



Swedish University of
Agricultural Sciences

Department of Animal Breeding and Genetics
Department of Food Science

Candidate genes for beef quality – allele frequencies in Swedish beef cattle

by

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Supervisors:

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Examensarbete 301

2008

Examensarbete ingår som en obligatorisk del i utbildningen och syftar till att under handledning ge de studerande träning i att självständigt och på ett vetenskapligt sätt lösa en uppgift. Föreliggande uppsats är således ett elevarbete och dess innehåll, resultat och slutsatser bör bedömas mot denna bakgrund. Examensarbete på D-nivå i ämnet husdjursgenetik, 20 p (30 ECTS).



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Agrovoc: Meat quality, Beef, Leptin, Calpain, Calpastatin och Diacylglycerol O-acyltransferase

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ABBREVIATIONS

DFD- Dark, Firm and Dry meat, can also be named “high pH” meat. DFD can result from chronic stress, where the animal has depleted the muscle glycogen reserves prior to slaughter and the meat therefore do not show a normal pH drop.

Haplotypes- A set of alleles at closely linked loci, or a set of single nucleotide polymorphisms (SNPs), that are transmitted together.

Linkage disequilibrium- The non random Combinations of alleles or genetic markers that occur more frequently in a population than would be expected from a random formation (see also Haplotypes).

Marbling- Marbling is small streaks of fat that are found within the muscle, i.e. intramuscular fat, and give somewhat the appearance of a marble pattern.

MAS- Marker Assisted Selection, selection of wanted/unwanted properties using a marker strongly associated with the phenotype.

Palatability- Texture, tenderness, juiciness and flavour.

Pelvic Suspension- The carcass is suspended in the aitch bone instead of the Achilles tendon which is a more common procedure.

PSE- Pale, Soft and Exudative meat can result from acute stress and gives a rapid initial acidification, i.e. a fast pH drop. The proteins in the meat denature and its water holding capacity is reduced.

Technological quality- Fat colour, amount of marbling, texture, meat colour and water holding capacity of meat.

SNP- Single Nucleotide Polymorphism, a point mutation changing one nucleotide to another. Occurring on average once every 10 kb in the genome.

SAMMANFATTNING

Det råder idag stor variation i köttkvalitet mellan enskilda djur vilket avspeglas i en ojämn kvalitet i butikernas styckningsdetaljer. Konsumenten kan inte garanteras en god ätkvalitet, vilket kan leda till minskad förtroende för svenskt kött och därmed påverka efterfrågan. Köttkvaliteten är därför av ekonomiskt betydelse för den svenska nötköttsproduktionen.

Köttkvaliteten styrs av djurets genuppsättning i kombination med miljöpåverkan som t.ex. foder och slakthantering. Resultat från internationella forskargrupper pekar på betydande inflytande av enskilda gener på mörhet och andra egenskaper som är av betydelse för ätkvaliteten. Dessa geners effekter bör även skattas för svenska produktionsförhållanden för att i framtiden bl.a. kunna utnyttjas i avelsprogram för förbättrad ätkvalitet hos svenskt kött i kombination med bra produktionsegenskaper hos de köttproducerande djuren.

Litteraturstudien i detta arbete omfattar framförallt gener som påverkar mörhet och marmorering. De gener som är undersökta för sin eventuella inblandning i marmoreringsprocessen är främst de som kodar för enzymer med direkt effekt på fettmetabolismen; leptin, thyroglobulin och DGAT (acyl-CoA: diacylglycerol acyltransferas). Samtliga dessa gener innehåller en eller flera enskilda mutationer som i olika studier visat sig påverka fettrelaterade egenskaper. Vad gäller köttets mörhet är de gener som styr uttrycket av det proteolytiska enzymet calpain och dess inhibitor calpastatin av särskilt intresse. Även dessa gener innehåller en rad mutationer som anses inverka på slutproduktens kvalitet. Litteraturstudien omfattar förutom ovan nämnda gener även gener vilkas effekter endast analyserats i ett fåtal studier, t.ex. *DNAJAI* (ett s.k. heat shock protein) och *NATI* (Novel APOBEC-1 target-1).

Studien inkluderar en analys av allelfrekvenser för några mutationer i de mest undersökta kandidatgenerna. Totalt har ca 400 kött djur genotypbestämts av det engelska företaget Igenity. De genotypade djuren härrör från svenska besättningar och representerar de fem olika raserna Charolais, Angus, Hereford, Limousin och Simmental. Allelfrekvenserna för de olika mutationerna i kandidatgenerna är i huvudsak överrensstämmande med värden i tillgänglig litteratur. Den största rasskillnaden i allelfrekvens i vår studie observerade för markören CAPN1-4751 i genen som kodar för det proteolytiska enzymet calpain. Den ur mörhetssynpunkt ofördelaktiga T allelen (vanligt förekommande i *Bos indicus* rasen) hade i vår undersökning en högre frekvens i rasen Simmental såväl jämfört med de övriga undersökta raserna, som de värden som anges för rasen i tillgänglig litteratur. Den högre allelfrekvensen kan möjligen hänföras till "sampling effekter" på grund av det begränsade djurmaterialet, dvs. att de genotypade djuren inte är representativa för populationen som helhet.

Vid fortsatta studier av enskilda geners effekt på köttkvalitet i den svenska populationen av kötttrasadjur krävs noggrann planering vad gäller logistiken vid provinsamling och vid valet av testparametrar. På grund av den låga andelen renrasiga kötttrasadjur som slaktas i Sverige är man hänvisad till att genomföra studier av köttkvalitet på korsningsdjur. För att kunna ta hänsyn till variationsorsaker som slakteri, foderstat, kön och ålder vid analysen av data, bör dessa inte tillåtas variera allt för mycket, särskilt om man arbetar med ett begränsat djurmateriale. Data från enstaka, stora gårdar med jämn fördelad slakt över säsongen är att föredra. När effekterna av de utvalda kandidatgenerna har skattats i den svenska populationen av kötttrasadjur bör allelfrekvenserna i dessa gener bestämmas i de renrasiga populationerna.

ABSTRACT

In Swedish beef retail cuts there are marked differences in meat quality, e.g. tenderness and marbling, between individual animals. A proportion of these differences are explained by the animals' genetic make up. A number of polymorphisms in key genes have been reported for their association with meat quality traits and the major candidate genes in this paper are *DGAT1*, *Leptin*, *TG*, *CAPN1*, *CAST*, and *DNAJ1*. Genotype information on 400 individuals from five Swedish beef breeds (Charolais, Hereford, Angus, Simmental and Limousin) regarding eight polymorphisms located in four candidate genes was compared to literature reports. The results from frequency analyses of these eight polymorphisms revealed only minor deviations from previous reports. However, the Simmental individuals in this study differed from both the other *Bos Taurus* breeds included in the study and from results in the literature in that a lower frequency of the "tender" T allele was observed for the marker CAPN1-4571 in the gene encoding the proteolytic enzyme calpain. This could be a result of "sampling effect" due to small sample size i.e. the samples are not representative for the overall population.

In an upcoming study regarding genetic effects on meat quality in Swedish beef breeds samples from pure beef breeds need to be complemented with crossbreeds in order to achieve adequate sample sizes. Systematic effects of gender, feed, age, and slaughter treatment should be minimized, especially considered the limited animal material available. Potential breeding animals may, when associations between the candidate genes and meat quality traits are well established, be genotyped to add information about their genetic potential for producing high quality beef.

INTRODUCTION

Skeletal muscle has been used in the human diet for thousands of years and was one of the first traits to motivate domestication of animals (Hocquette *et al.*, 2005). In urbanized countries the development in farm animal production has during a long period had a main focus on extensive rearing systems. In a historic perspective, the focus on quantity has been necessary to support the growing population with meat at low costs. Today, in the developed countries there is no longer a shortage of animal products, and hence producers have the last decade started to focus on quality. These quality oriented rearing systems have developed from consumers' higher demands on the products and attention has been centred to quality reinsurance and animal health (Jeon *et al.*, 2006). Research has worked in the same direction, and has expediently contributed to the rapid progress in the development of high quality parameters.

