a tool more closely related to sensory analysis compared to shear force analysis (Ruiz de Huidobro *et al.*, 2005). It was therefore included in Paper IV to see if it would better differentiate the results from suspension methods.

The *longissimus* samples aged for both 7 and 14 days from the 18-month group hung by the pelvic bone had lower values for the compression parameters compared with the achilles-suspended group, with a larger difference between suspension methods for the 7-day samples. These results were in agreement with shear force values. For the compression peak the decrease in within-animal variation was evident after 7 days of ageing but not 14.

For shear firmness (the slope of a line from the origin of the curve to its peak), the difference between suspension methods was only significant for samples aged 7 days. Cattle fed for 22 months had shear and total energy values similar to the pelvic group at 18 months. No differences were found between samples from pelvic-suspended sides from 18-month-old heifers and achilles-suspended sides from 22-month-old heifers, except for springiness and cohesiveness after both 7 and 14 days of ageing, and compression area 2 and gumminess after 7 and 14 days, respectively.

When evaluating the effects of 7 vs. 14 days of ageing for the *longissimus,* samples from achilles-suspended sides aged 14 days were more tender with reduced peak force and total energy compared with those aged 7 days. Moreover shear firmness and the compression parameters except gumminess were lower after 14 days. Peak force and total energy from the pelvic-suspended sides aged for 7 days were not different from those aged 14 days, but shear firmness and all compression parameters except cohesiveness were reduced after ageing. The extent of ageing was more evident for samples from the achilles-suspended sides from 18-month-old heifers than from the 22-month-animals, where peak force was similar after 7 and 14 days of ageing (P = 0.267). However, total energy and all compression parameters except gumminess showed effect of ageing also in the 22-month samples.

Sensory analysis

Sensory analysis performed on *longissimus* samples aged 14 days (III and IV) showed that suspension method strongly affected all sensory traits except juiciness (III), fat taste (III and IV) and acidity (IV). Muscles from pelvic-suspended sides had higher tenderness (III and IV), lower bite resistance (III and IV), lower threadiness (IV), higher juiciness (IV), stronger meat taste and less visible marbling (III and IV) than meat from achilles-suspended sides at both 18 months (III and IV) and 22 months (III). For *longissimus* tenderness an interaction was found between slaughter age and suspension method where the difference between pelvic and achilles suspension was larger at 18 months than at 22 months (III). Harrison *et al.* (1978) and Vestergaard *et al.* (2000b) both showed the beneficial effect of prolonged feeding on sensory attributes, but in our studies pelvic suspension reduced the need for finishing. A finishing system with higher energy inputs could maybe have influenced the rate of marbling and hence improved sensory quality further as reported by Harrison *et al.* (1978). The need for improved quality can, however, be discussed if the limits for consumer acceptance presented in relation to shear forces are considered (Miller *et al.*, 2001).

Prolonging the feeding period from 18 to 22 months influenced Charolais (III) and Angus (IV) heifers differently. Angus heifers responded to prolonged feeding with higher tenderness and lower bite resistance as well as higher scores for visible marbling and increased fat taste. Charolais heifers only increased scores for visible marbling and acidity.

The stretching effect of the muscle may not affect only the myofibrils but also other structures in the muscle. Eisenhut *et al.* (1965) showed, except for the evidence that stretching of muscles change fibre angles in relation to the spine, also that marbling scores were higher for achilles- sides compared to pelvic-suspended sides. They suggested that the difference in marbling may be due to the shortening of sarcomeres in achilles-suspended sides producing larger globules of intramuscular fat. This finding has, to our knowledge, not been further investigated but has been reported also in Aalhus *et al.* (2000) and is now confirmed in Paper III and IV. Since some consumers prefer to buy meat with low marbling even though the taste of meat with higher marbling is preferred (Grunert, 1997; Ngapo & Dransfield, 2006), use of pelvic suspension could benefit from this fact depending on how the fat is distributed within the muscle.

Relation between instrumental and sensorial measurements

Relations between sensory and instrumental data have been widely discussed. In the late 1960's Marsh *et al.* (1966) described similar correlations between peak force and sensory evaluations, and between total energy and sensory evaluations. Ruiz de Huidobro *et al.* (2005) found that compression tests on raw meat were the best predictor of sensory tenderness of cooked meat. They concluded that shear force was not the best method to analyse meat tenderness. This is a common conclusion (Dransfield, 1996) and still shear forces are the most commonly used method to analyse meat tenderness.