A problem for the consumers is that cooked beef may vary in taste, tenderness and appearance from time to time, even if you prepare the meat exactly the same way, due to large variation in the quality of beef retail cuts. This variation results from several factors, e.g. nutrition, gender, age, rearing system, slaughter procedures, etc. Therefore today's standardised way to breed and slaughter cattle should provide the necessary conditions for obtaining high and reproducible beef quality, something that would be appreciated by the consumer. However, the consumer's perception of eating quality is also dependent on the individual animal's genetic make up why quality of retail cuts will differ between individuals.

Generally, in commercial production the endeavour to minimize cost and maximize income plays an essential role. High and reliable quality products are crucial to establish consumers' confidence and to stabilize the company's income, something that is also applicable to the beef production. To accomplish this, factors that may impact on the meat hygiene have to be optimized. Meat hygiene is often controlled by an "in-house control system", e.g. a Hazard Analysis and Critical Control Points (HACCP) program which marks critical steps in the production line that may compromise human health, and is in the majority of countries regulated by law (Warris, 2004). However, control programs for eating quality is not compulsory and exist only for a few fresh meat products in Sweden. An additional obstacle for improving eating quality is the fact that in modern industry a fast turnover rate is one of the most important factors for a profitable business. Storage time is expensive and thus the time allowed for tenderisation is often minimised (Edlund *et al.*, 1999), with a negative effect on tenderness (Warris, 2004).

This study gives an overview of the research on candidate genes and their effect on the eating quality of meat. It also outlines how some of the variation in final meat quality can be explained by individual differences in the genetic make up. In addition, the paper includes a frequency analysis in Swedish beef cattle for some of the polymorphisms identified in the candidate genes dealt with in the literature overview. The results should be indicative of possible focal points in future studies.

SELECTION FOR MEAT QUALITY

Apart from the logical association to sensory meat characteristics, the term meat quality may also refer to several other aspects. The wholesomeness in meat quality is an important aspect which may refer to the products' nutritional value and to microbial and/or chemical food safety. High meat quality may also refer to a carcass with a favourable conformation and an optimal ratio of fat and lean meat. Also, perceived meat quality may include ethical aspects, like animal welfare and sustainable production systems. The latter aspects are, however, not further addressed in this study in which meat quality is referred to as technological and sensory quality/palatability (see Abbreviations).

The perception of eating quality can be affected anywhere in the product chain, all the way from the set of genes the calf inherited from its parents to the post-mortem treatment of the meat, apart from the price the meat attributes that are the most important for the consumer are tenderness, juiciness, flavour and colour (Bernard *et al.* 2007). However, these are not the same parameters that are of highest economic importance to the Swedish beef breeders, i.e. growth, feed efficiency and maturity time. In order to accomplish a parallel improvement of quality and production efficiency it is of great importance that the two categories of traits are not negatively correlated, i.e. selection for improved meat quality must not conflict with the traits that are of economic importance to the breeder (Christensen and Therkildsen, 2006).

The major meat quality parameters have an intermediate to high heritability and thus allow for genetic selection to achieve an overall improvement of the meat quality. In data taken from 7179 beef cattle representing 14 breeds, the estimated heritability for Warner-Bratzler shear force (WBSF) test was 0.40. In the same study, 2320 steak samples were tested by a trained sensory panel who estimated the heritability for tenderness and juiciness to 0.37 and 0.46, respectively (Dikeman *et al.*, 2005). Estimated heritabilities of marbling (see, Abbreviations) score were ranging between 0.30-0.57 (see review by Utrera and Van Vleck, 2004). However, the beef quality parameters that generally are of importance for consumer acceptance are expensive to measure with available methods. Thus, it would be advantageous

if the eating quality of meat of an animal could be assessed based on its genotype (see review by Garnier *et al.*, 2003).

Modern techniques enable more detailed studies regarding the genetic influence on meat quality. For example, molecular techniques make it possible to search for functional (causative) mutations and linked markers. However, to genetically improve beef quality a first step is to define the economic values of the different meat characteristics in order to set up for selection priorities. For marker-assisted selection, MAS, the genetic markers should be well documented for their effect in the target population. Today only a limited number of markers have been confirmed to have a functional effect (Kühn *et al.*, 2005). According to Dekkers (2004) there are three different types of genetic markers that can be used in MAS 1) direct markers, coding for a functional mutation 2) linkage disequilibrium (LD, see Abbreviations), markers that are in population-wide LD with the functional mutation. (The distance between the LD marker and the functional mutation is dependent on the degree of LD, which in turn is dependent on the history and structure of the population) 3) linkage equilibrium (LE) markers that are in population-wide equilibrium, i.e. random combination of alleles at marker loci, but sufficiently close to the functional mutation to show low recombination fraction. Direct and LD markers may be used regardless of family background whereas LE markers function best within families, due to the occurrence of different coupling phases between families in a population. This means that the economic cost for selection based on direct and LD markers can be lower, at the same time as the accuracy is higher. The most practical and commercially viable system would be gene assisted selection with direct markers (GAS), although these markers can take many years to identify (Mullen *et al.*, 2006).

Approximately 200 bovine genetic markers are presently being investigated by the European Union project, GeMQual, where candidate genes are being associated to meat quality traits by measurements in 15 different European breeds. Swedish beef populations are however not included. The traits most studied are tenderness and marbling, and several single nuclear polymorphisms, SNP, (see Abbreviation) have been associated both with tenderness (Page *et al.*, 2002, Costello *et al.* 2007, Schenkel *et al.* 2005, Casas *et al.*, 2006 and Morris *et al.*, 2006) and marbling (Thaller *et al.*, 2003, Barendse *et al.*, 2004, Schenkel *et al.*, 2006a, Schenkel *et al.*, 2006b and Nkrumah *et al.*, 2006). There are DNA tests available for genotyping and validation of the haplotypes' (see, Abbreviations) effects on desired traits. Several of the tests have been evaluated by the National Beef Cattle Evaluation Consortium, NBCEC in USA, and some are confirmed to reflect meat quality (Quaas *et al.*, 2006), whereas others showed no significant association with the claimed characteristics (Quaas *et al.*, 2006 Rincker *et al.*, 2006). Colour and colour stability have also been stated as important meat attributes and should therefore be relevant to consider when discussing meat quality (Bernard *et al.*, 2007).

Marbling

The term marbling refers to the amount of intramuscular fat (IMF) that is present within the meat. In the growing animal fat deposition takes place at different growth stages. The main onset of fat deposition occurs at puberty and the degree of marbling at slaughter is thus highly dependent on when in relation to sexual maturity it takes place. The onset of puberty is to a large extent dependent on breed. At equal age the late maturing breeds Limousin, Simmental and Charolais tend to be leaner than early maturing breeds like Angus and Hereford (Wheeler *et al.*, 2005). Sex and castration are also important factors for the final fat deposition, through affecting the level of male sex hormones and thereby also the anabolic effect on muscle growth (Warris, 2000).

To be able to study the degree of marbling it is necessary to grade the amount of IMF present within the meat at a defined cut. There is no standardised method, but fat bound within meat can be extracted by organic solvents. This approach to calculate the IMF is both expensive and time consuming, and for that reason alternative methods have evolved (Warris, 2000). One modern technique to estimate IMF level is image analysis. Digital images give information on marbling; by scanning of the picture and computation of the fraction of fat particles (Faucitano *et al.*, 2005).

In the beef industry there is no world-wide grading system that is being applied for marbling, but the USA, Canada and Japan have all developed systems which involve visual marbling grading (Strong, 2004). This visual grading is often performed on a defined cut, for example on the *Musculus longissimus* at partitioning of the carcass at the 10/11th rib site. There have been many attempts to develop an objective form of IMF grading, but no methods have so far been generally accepted.

The level of IMF is of relevance in terms of palatability. A low level of IMF reduces the eating quality and is reported to have a negative impact on both perceived tenderness and juiciness. A high level IMF will give a high overall score in a sensory panel, but this is only true at levels of fat exceeding 2% (Warris, 2004). Finding associations between marbling and genetic polymorphisms are more complicated than finding genetic associations with tenderness because of the often subjective way of measuring marbling. This means in practice that larger amount of registrations are needed to get high enough accuracy in estimations of the genetic effects (Hocquette *et al.*, 2007).

Candidate genes

Listed below are the genes that carry alleles that may, single wise or grouped in haplotypes, have impact on marbling characteristics.

Diacylglycerol O-acyltransferase (DGAT)

DGAT is an enzyme which catalyzes the final step of the triglyceride synthesis (Cases *et al.*, 1998; Winter *et al.*, 2002). Mutated mice with *DGAT1* genes totally silenced can no longer synthesise milk. The depletion of milk secretion is likely due to the lack of triglyceride anabolism in the mammary gland. Mice with silenced *DGAT1* genes still produce triglycerides in other tissues, but to a lower extent and do also resist diet-induced obesity (Smith *et al.*, 2000). Sorensen *et al.* (2006) suggest that genotyping for the mutation that changes the structure of the DGAT enzyme, rather than quantitative measures of serum levels of the enzyme DGAT, would be a possible tool in selection for marbling properties. So far, there are only a few indications of an association between the level of DGAT enzyme and muscular fat content (Sorensen, *et al.*, 2003).

Genetic polymorphism in the DGAT1 gene

The *DGAT1* gene is found on bovine chromosome 14 and has in dairy cattle been identified as a candidate gene for fat content in milk. A dinucleotide polymorphism in exon eight causes a change in the amino acid sequence of the protein from a lysine (K) to an alanine (A) in the position 232 (K232A) (Grisart *et al.*, 2001). *In vitro* assays from virus expression systems indicate that the lysine variant gives a catalytically more active enzyme (Grisart *et al.*, 2004).

The association of this polymorphism with fat content in meat has so far not been closely studied. Thaller *et al.* (2003) found an association with this SNP and intramuscular fat content in *M. semitendinosus* whereas Moore *et al.* (2003) found no association with backfat (for allele frequencies of the K232A polymorphism see Table 1). In the Korean breed, Hanwoo, no effect was found for the K232A substitution alone, but in combination with another SNP in the *DGAT1* gene, named T11993C, the two polymorphisms showed a significant effect ($P < 0.005$) on marbling (Kong *et al.*, 2007).

Table 1. Allele frequencies of the K232A polymorphism in the *DGAT1* gene.

Breed	n	A ²	G ³	Literature cited
Crossbred ¹	36	0.29	0.71	(Sorensen <i>et al.</i> , 2006)
Holstein (Dairy breed)	79	0.42	0.58	(Kaupe <i>et al.</i> , 2004)
Jersey (Dairy breed)	10	0.65	0.35	(Ripoli <i>et al.</i> , 2006)
	47	0.69	0.31	(Kaupe <i>et al.</i> , 2004)
Hanwoo (Korean Breed)	200	0.25	0.75	(Kong <i>et al.</i> , 2007)
Angus	75	0.09	0.91	(Ripoli <i>et al.</i> , 2006)
	43	0.13	0.87	(Kaupe <i>et al.</i> , 2004)
Charolais	10	0.15	0.85	(Ripoli <i>et al.</i> , 2006)
	27	0.11	0.89	(Thaller <i>et al.</i> , 2003)
	31	0.08	0.92	(Kaupe <i>et al.</i> , 2004)
Simmental	126	0.06	0.94	(Kaupe <i>et al.</i> , 2004)
Hereford	10	0.00	1.00	(Ripoli <i>et al.</i> , 2006)
	50	0.00	1.00	(Kaupe <i>et al.</i> , 2004)

¹*Bos taurus* (Charolais and Holstein)

²The A nucleotide in position 232 gives rise to a lysine

³The G nucleotide in position 232 gives rise to an alanine

Thyroglobulin, (TG)

Thyroglobulin is one of the largest proteins in the body and is a dimeric glycoprotein hormone (660kDa) synthesized by the epithelial thyroid cells. It is secreted to the follicular lumen and is together with iodine a precursor for the thyroid hormones triiodothyronine (T3) and thyroxin (T4). These hormones have several functions, they have an impact on metabolic rate and are involved in the body growth, by regulating growth hormone secretion and thereby skeletal growth. T3 and T4 are involved in the development of several functions e.g. in the central nervous system. They are also important for normal function of the reproduction organs; deficiency may cause disturbances in the female reproduction cycle and in males lead to reduced sperm production. The thyroglobulin also serves as a reservoir for iodine and inactive thyrodine hormones (Sjaastad *et al.*, 2003).

Wagyu (Japanese Black) cattle are a breed known for its high marbling score. There are indications of higher marbling score in Wagyu crossbreeds compared to individuals without Wagyu genes. However, purebred Wagyu individuals have lower marbling scores than crossbreeds (Mears *et al.*, 2001). The same study reports that, independent of breed,

individuals with higher plasma levels of the thyroglobulin hormones T3 and T4 receive higher marbling scores. Partly based on this assumption the *TG* gene has been assigned a candidate gene for marbling in beef cattle.

Polymorphism in the TG gene

Polymorphism in the 5' untranslated leader sequence (-537bp) of the thyroglobulin gene has been associated with marbling score in Angus and Shorthorn cattle (Barendse *et al.*, 2004). In contradiction, a confirmation study of the GeneSTAR[®] marbling test gave no significant results for the association of this polymorphism in the *TG* gene with marbling score (Rincker *et al.*, 2006). Moore *et al.* (2003) found no association between degree of backfat and polymorphism in the same position.

Leptin

Leptin is a peptide hormone primarily produced by white adipose tissue and in lesser extent in the placenta and skeletal muscle. This peptide hormone plays a role in the synthesis of glycogen and the glucose transport (Berti *et al.*, 1997) and is particularly active in the brain tissue in the region of the hypothalamus. This area of the brain takes part in regulating eating behaviour, and the presence of leptin decreases hunger and depresses willingness for food. When adipose tissue grows larger correspondingly larger amounts of leptin are produced (Alberts *et al.*, 2002). The today classical experiment on mice by Ingalls *et al.*, (1950) revealed the function of leptin and its importance in appetite regulation. With the leptin gene knocked out, the mice's eating behaviour changed rapidly and they soon became obese. This gene is therefore often referred to as the "obese" gene. A prime candidate for an endocrine regulation of the leptin expression is insulin. Insulin appears to increase the leptin secretion; a possible cause of action could be that increasing levels of insulin cause an increased metabolism and glucose transport. If the reverse is true, i.e. if increasing levels of leptin also cause increasing levels of insulin still remains uncertain. The hypothesis has been tested in several different studies but with conflicting results, as reviewed by Margetic *et al.* (2002). The leptin gene is located on bovine chromosome 4 and transcribed into a 167 amino acid peptide. The sequence includes a 21 amino acid long signal sequence, which is cleaved off during translocation of the leptin into microsomes. Therefore the final protein circulating in the blood contains only 146 amino acid residues (Margetic *et al.*, 2002). The protein circulates in the serum bound to other proteins. These "transporting proteins" may impact on the half life and the biological activity of leptin (Houseknecht *et al.*, 1998).

Although persons with leptin deficiency suffer from severe obesity, it is not a major cause of human obesity as it is a rare disorder (see review by Houseknecht *et al.*, 1998). A stronger candidate gene for human obesity is the leptin receptor gene (Houseknecht *et al.*, 1998). Thus, in addition to the leptin gene, also the leptin receptor gene and its polymorphisms are of main interest in the study of the leptin hormone and its action on fat deposition in cattle.