In Paper III, sensory analysis of *longissimus* showed large differences in both tenderness and bite resistance due to pelvic suspension, whereas no differences were found in the instrumental tenderness measurements after 14 days of ageing. Total energy was, however, reduced due to pelvic suspension in samples aged 7 days. Shear force did also show an increased tenderness due to aging in achilles-suspended sides although the differences between 7 and 14 days were similar to the differences between achilles- and pelvic-suspended samples. Novakofski & Brewer (2006) claim sensory methods to have low detection limits and an upper measurable limit leading to curvilinear responses not seen in the instruments, where linear responses are more common. They also state that humans may be more sensitive in certain response regions and that the perception of tenderness involves parameters such as juiciness and firmness together with other parameters involved in the process of breakdown before swallowing. This complex experience can, according to them, not be measured by objective tests such as the shear force method. It should be noted that sensory analysis was more effective in detecting differences than instrumental measurements in our studies.

In Paper IV, more instrumental parameters were analysed, as texture profile analysis by compression were included. The sensory samples (14 days ageing) in Paper IV showed differences between suspension methods of similar size as the shear forces from samples aged 7 days. In this study, peak force was decreased by pelvic suspension also after 14 days. The compression variables demonstrated the lowest, but significant, differences between suspension methods. Thompson *et al.* (2006) suggested that the larger responses in shear force compared to compression variables indicate that pelvic suspension affects the myofibre axis rather than connective tissue. Cooking temperature is also important for the outcome. Eikelenboom *et al.* (1998) found that pelvic-suspended samples were tougher than or equal to achilles-suspended samples at end-point temperatures below 65°C. The rigidity of the myofibrillar compartment is believed to increase with cooking temperature but is probably also accompanied by a reduction in the effect of connective tissue (Bouton *et al.*, 1975b). At temperatures above 65°C the collagen gelatinise and therefore contributes less to the resistance in meat (Bailey & Light, 1989; Lepetit & Culioli, 1994). The end-point temperature used in our study (70°C) is probably minimising the influence of connective tissue on the samples and therefore not providing any substantial differences in compression values between suspension methods.

Bouton *et al.* (1975a) demonstrated stronger relationships between compression parameters and sensory analysis. In our study, correlations between the different instrumental measurements and sensory tenderness for *longissimus* were considerably higher for carcasses suspended by the achilles tendon compared with those hung by the pelvic bone, where instrumental texture data were not significantly correlated with sensory evaluated tenderness. For the samples from heifers slaughtered at 18 months and achilles-suspended, more correlations between sensory evaluated tenderness and shear variables were significant after 7 days of ageing than after 14 days, whereas this difference between ageing times was absent in the 22-month heifers. These differences in correlations are most likely dependent on the differences in variation within the materials as also documented by others (Peachey *et al.*, 2002; Szczesniak, 1968).

Brady & Hunecke (1985) reported that both peak force and shear firmness correlated well with sensory tenderness. Their results showed no correlations between compression variables and sensory analysis. In Paper IV shear firmness was included, but did not increase any correlations compared to other shear or compression variables. Our data does not support the evidence that this measure would be better than shear values or sensory evaluation.

The conclusions from our studies show that a combination of peak force and total energy is a good predictor of tenderness and that it could be used as a measurement when tenderness is the only goal for evaluation.

Age and gender

Age and gender have long been known to influence tenderness of beef. Field *et al.* (1966) found steers and heifers more tender compared to bulls and similar conclusions have been made more recently by Ruiz de Huidobro *et al.* (2003). Fisher *et al.* (1994a) found that meat from barley-silage fed bulls had higher shear force values than both heifers and steers on the same feeding. Studies of pelvic suspension including more than two gender categories are limited but evidence to support our findings can be found for each gender category. Young bulls show a substantial response to pelvic suspension and other stretching techniques (Eikelenboom *et al.*, 1998;

Wahlgren *et al.*, 2002). The larger effect found in bulls of higher age (II) has not been reported elsewhere. Heifers have been more thoroughly evaluated and are generally reported to show significant effects to pelvic suspension, especially in the *longissimus*, in tenderness and sarcomere length (Jeremiah *et al.*, 1984a; & 1984b; Joseph & Connolly, 1977; Mooney *et al.*, 1999). The effects of pelvic suspension in cows have, as in Paper II, been found to be limited. *Enfält et al.* (2004) found no differences between suspension methods for cow *longissimus* samples aged from 2 to 21 days. TenderCut have however been shown to decrease shear force and increase tenderness of cow *longissimus* muscle (Claus *et al.*, 2001; Mandell *et al.*, 2006; Tribot Laspière & Chatelin, 2006).