Leptin activity

To determine if serum concentration of leptin is an indicator of fat characteristics, studies conducted on correlations between leptin activity and various aspect of fat have been made. Serum concentration measured before slaughter is associated with marbling, back fat depth and intestinal fat content (Geary *et al.*, 2003; Nkrumah *et al.*, 2007). Also leptin serum

concentrations measured at slaughter appear to be highly correlated to carcass fat features (Brandt *et al.*, 2007).

Polymorphisms in the leptin gene

Several SNPs in the leptin gene have been found to be associated with bovine fat properties (Figure 1). A SNP in exon 2 with a transition from a cytosine (C) to thymine (T) changes an amino acid from arginine to cysteine, referred to as marker Ex2FB (Buchanan *et al.*, 2002). Results indicate that the T allele is associated with high grade fat and average backfat (Buchanan *et al.*, 2002), subcutaneous fat (Schenkel *et al.*, 2006) ultrasound backfat gain and carcass grade fat (backfat) (Nkrumah *et al.*, 2004). Kononoff *et al.* (2005) found an association between highly marbled individuals and animal homozygous for the T allele in this SNP. However, the T/T was not associated with marbling scores in the study by Schenkel *et al.* (2005), nor by Nkrumah *et al.* (2004). The difference in total carcass fat was significant between the two homozygous genotypes (C/C and T/T) whereas there was no difference between the heterozygous C/T and the homozygous T/T genotypes. This indicates a large degree of dominance of T over C (Schenkel *et al.*, 2005). In samples of the major European beef breeds, Buchanan *et al.* (2003) observed that the British breeds Aberdeen Angus and Hereford had a higher frequency of the T allele than the continental breeds Charolais and Simmental (Table 2).

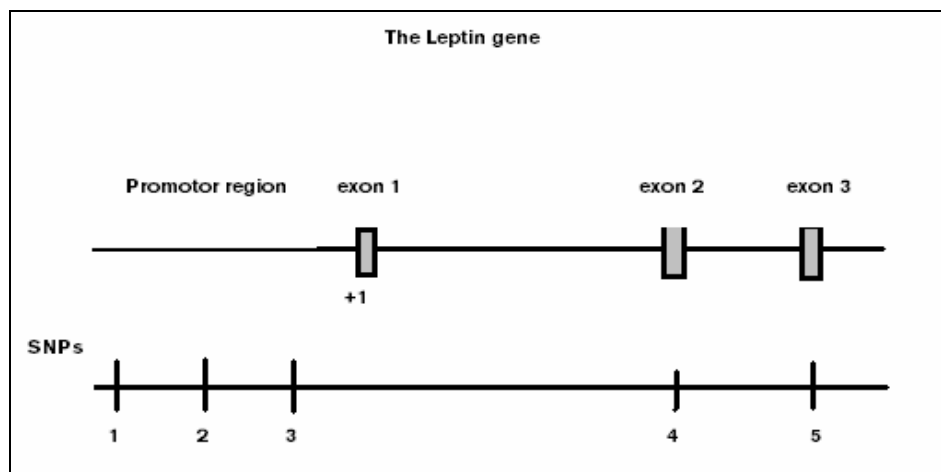


Figure 1. The marker position in the leptin gene.¹ Marker UASMS1 (207),² Marker UASMS2 (528),³ Marker UASMS3 (1759),⁴ Marker Ex2FB (exon 2),⁵ Marker A59V (exon 3).

Another SNP (E2JW) in the same exon (exon 2) has been associated with fat, i.e. lean yield and grade fat (not with IMF). The mutation causes a nucleotide substitution from an A to a T, where individuals homozygous for the A allele have fatter carcasses. The T allele in marker E2JW is very rare. Together the two SNPs in exon 2 (Ex2FB and E2JW) are particularly significant for tenderness (see the section Candidate genes for tenderness) (Schenkel *et al.*, 2005).

Table 2. Allele frequencies of the marker Ex2FB in the leptin gene in Canadian beef cattle

Breed	N	C	T	Literature cited
Crossbreed	952 ¹	0.58	0.42	(Schenkel <i>et al.</i> , 2005)
	1577 ²	0.50	0.50	(Kononoff <i>et al.</i> , 2005)
Angus	60	0.42	0.58	(Buchanan <i>et al.</i> , 2002)
	26	0.29	0.71	(Nkrumah <i>et al.</i> , 2004)
	43	0.45	0.55	(Schenkel <i>et al.</i> , 2005)
Limousin	30	0.52	0.48	(Schenkel <i>et al.</i> , 2005)
Charolais	55	0.66	0.34	(Buchanan <i>et al.</i> , 2002)
	30	0.58	0.42	(Nkrumah <i>et al.</i> , 2004)
	11	0.55	0.45	(Schenkel <i>et al.</i> , 2005)
Simmental	17	0.68	0.32	(Buchanan <i>et al.</i> , 2002)
	30	0.53	0.47	(Nkrumah <i>et al.</i> , 2004)
	68	0.59	0.41	(Schenkel <i>et al.</i> , 2005)
Hereford	22	0.45	0.55	(Buchanan <i>et al.</i> , 2002)
	30	0.45	0.55	(Nkrumah <i>et al.</i> , 2004)

¹*Bos taurus* crosses (Angus, Charolais, Simmental and Limousin)

²*Bos taurus* crosses (Angus, Charolais, Hereford, Simmental and Limousin)

Four additional SNPs in the leptin gene have been associated with fat deposition. Three of these are located in the promoter region of leptin and have in several studies been associated to fat characteristics (Schenkel *et al.*, 2006 and Nkrumah *et al.*, 2005). These SNPs are named UASMS1, UASMS2 and UASMS3. Alleles at UASMS1 and UASMS3 were found to be in complete LD (Nkrumah *et al.*, 2005; Schenkel *et al.*, 2006). The fourth SNP, named A59V, is located in exon 3 of the leptin gene.

The (C/T) UASMS2 marker is located at position 528 in the leptin gene. Animals with the homozygous genotype T/T show higher marbling scores than those carrying the heterozygous C/T and the homozygous C/C genotypes (Nkrumah *et al.*, 2005). Moreover, another study reported similar results, where the genotype T/T in combination with the genotype C/C in marker Ex2FB was associated with high back fat levels after 150 days on corn based feed

(Lusk, 2007). Contrasting results were reported by Schenkel *et al.* (2005) where no significant association was found between marker UASMS2 and any of the analysed fat traits. The C allele has been shown to have the highest frequency in all analysed breeds (Table 3).

Table 3. Allele frequencies of the marker UASMS2 in the leptin gene

Breed	N	C	T	Literature cited
Cross breed	150 ¹	0.79	0.21	(Nkrumah <i>et al.</i> , 2006)
	952 ²	0.74	0.26	(Schenkel <i>et al.</i> , 2005)
Angus	160	0.89	0.11	(Nkrumah <i>et al.</i> , 2006)
	43	0.73	0.27	(Schenkel <i>et al.</i> , 2005)
Hereford	-	0.74	0.26	(Nkrumah <i>et al.</i> , 2006)
	30	0.66	0.35	(Schenkel <i>et al.</i> , 2005)
Limousin and Gelbvieh	-	0.83	0.17	(Nkrumah <i>et al.</i> , 2006)
Limousin	11	0.77	0.23	(Schenkel <i>et al.</i> , 2005)
Charolais	-	0.75	0.25	(Nkrumah <i>et al.</i> , 2006)
	68	0.70	0.30	(Schenkel <i>et al.</i> , 2005)

¹*Bos taurus* crosses (Angus x Charolais)

²*Bos taurus* crosses (Angus, Charolais, Simmental and Limousin)

The UASMS1 SNP consists of a substitution from cytosine (C) to thymine (T) (with a corresponding nucleotide substitution from C>G for the UASMS3 marker). Marker UASMS1 (with position 207) has in two studies been found to be significantly associated with ultrasound backfat thickness and fat yield (Nkrumah *et al.*, 2005; Schenkel *et al.*, 2006). Frequencies of the marker UASMS1 alleles are given in Table 4. Moreover, pair wise comparison of the genotypes in markers UASMS3 and UASMS2 demonstrated significant LD between the two polymorphisms (P= 0.002) (Nkrumah *et al.*, 2005). All three alleles (UASMS1, UASMS2 and UASMS3) are probably in LD.

Table 4. Allele frequencies of the marker UASMS1 in the leptin gene

Breed	n	C	T	Literature cited
Cross breed	952 ¹	0.38	0.62	(Schenkel <i>et al.</i> , 2005)
Angus	43	0.49	0.51	(Schenkel <i>et al.</i> , 2005)
Hereford	30	0.48	0.52	(Schenkel <i>et al.</i> , 2005)
Limousin and Gelbvieh	11	0.45	0.55	(Schenkel <i>et al.</i> , 2005)
Charolais	68	0.35	0.65	(Schenkel <i>et al.</i> , 2005)

¹*Bos taurus* crosses (Angus, Charolais, Simmental and Limousin)

The fourth identified polymorphism in the leptin gene, marker A59V, is a recently identified SNP, located in exon 3. This polymorphism has been found to be associated with fat deposition. Similar to the UASMS2 marker, the homozygous T genotype is the most favourable for several of the fat characteristics, e.g. ultrasound backfat level, average carcass backfat and carcass grade fat. The combined genotype TT/TT in these two polymorphisms (A59V and UASMS2) seems to have a larger effect on high fat grades (+3.56 mm ultrasound

backfat and +3.85 mm carcass grade fat) than the effect of each locus taken separately (Nkrumah *et al.*, 2006).

There is a SNP found in the bovine leptin receptor gene, where the heterozygous genotype C/T in Canadian beef cattle showed a tendency to reduced inter-muscular fat (-0.67%), fat yield (-1.22%), subcutaneous fat (-0.65%) and grade fat (-1.08%) compared to the C/C genotype. However, the T allele seems to be rare (Schenkel *et al.*, 2006b). The SNP changes a cytosine to a thymine and is located in exon 20 of the leptin receptor gene (Liefers *et al.*, 2005).

Novel “marbling” genes

Novel APOBEC-1 target-1 (NATI) gene. This gene is a translational suppressor and is believed to be involved in the synthesis of intramuscular fat, through suppressing certain genes during early stages of adipose development. *NATI* mRNA is particularly abundant in intramuscular adipocytes of young and lean animals (Childs *et al.*, 2002).

Retinoic acid receptor-related orphan receptor C (RORC) gene. This gene has been associated with fatness in 1750 cattle in a study by Barendse *et al.* (2007). The *RORC*:g.3290 T>G SNP shows a strong association with marbling and the gene is believed to be involved in the maintenance of adipocytes and their ability to process glucose. The gene is located on bovine chromosome 3 and might be identical to the QTL for fat depth and marbling that Casas *et al.* (2003) identified in the same region.

In *mitochondrial DNA*, Mannen *et al.* (2003) found a polymorphism associated with marbling score and longissimus muscle area in Japanese Black cattle. The G2232A polymorphism may alter the function of the mitochondrial ribosome and thereby the rate of the mitochondrial protein synthesis. This information may, according to the author, be utilized as measure of maternal effects on genetic variation in beef cattle.

Mitochondrial transcription factor (TFAM) gene is a nuclear gene that encodes a regulating protein that is believed to regulate the initiation of transcription and replication of mitochondrial DNA. The gene has been proposed to be involved in the energy metabolism and body fat deposition and two SNPs located in the promoter region of this gene have been associated with marbling (Jiang *et al.*, 2005).

The fatty acid binding protein 4 (FABP4) gene plays an important role in the lipid metabolism and homeostasis in adipocytes. Being expressed in the adipose tissue it interacts with peroxisome proliferator-activated receptors and binds to lipase. A SNP with an allele substitution from G>C is associated with marbling score and subcutaneous fat depth. Individuals' homozygous for the G allele show the highest marbling scores (+0.42 in marbling score compared to homozygous CC individuals). The *FABP4* gene falls into a significant QTL interval for beef marbling on bovine chromosome 14 (Michal *et al.*, 2006).

The growth hormone 1 (GHI) gene harbours a missense mutation that replaces a leucine for a valine. The leucine allele has a frequency of 0.77 and has in a study on 1027 Australian cattle been associated with lower marbling scores (Barendse *et al.*, 2006).

The Titin-cap (TCAP) gene encodes a protein in striated and cardiac muscle. The protein binds to titin domains and is a substrate of titin kinase. The interactions are believed to be

important for sarcomere assembly. In exon 2 a common (frequency 0.34) 6-bp deletion causes a double amino acid deletion (Leu-Gln). This polymorphism is associated with Korean Beef marbling standard score. Animals homozygous for the deletion allele have the highest marbling score (2.26) whereas the genotype the homozygous for the insertion allele was associated with the lowest (2.02). Additionally, another associated polymorphism is located in intron 1 of the *TCAP* gene and gives a nucleotide substitution from A to G (Cheong *et al.*, 2007).

Tenderness

The tenderness in beef is predominantly dependent on three factors, 1) background toughness, 2) toughening phase, and 3) tenderisation phase. It is established that consumers can feel the difference between tough and tender meat as determined by Warner-Bratzler shear force tests (Hauffman *et al.*, 1996) and that they also are willing to pay more for tender meat (Boleman *et al.*, 1997). The difference in tenderness perceived by the consumer depends mainly on processes during the tenderisation phase (Veiseth, and Koohmaraie, 2005).

Background toughness. In cattle there is considerable variation in tenderness between different types of muscles. The function of the muscle in the living animal has a key role for this variation. The fore shank and heel of the round are used extensively for movement and therefore contains more connective tissue. Considered the finest and most tender retail cut of beef is often the fillet, also called the tenderloin. This muscle is in Latin named *M. psoas major* and its main function is to stabilise the back, with no distinct movement. *M. psoas major* has consequently low amounts of connective tissue and is therefore more tender. Tenderness decrease with age, why meat from younger animals is more likely to be tender (Reagan *et al.*, 1976). This age related decrease in tenderness is mainly due to increased collagen cross-links (Lepetit, 2007), or reduced levels of soluble collagen.

Toughening phase. When comparing animals of the same age and breed, differences in tenderness can be due to contraction level of the sarcomeres at the onset of rigor mortis. Stressing animals before slaughter may result in dark firm and dry (DFD, see, Abbreviations) meat. The somewhat opposite result shows when carcasses have a fast drop in pH and inadequate cooling, the meat becomes pale, soft and exudative (PSE, see, Abbreviations).

To minimize toughness electrical stimulation is a possible tool (Warris, 2004). There are abundant theories about why electrical stimulation has an improving effect on tenderness. The intense contraction level may loosen up the muscle structures (at high volt electrical stimulation), the increase in calcium maybe stimulates the proteolytic process or perhaps the electricity makes the muscles contract more intensely and the energy resources empty faster and thereby minimize the connections in the actin/myosin filaments (Lawries and Ledward, 2006). High demands on hygiene requires fast carcass chilling which may lead to tougher meat due to cold shortening (Figure 2). The electrical stimulation prevents cold shortening by promoting a rapid pH fall and an early onset of rigor mortis. The mechanism behind this is believed to be as follows: at high adenosine triphosphate- (ATP) levels combined with cold carcasses (below 10°C), the calcium pump ceases to function optimally and the accumulated calcium ions can activate ATP-ase and thereby the muscle contraction mechanism (Warris, 2004). In addition, pelvic suspension has a positive effect on tenderness by stretching the muscles and reducing the connection sites between the muscle filaments (Figure 2).