As described earlier, stretching of sarcomeres has been suggested to increase proteolysis *post mortem* due to increased availability of binding sites for the calpains (Weaver *et al.*, 2008). In Paper II the effect of pelvic suspension on sarcomere elongation was limited in heifers for all muscles. This was also accompanied by unaffected peak forces and a question arises as to whether this lack of response is due to inhibited proteolytic activity. The assumed effect of sarcomere length on proteolysis does, however, not match our results from cows. Peak force values were similar to those of heifers and unaffected by pelvic suspension. The sarcomeres were, however, significantly elongated for all cow muscles except *psoas* (II).

There are apparent gender differences in tenderness development after slaughter. Huff-Lonergan *et al.* (1995) demonstrated *post mortem* protein degradation to be slower in older cows and young bulls compared to steers. The tenderness of the samples in the above mentioned study did, however, show similar values for young bulls and steers, while cows had more tough meat (Huff & Parrish, 1993).

In addition to the difference in proteolysis genders also differs in connective tissue (Prost *et al.*, 1975), where males have higher content than females. Older animals have also been shown to have higher connective tissue content (Purslow, 2005). The highest shear force values in our studies were indeed found in samples from male animals, whereas, the age of the animal seemed to be of less importance, since cows (47-88 months) were as tender as heifers (24-28 months). It is possible that the end-point temperature used in our studies (70°C) was too high to demonstrate clear differences in tenderness related to connective tissue (Bouton *et al.*, 1975b; Eikelenboom *et al.*, 1998).

The effect of pelvic suspension are in our studies combined with the effect of electrical stimulation. Pelvic suspension has, however, been shown effective in prevention of shortening in un-stimulated young bull carcasses in chilling conditions similar to ours (Sørheim *et al.*, 2001). These authors also showed decreased variation in tenderness between samples after pelvic suspension. The additive effects of electrical stimulation and pelvic suspension are low but never negative (Eikelenboom *et al.*, 1998; Fisher *et al.*, 1994b). Derbyshire *et al.* (2007) also demonstrated positive effects of electrical stimulation and pelvic suspension, as well as the combination of the two treatments. The positive effects of pelvic suspension can be of special advantage for smaller slaughterhouses that do not use electrical stimulation (Derbyshire *et al.*, 2007).

Based on the results in our studies we would recommend the use of pelvic suspension as a powerful tool in reducing the variation in tenderness between animals of different background and gender. The most obvious differences between suspension methods were found for males, but among the beef heifers included in our studies individual samples with extreme toughness occurred in achilles suspended carcass sides (III and IV). Shear force values of this magnitude are not often reported in heifers and may be considered to be outliers. The importance of the existence of such extremes should, however, not be neglected. Any occurrence of tough meat is negative for the meat industry. We have not found any explanation for these extreme values, but they are effectively eliminated by pelvic suspension. Since all carcasses in our studies were electrically stimulated it can be concluded that this method alone was not sufficient for producing meat with a consistent tenderness.

Conclusions and implications

Pelvic suspension compared to achilles suspension will result in the following conclusions:

Pelvic suspension will effectively reduce differences in tenderness between genders. Tenderness will be positively affected in the *longissimus*, *semimembranosus*, *adductor* and *gluteus* muscles from pelvic- compared to achilles-suspended young bulls and bulls. Heifers will respond differently to pelvic suspension depending on breed, where *longissimus* tenderness will improve more for beef heifers versus dairy heifers. Pelvic suspension will have the least affect when used for cows.

- The change in suspension method should be relatively easy to implement and would improve the overall quality of Swedish beef meat.
- Lower tenderness levels in meat from males could be improved to tenderness equal to meat from heifers and cows.

The tenderness variation within and between muscles in carcasses from a variety of production and nutritional backgrounds will decrease and the influence of interactions between breed, gender, age and production systems will be reduced.

- Advantages of widespread adoption of pelvic suspension would provide more flexibility for Swedish beef producers to adapt their production systems to their natural prerequisites.
- Fewer complaints about unacceptably tough meat should occur.
- Less variation in quality would have both in-country and export advantages.

Post mortem ageing time can be reduced to 7 days.

- Higher throughput in the industry will lead to better flexibility in processing and storing.
- Addition of value to selected cuts could be done without major requirements of time and space for carcass ageing.

Yield will be increased in some major muscles through either altered shape or decreased purge during ageing.

- Improved yields are closely related to profitability and should be of interest for the industry.

Numerous meat quality traits will be improved, such as reduced purge, improvements in many sensory and textural profile traits, and lower shear force values.

Industry adoption of pelvic suspension can meet resistance because of resistance to change, not realizing the benefits of the technique, perception of increased labour cost and increased need for space in the coolers.

- Each company has to make a choice based on a balance between cost and benefit and the contribution to overall improvement of Swedish beef meat.
- Systems containing advantages for value-addition may have to be developed.
- Pelvic suspension could be a valuable tool to remain competitive in the market.

Future perspectives

The papers in this thesis have shown pelvic suspension to be an effective tool to increase tenderness and reduce drip. The variation in tenderness both within and between animals can also be decreased. Furthermore, the response to pelvic suspension has been shown to vary between genders with smaller effects in female individuals. Further knowledge would, however, be appreciated in the following areas:

- The elongation of sarcomeres due to pelvic suspension could differ depending on the method used for stretching. The predominantly used method for pelvic suspension in Sweden is with a rope from the pelvic bone. Further studies should focus on the elongation of sarcomeres in different sections of the loin when using several stretching techniques.

- The additive effects of electrical stimulation and pelvic suspension needs to be further evaluated. As electrical stimulation is generally not used in smaller slaughterhouses pelvic suspension could be a good tool to obtain tender meat.

- The yield of the loin was affected by suspension method and it would be of major importance to investigate the reason for this and evaluate the benefit obtained.

- Although results have been put forward to show benefits of pelvic suspension on "average" meat not from the higher quality classes, the method is only implied as a quality assurance method on high quality meat from young animals. The hesitant behaviour from the industry is explained by the fact that pelvic-suspended carcasses take more space in the coolers and therefore make storing of carcasses inefficient. Therefore it would be of interest to investigate the effects of a stretching method such as the TenderCut, not associated with altering the position of the hind leg but still effective in stretching the loin muscle.

- Future studies should also focus on further understanding of the differences in tenderness between genders. This would imply deeper studies on connective tissue, sarcomere length, fibre diameter and intramuscular fat.

- The interaction between cooking temperature and effect of stretching especially on connective tissue is still not fully explained. This could be evaluated using compression and extended shear measures to gain more knowledge on optimal utilisation of instrumental tenderness.

- The possible difference in rate of proteolytic degradation depending on muscle stretching needs to be further investigated. Fundamental chemical, physical and biological changes in the muscle protein matrix due to stretching still await an explanation.

The outermost goal of further work in the area must, however, be to gain knowledge for the industry on how to apply the research on pelvic suspension in the production. This may be achieved by development of programs for value-added products or systems for quality assurance.

There are great advantages to gain both for the university and the industry if research on meat quality is done in cooperation. The mutual vision is to continuously provide the Swedish consumers with high quality Swedish meat.

Svensk sammanfattning

Svensk nötköttsproduktion

I Sverige äter vi ca 28 kg nötkött per person och år, där 58% av det nötkött vi äter kommer från en inhemsk produktion. Svenskt nötkött kommer från djur såväl av köttras som mjölkras och de föds upp på många olika sätt. Nötköttsproduktionen i Sverige är generellt sett grovfoderbaserad. På somrarna betar de allra flesta djuren ute och på vintern föds många djur upp med en kombination av vallensilage och kraftfoder på stall. Den kvalitetsparameter som är viktigast när det gäller nötkött är mörhet. Mörheten varierar med djurets ålder och kön där yngre djur och djur av honkön generellt sett betraktas som mörare än äldre djur och djur av hankön. Mörheten beror också på olika faktorer under slakten och under tiden från slakt till konsumtion, även kallad mörningstid. De många uppfödningssätten och raserna som används leder till en stor variation mellan slaktkroppar och muskler.