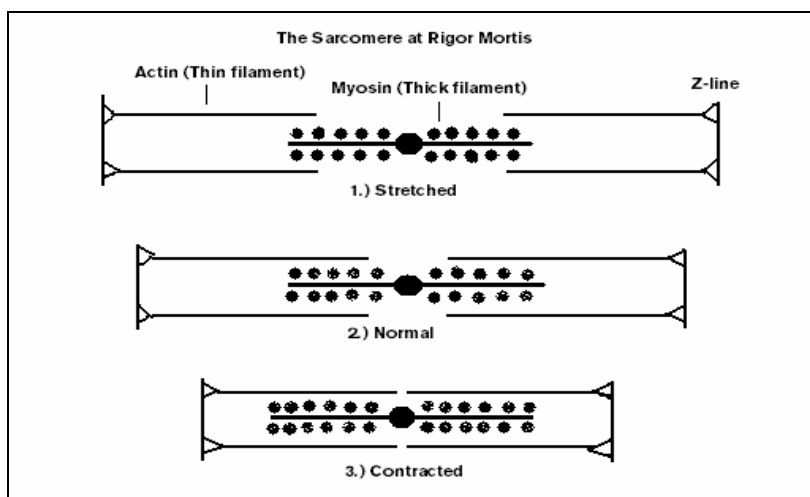


Figure 2. The sarcomere at rigor mortis. 1) Stretched muscle, low level of connection sites between actin and myosin (low shear force values). 2) Normal muscle. 3) Contracted muscle, high level of actin/myosin binding, e.g. cold shortening (high shear force values).

Tenderisation phase. The ultimate tenderness is also affected by post-slaughter treatment, e.g. chilling time, *post mortem* tenderisation and cooking methods. *Post mortem* tenderisation is a result of the proteolytic enzyme activities that are present within the muscles and activated at slaughter (Geesink *et al.*, 2006; Veiseth and Koochmaraie, 2005). This tenderisation process is highly time dependent and at ~72 h after slaughter the major part of the tenderisation process has been completed (Koochmaraie *et al.*, 2006). However, a minor improvement in tenderness is achieved also during the period from 72 hours up to three weeks. The main functional substances in the proteolytic process are the calpain enzymes and their repressor, the protein calpastatin. The genes coding for these enzymes are considered as candidate genes for the major meat quality parameter, tenderness (Young *et al.*, 2001).

Despite the industry processes to optimise tenderness, there is still a large variation between individuals. This final variation can mainly be explained by heritable factors. There are more standardised ways to measure toughness than for marbling. One of the most popular methods is the Warner-Bratzler shear force test. This method was developed in the beginning of the 1930s and is based on the force, in kilogram, needed to incise a test object into two pieces (Warris, 2004).

Candidate genes

The calpain family comprises a minimum of eight closely related subgroups but also several other calpain subgroups named “calpain-like” molecules due to their similarity with the first found calpain molecule. This text refers to the calpains involved in the so called calpain system and which are believed to be the most important enzymes in the proteolytic tenderisation process (Koochmaraie and Geesink, 2006).

Calpain

The Calpain proteolytic enzyme is composed of two subunits with molecular weights of 28 and 80 kDa, respectively (Figure 3). Two types of this enzyme are named after their requirement of calcium ions to become active. At calcium concentration around 1-2mM the form m-calpain is activated, where the m- stands for millimolar. When lower concentrations (50-100 μ M) of the calcium ion are present, the main calpain enzyme that is active is written

with a micromolar symbol, μ -calpain (Goll *et al.*, 2003). The third thoroughly investigated form of a protease in the calpain system is named calpain 3, but it is, according to Veiseth and Koohmaraie (2005), not likely to be involved in the tenderisation process. As regards the role of the m-calpain there is little evidence that measurable concentrations of intracellular free Ca^{2+} ever occur in post-mortem muscles, why most likely the μ -calpain is the active form after slaughter. In addition, so far no studies have been able to show that m-calpains are autolyzed during *post mortem* storage, as would be expected if the enzyme was active (Boehm *et al.*, 1998).

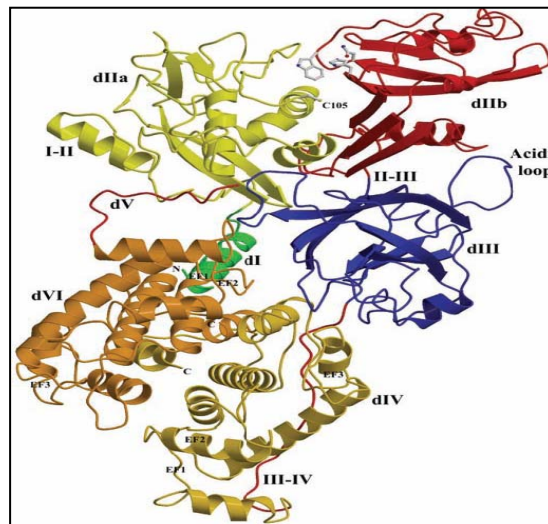


Figure 3. Crystallographic structure of human m-calpain. The domains are labeled dI, dIIa, dIIIb, dIII, dIV, dV, and dVI (Reverter *et al.*, 2001).

Much of the evidence for the calpains' involvement in tenderisation of meat has come from studies showing the effect of calcium ions on the process (calcium is needed for the activation of calpain). Enhancement with calcium chloride to beef samples has in more than one study showed significant effects on shear force, with results up to 32% more tender meat than corresponding controls (Boleman *et al.*, 1995; Lawrence *et al.*, 2003; Wheeler *et al.*, 1991). To support these studies, trials with expected opposite effect have also been performed, where injections with zinc chloride (ZnCl_2), a calcium inhibitor, of beef muscle resulted in samples with drastically higher shear force values (Lawrence *et al.*, 2003). Finally, in year 2006, the first knock out of the μ -calpain gene was performed in mice, where the results further support the role of the calpain gene in tenderisation of meat (Geesink *et al.*, 2006).

μ -calpain

According to cited literature (Geesink *et al.*, 2006), μ -calpain is essential for the *post mortem* enzymatic activity. This enzyme is thought to act on the proteins in the sarcomeres and is found mainly on or next to the Z discs (Kumamoto *et al.*, 1995). By degrading the proteins tropomyosin and titin, which are also located in the area of the Z discs, the rigid structure of the myofibrils becomes unstable and the structure loosens up. In the living muscle the inhibitor calpastatin prohibits the action of the calpain by binding to the enzyme (Goll *et al.*, 2003). In order for the calpains to become active *post mortem* a probable cause of action is as follows: when energy, ATP, is depleted in the muscle and *rigor mortis* sets in, there is no longer a reabsorption of the calcium ions by the calcium pump back to the sarcoplasmic reticulum. Instead the calcium is released into the sarcoplasm where it inhibits calpastatin/calpain bindings. When ATP is depleted and the calcium levels in the sarcomere

increase above the threshold level the calpastatin no longer attaches to the calpain and the proteolytic enzyme becomes active (Warris, 2004).

Calpain activity

There are today methods available to measure calpain activity with a monoclonal antibody specific for the 28-kDa subunit common to both calpain variants. An immuno affinity column containing the antibody will bind both forms. The bound calpains can be eluted either by flushing the column with high salt or with alkaline solutions at pH 9.0–9.5, conditions that do not affect the activity of the calpain. To measure the proteolytic activity of calpain a protein substrate which bind calcium ions is often needed, e.g. casein (Goll et al., 2003).

Within the European GEMQUAL project, in a study performed by Ertbjerg *et al.* (2006), the effect of the calpain system on tenderness and its potential association with shear force was analysed. The relationship between these two parameters seemed to be highly dependent on breed. Of the variation in shear force value 49% could be explained by μ -calpain activity in the Casina breed, whereas no similar effects were found in any of the other analysed breeds.

Polymorphisms in the calpain gene

The *CAPNI* gene that codes for μ -calpain is located on bovine chromosome 29. Two SNPs in the *CAPNI* gene located in exon 9 and 14 were evaluated by Page *et al.* (2002). These two polymorphisms resulted in two amino acid substitutions, alanine/glycine and isoleucine/valine, respectively. Further analyses of crossbreds showed that a higher shear force was value associated with haplotypes that coded for glycine and isoleucine, i.e. the genotypes with Guanine (G) and Adenine (A) at positions 316 (exon 9) and 530 (exon 14), respectively (Page *et al.*, 2002). Costello *et al.* (2007) found similar results regarding the *CAPNI* gene, marker CAPN1-316, but conflicting results for marker *CAPNI*-530. The favorable allele C in exon 9 of the *CAPNI* gene is present in the most common *Bos taurus* beef breeds, but at low to intermediate frequencies (Page *et al.*, 2004) (Table 5). The polymorphism in position 316 is influencing the final peptide located in the second domain and the SNP in exon 14 is affecting the amino acid chain in domain 3 (Page *et al.*, 2002) (Figure 2).

Table 5. Allele frequencies of the *CAPNI* gene, marker 316

Breed	N	C	G	Literature cited
Cross breed	597 ¹	0.22	0.78	(White <i>et al.</i> , 2005)
	532 ²	0.20	0.80	(White <i>et al.</i> , 2005)
	281 ³	0.17	0.83	(Costello <i>et al.</i> , 2007)
	362 ³	0.17	0.83	(Page <i>et al.</i> , 2004)
	564 ²	0.20	0.80	(Page <i>et al.</i> , 2004)

¹*Bos indicus* x *Bos taurus*

²*Bos taurus* cross-(Hereford, Angus, R. Angus, Simmental, Gelbvieh, Limousin and Charolais)

³*Bos taurus* cross (Simmental x Angus)

Table 6. Allele frequencies of the *CAPNI* gene, marker 530

Breed	N	A	G	Literature cited
Cross breed	600 ¹	0.14	0.86	(White <i>et al.</i> , 2005)
	362 ²	0.37	0.63	(Page <i>et al.</i> , 2004)
	564 ³	0.28	0.72	(Page <i>et al.</i> , 2004)

¹*Bos indicus* x *Bos taurus*

²*Bos taurus* cross-(Hereford, Angus, R. Angus, Simmental, Gelbvieh, Limousin and Charolais)

³*Bos taurus* cross (Simmental x Angus)

The largest breed differences in tenderness are found between *Bos taurus* and *Bos indicus* breeds. The breeds descending from *Bos indicus* are known for their tough meat, regardless of applied meat treatment and breeding method (Gazzola *et al.*, 1999). In a study investigating the genetic variation in the *CAPNI* gene in *Bos indicus* breeds a SNP (marker 4751) was found to be associated with variation in shear force at 7 and 21-day post-mortem. The T allele was associated with higher shear force values and occurred at a higher frequency in the *Bos indicus* breeds. This marker was therefore also tested on the *Bos taurus* breeds where the more frequent C allele was associated with lower shear force values (White *et al.*, 2005).

Table 7. Allele frequencies of the *CAPNI* gene, marker 4571

Breed	N	C	T	Literature cited
Cross breed	597 ¹	0.64	0.36	(White <i>et al.</i> , 2005)
	554 ²	0.58	0.42	(White <i>et al.</i> , 2005)
Angus	19	0.84	0.16	(White <i>et al.</i> , 2005)
Braham	504	0.11	0.89	(White <i>et al.</i> , 2005)

¹*Bos indicus* x *Bos taurus* crosses

²*Bos taurus* crosses (Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin and Charolais)

Calpastatin

The name calpastatin was proposed by Takashi Murachi (1979), and the enzyme was discovered during a purification process of the m-calpain. The calpastatin enzyme exists in a minimum of eight isoforms, resulting from either alternative splicing or different promoters and the molecule range in size from 17.5 -87.0 kDa (Goll *et al.*, 2003). The gene harbours 35 exons and spans over at least 130kb genomic DNA (Raynaud *et al.*, 2005).

Function

The calpastatin is bound to calpain to prevent its activity, and therefore a process is required to transfer calpain *post mortem* from inactive form to active in order to initiate the tenderization process. Calcium ions are required for the activation of calpain, but also for its inactivation (the calpastatin inhibition of calpain), but at different concentrations. If the action of calcium ions at various concentrations would be the same, the raised levels of Ca²⁺ will only cause further calpastatin/calpain bindings (Kaprell *et al.*, 1989). How the calpain activation and its translocation from the calpastatin occur is not completely understood (Goll *et al.*, 2003).

Calpastatin activity

Calpastatin concentration is difficult to measure because the process requires purification, during which significant fractions may be lost. Moreover it is sometimes difficult to discriminate between degraded polypeptides and the various isoforms that exist. Quite a few purifying methods make use of the heat stability of calpastatin and hence include heating. Another method is based on immuno-affinity columns with calpastatin IV domain specific antibodies. For unknown reasons, this method suffers from suboptimal affinities for the specific antibodies (Goll *et al.*, 2003).

Despite many years of investigations there are still different opinions concerning the effect of calpastatin on tenderness. To test the hypothesis that the initial level of calpastatin has an effect on post-mortem proteolysis and hence tenderness, Geesink and Koohmaraie (1999) tested incubation of purified calpastatin in muscle cells. The outcome was a gradual decrease of proteolysis with increasing concentrations of calpastatin, thus supporting the hypothesis. The genetic influence on the difference in tenderness between *Bos indicus* and *Bos taurus* has been studied, where meat from *Bos indicus* breeds was found to be tougher and contain higher levels of calpastatin (O'Connor *et al.*, 1997). However, within the European GEMQUAL project a study performed by Ertbjerg *et al.* (2006) found significant associations between level of calpastatin and tenderness in only two, Pirenaica and Highland, out of 15 European breeds. The analysis had been performed on calpastatin activity 45 min post-mortem and after ten days aging time.

Polymorphisms in the calpastatin gene

In a quantitative trait loci (QTL) study a region on bovine chromosome 7 was found to be associated with tenderness (Drinkwater *et al.*, 2006). The genes coding for calpastatin (*CAST*) and lysyl oxidase (*LOX*) are both located on chromosome 7 (Gu *et al.*, 2000), less than 50cM apart (Drinkwater *et al.*, 2006). Several studies have found an association between a SNP in the *CAST* gene and tenderness (Casas *et al.*, 2006; Drinkwater *et al.*, 2006; Schenkel *et al.*, 2006). However, there are also studies in which no association between the *CAST* gene and tenderness was observed (Chung *et al.*, 2001; Lonergan *et al.*, 2001). A polymorphism in the *CAST* gene was found by Barendse *et al.* (2002), which was further examined by Casas *et al.* (2006). The SNP is located in the 3' untranslated region, and consist of a nucleotide substitution from C to T. The SNP is patented and utilized in the commercial GeneSTAR® tenderness test. The genotype that is homozygous for the T allele is the most favourable as it gives a better score in WBSF (-0.3-0.5 kg) and panel (+0.2-0.3 sensory score) tests than genotypes homozygote for the C allele and the heterozygous C/T genotypes (Casas *et al.*, 2006). Data on frequency analyses of the alleles are mainly available for crossbreds, for which 80 percent of the alleles consist of the favourable T variant (Table 8).