Slakt och distribution

Storleken på svenska slakterier varierar väldigt mycket och i statistiken finns slakterier med årlig slakt från 2 till 78 400 nötkreatur. Under 2007 slaktade de fyra största boskapsslakterierna i Sverige mer än hälften av de 420 000 nötkreatur som slaktades under året. Tio slakterier slaktade mer är 200 nötkreatur i veckan och åtta av dessa slakterier använde sig av elstimulering i slakten. Elstimuleringen kombineras med kylning med hög lufthastighet och temperaturer runt 0°C i 1–2 timmar innan slaktkropparna flyttas till en lagerkyl som håller 2–5°C. De slakterier som inte använder elstimulering kyler i 2-5°C men med högre lufthastighet under första dygnet. Sedan detta doktorandarbete påbörjades har bäckenhängning introducerats i Sverige och det används som kvalitetssäkringsmetod på ca 3% av slaktkropparna. Största delen av köttet i Sverige möras i vakuumförpackning efter att det styckats ner från slaktkroppen ungefär två dygn efter slakt. En stor förändring för hur kött säljs i Sverige har skett under de senaste fem åren. Tidigare distribuerades köttet till butik i vakuumpåsen, styckades upp i mindre bitar och skivor i affären och såldes till konsumenterna i tråg svepta i plastfolie. Nu möras köttet fortfarande i vakuum, men sedan skivas och packas det centralt i större anläggningar. Köttet kommer sedan till butiken i färdiga konsumentförpackningar.

Omvandling från muskel till kött

Kött består av 75% vatten och 20% protein, de resterande 5 procenten är huvudsakligen fett, glykogen och mineraler. Muskelproteinerna har olika funktioner. Proteinerna aktin och myosin möjliggör kontraktion av musklerna medan de strukturella proteinerna kollagen och elastin bildar bindväv och ger musklerna stöd. Mängden bindväv och bindvävens styrka ökar med djurets vikt och ålder. Aktin och myosin är organiserade i strukturer som kallas sarkomerer. De kan förlängas eller förkortas beroende på hur många bindningar som bildas mellan aktin och myosin. I det levande djuret stimuleras musklerna av nervsignaler som gör att musklerna kontraherar. Energi behövs både för att styra kontraktion och avslappning av musklerna. Energin produceras via glykolysen från glykogen i musklerna. Efter slakt börjar omvandlingen från muskel till kött. Muskeln fortsätter då producera energi men eftersom blodet inte längre kan transportera bort avfallsprodukter så ansamlas mjölksyra och pH-värdet i muskeln sjunker. Glykolysen stannar när glykogenet tar slut eller när enzymerna som styr den inaktiveras av det låga pH-värdet. När ingen mer energi produceras kan inte musklerna hållas avslappnade. *Rigor mortis* (likstelhet) inträffar när energin tagit slut och permanenta bindningar mellan aktin och myosin bildats. Sarkomerernas längd bestäms av graden av överlappning mellan aktin och myosin. När *rigor* inträtt påbörjas mörningsprocessen där proteiner bryts ner och ger mörare kött. Den slutliga mörheten blir bättre om mörningsprocessen kan starta tidigt. Därför används elstimulering som sker genom att ström leds genom slaktkroppen. Detta görs för att få musklerna att kontrahera och konsumera energi. *Rigor* inträder tidigare och mörningsprocessen kan starta snabbare. Elstimulering kan också förhindra den kraftiga kontraktion som bildas om slaktkroppen kyls för hårt. Kylsammandragning eller "Cold shortening" ger helt överlappande myosinfilament och kött som är omöjligt att möra. Därför rekommenderas elstimulering om väldigt kraftig kyla används. Mörheten i en muskel blir bättre om muskeln går in i *rigor* med sträckta sarkomerer. Vanlig hängning från akillessenan i bakbenet gör att många muskler i bakdelen får en chans att dra ihop sig och ryggen svankar och kan därför dra ihop sig och ge korta sarkomerer. Sträckning kan uppnås i flera muskler i bakdelen och ryggen om man hänger slaktkroppen i bäckenbenet (pelvic bone) istället för i akillessenan på bakbenet. Om slaktkroppen bäckenhängs får den en ställning som efterliknar den hos det levande djuret. Musklerna i bakdelen får en mer naturlig form och ryggen sträcks. Sarkomererna går in i rigor i ett mer sträckt tillstånd, de kan då hålla mer vätska och köttet blir mörare.

Mörning av kött sker genom nedbrytning av proteiner (proteolys), men bindningen mellan aktin och myosin som bildas vid *rigor* försvinner inte. Det är därför viktigt att muskeln ej går in i *rigor* i ett kontraherat tillstånd. Mörningen av kött beror på aktiviteten av de proteolytiska enzymerna, bla. calpainerna, i muskeln och längre mörningstid leder till ökad mörhet. Enzymerna som driver mörningen är aktiva tills köttet upphettas vid tillagning. Mängden bindväv påverkar också mörheten och varierar mycket mellan olika muskler.

Avhandlingens syfte

Syftet med avhandlingen var att öka kunskapen om bäckenhängning och dess påverkan på olika muskler från nötkreatur av olika kön. Bäckenhängningens effekt undersöktes på djur av såväl köttras som mjölkras, och dess effekt i kombination med olika slutuppfödning och mörningstider undersöktes också.

Kvalitetsanalyser

Köttkvaliteten utvärderades med hjälp av följande analyser:

Vattenhållande förmåga – vätskeförlust i vakuumpåsen samt förlust vid frysning och kokning registrerades.

Sarkomerlängd – längden på sarkomererna mättes i mikroskop.

Skärmotstånd – den kraft som krävs för att skära igenom ett köttprov av standardiserad storlek. Ett lågt värde betyder mörare kött.

Sensorik – en tränad smakpanel bedömer köttets mörhet, bitmotstånd och saftighet mm. Högre värden är kopplade till högre intensitet.

Artikel I och II

Effekten av bäckenhängning undersöktes i kött från djur av mjölkras, slumpmässigt utvalda på ett slakteri. Ungjurar (22-28 månader), tjurar (32-35 månader), kvigor (24-28 månader) och kor (47-88 månader) ingick i studierna. Fem muskler analyserades; biff (*longissimus*), innanlår (*semimembranosus*), adduktor (*adductor*), rostbiff (*gluteus*) och filé (*psoas*).

Alla muskler utom filén sträcktes med bäckenhängning i jämförelse med normal hängning i akillessenan. Sarkomerlängden ökade i biffen, innanlåret, adduktor och rostbiffen för bäckenhängda ungtjurar, tjurar och kor. Ingen skillnad fanns för kvigor av mjölkras. Utbytet ökade för biffen, där bäckenhängda sidor vägde 9% (400g) och 7% (270g) mer för handjur respektive hondjur. Vätskeförlusten i vakuumpåsen under mörning minskade med bäckenhängning speciellt för biffen och innanlåret. Alla muskler utom filén fick minskat skärmotstånd i bäckenhängda prover från ungtjurar och tjurar. Denna skillnad fanns inte för kvigor och kor. Filén påverkas lätt negativt av bäckenhängning men var fortfarande den möraste styckningsdetaljen. Variationen i skärmotstånd, både inom djur och mellan djur, minskade tydligt med bäckenhängning. Skillnaden mellan könen minskade också och kött från ungtjurar och tjurar nådde samma mörhetsnivå som kött från kvigor och kor.

Artikel III och IV

Charolaiskvigor och anguskvigor uppfödda på Götala försöksstation i Skara ingick i studien. De föddes upp gemensamt från 8 månaders ålder och slaktades antingen vid 18 månaders ålder direkt efter bete eller vid 22 månaders ålder efter slutuppfödning med huvudsakligen ensilage på stall. Biffen analyserades efter 7 och 14 dagars mörning. För anguskvigorna resulterade bäckenhängning i lägre skärmotstånd både efter 7 och 14 dagars mörning. Bäckenhängningen gjorde också kött från kvigor slaktade vid 18 månaders ålder lika mört som normalhängt kött från kvigor slaktade vid 22 månaders ålder. Däremot hittades inga skillnader i skärmotstånd mellan hängningsmetoder för charolaiskvigorna. För både charolais- och anguskvigor minskade bäckenhängning betydelsen av mörningstid. Bäckenhängt kött var lika mört efter 7 dagars mörningstid som normalhängt efter 14 dagars mörning. Även variationen i mörhet minskade för båda djurgrupperna. Den sensoriska analysen visade att mörheten var högre för bäckenhängda sidor jämfört med normalhängda sidor för både charolaiskvigor och anguskvigor. En spännande iakttagelse var också att den synliga marmoreringen minskade i bäckenhängda jämfört med normalhängda sidor.

Slutsats

Bäckenhängning är en effektiv metod för att öka mörheten i nötkött och minska variationen inom och mellan djur. Den ökade sarkomerlängden gör att muskeln kan hålla mer vatten vilket leder till minskade vätskeförluster under mörningen. Sträckning av musklerna leder också till en mer utsträckt form och i denna studie till och med ökad vikt för biffen. Detta sker förmodligen på bekostnad av någon annan detalj, men kan ändå vara av betydelse eftersom biffen är en av de dyraste styckningsdetaljerna. Användning av bäckenhängning minskar behovet av lång mörningstid och slutgödning av djur och bör därför vara av stort intresse för industrin.

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