Table 8. Allele frequencies of the *CAST* gene, marker C/T

Breed	N	C	T	Literature cited
Cross breed	580 ¹	0.17	0.83	(Casas <i>et al.</i> , 2006)
Cross breed	539 ²	0.20	0.80	(Casas <i>et al.</i> , 2006)
Braham	444	0.28	0.72	(Casas <i>et al.</i> , 2006)

¹*Bos indicus* x *Bos taurus* crosses

²*Bos taurus* crosses- (Hereford, Angus, Red Angus, Simmental, Gelbvieh, Limousin and Charolais)

The second *CAST* gene polymorphism that has been associated with tenderness is positioned in exon 3. Individuals homozygous for the C allele show an overall reduction in WBSF value of 0.32 kg compared to individuals homozygous for the G allele. The reduced tenderness for the G allele was consistent over the *post-mortem* aging days. The heterozygous genotype CG showed an intermediate tenderness score. The C allele, positively associated with tenderness, also showed a tendency to reduce *M. longissimus* area and increase total fat yield (Schenkel *et al.*, 2006a). The prevalence of the C allele is according to Schenkel *et al.* (2006a) highest for the Limousin breed (0.73) (Table. 9).

Table 9. Allele frequencies of the *CAST* gene, marker C/G.

Breed	n	C	G	Literature cited
Cross breed	547 ¹	0.63	0.37	(Schenkel, <i>et al.</i> 2006a)
Angus	12	0.64	0.36	(Schenkel, <i>et al.</i> 2006a)
Limousin	28	0.73	0.27	(Schenkel, <i>et al.</i> 2006a)
Charolais	8	0.69	0.31	(Schenkel, <i>et al.</i> 2006a)

¹*Bos taurus* crosses (Angus, Charolais, Simmental and Limousin)

Novel “tenderness” genes

Leptin-Two polymorphisms in exon 2 (Ex2FB and E2JW) at the leptin gene being associated with fat have in combination a significant effect on tenderness. Shear force analysis has shown that genotype AT(Ex2FB2)/TT(E2JW) is associated with tougher meat, with a higher shear force value for genotype AT/TT, 6.98 kg (day 2) and 4.11 kg at day 21, compared to genotype AT/CT, 5.00 (day 2) and 3.42 (day 21) . Shear force values of the two SNPs tested separately were not conducted (Schenkel *et al.*, 2005).

DNAJA1-is the name of a gene encoding a heat shock protein of the Hsp40 family. The protein is a co-chaperone to a different protein family (Hsp70 family) and is believed to be involved in protein folding and mitochondrial protein import. The Hsp70/DNAJA protein complex inhibits apoptosis (Mosser *et al.*, 2000) and thus influences tenderness (Gotoh *et al.*, 2004). This would be in line with a proposed theory that apoptosis is an initial stage of proteolysis (Quali *et al.*, 2006). In a study made by Bernard *et al.* (2006), the transcriptome (mRNA level) from the *DNAJA1* gene was negatively correlated with tenderness. The study was performed on Charolais bulls and in which the *DNAJA1* gene explained 63% of the variation in tenderness.

Juiciness and flavour

The overall perception of juiciness in meat is affected by tenderness, marbling and water holding capacity, WHC. Each one of these parameters is of major importance for how the consumers' ultimately perceive the meat.

The WHC is determined by the exudation of natural occurring fluid (or the ability to accumulate fluid at elevated salt concentrations). Loss of fluid can be measured at three different stages. Fluid from fresh, not cooked meat is named "weep"; from thawed, uncooked meat "drip"; whereas losses in cooked meat are referred to as "shrink" (Lawries and Ledward, 2006). Meat with low WHC loses considerable amounts of fluid during cooking which may lead to non succulent meat, i.e. dry meat. The mechanism behind the WHC is depending on the proteins capacity to bind water and the muscle cell structure to entrap and retain water. Within the muscle cells' network water is mainly retained by steric effects (space forces). The myofibril proteins are the main responsible proteins for WHC and their ability to entrap water is affected by pH, ionic strength and oxidation (Huff-Lonergan and Lonergan, 2005). Poor WHC leads to weight loss and unattractive appearance of the retail cuts due to fluid accumulation in the tray or bag. Hence, the WHC is therefore an important economic trait from many aspects (Warris, 2000).

The taste of meat is closely related to aroma and can be highly unpleasant, e.g. boar taint in pork. Flavour is mainly determined by water-soluble molecules and odours from volatile fat soluble elements, which are often not detectable before heating. Meat aroma evolves during cooking, during which fat melts and becomes more susceptible to chemical reactions such as autoxidation and degradation, and forms e.g. carbonyl compounds and alcohols, unsaturated lipids can cause rancid odour. There are four major classes of precursors of heat induced meat flavours: amino acids, carbohydrates, amino acid-carbohydrate interactions (Maillard reaction), and nucleotides (Inosine 5 mono phosphate, IMP) (Bayliss, 1995).

Taken together these parameters reflect eating quality or palatability. The palatability is frequently determined by sensory panel tests, both professional and untrained.

Candidate genes

Juiciness and flavour are often difficult and expensive to measure because they often require a panel test (Warris, 2000). Below follows an introduction to genes which, based on current knowledge, may have an impact on flavour and/or juiciness characteristics.

The PKRAG genes

AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme that comprises three subunits, a catalytic α subunit and two regulatory subunits, β and γ . All isoforms ($\alpha 1$, $\alpha 2$ $\beta 1$, $\beta 3$, $\gamma 1$, $\gamma 2$ and $\gamma 3$) are encoded by different genes and can be combined in 12 different modes (Roux *et al.*, 2006). The AMPK family acts primly as a stress response system that regulates the cellular ATP production. The enzyme is activated by stress and inhibits the biochemical energy consuming pathways (McKay *et al.*, 2003). The *PKRAG3* gene is in swine coding for the subunit $\gamma 3$ and widely known as the *RN* gene. A naturally occurring mutation (R225Q) gives a higher skeletal muscle glycogen level and affects the final meat quality (Milan *et al.*, 2000). By a rapid decrease in pH the water-holding capacity and the yield of cooked cured ham from pigs with this mutation becomes considerably reduced (Bernard *et al.*, 2007). The

gene is, in hitherto studied species, only expressed in skeletal muscles. According to a study performed by Yu *et al.* (2005), beef breeds have seven polymorphisms in this gene, of which four appear in exons, and where two of them result in amino acid changes. There are also single mutations that give rise to alternative splicing sites. Moreover, Roux *et al.* (2006) recently found an additional five polymorphisms located in exons of the bovine *PKRAG3* gene, of which five change the amino acid sequence of the protein. In a study by Ciani *et al.* (2007) on the Chianina breed *PKRAG3* haplotypes showed association with meat quality.

The *PKRAG1* gene encodes the subunit $\gamma 1$ of the AMPK enzyme and contains number of polymorphisms which are proposed candidate markers for differences in the AMPK activity (Benkel *et al.*, 2005).

The “fish flavour” gene

The flavin containing monooxygenase 3 (FMO3) gene has been associated with off-flavours in both hen's eggs (Honkatukia *et al.*, 2005) and cow's milk (Lundén *et al.*, 2002). In human this gene is responsible for a recessive defect that gives a fishy body odour (Zhou and Shepard, 2005) and it has also recently been associated with off-flavour in pork (Glenn *et al.*, 2007). The overall effect of this gene in different species and products suggest that FMO3 may also be involved in the formation of off-flavours in beef and is thus an obvious candidate gene for future studies.

Colour

For the consumer, colour and appearance of the meat play very important roles for purchase preferences. Hence, a stable meat colour, that does not go greyish over time, is requested by the retailers. The red colour in meat derives mainly from the protein myoglobin, and the concentration and state of this protein have large impact on the colour appearance. The protein myoglobin consists of 153 amino acids and contains a porphyrin ring structure. Similar to the blood protein haemoglobin, myoglobin binds iron to its porphyrin structure (Young and West, 2001).

The occurrence of different hues (tones) of red in meat can primarily be explained by the affinity of the iron in the myoglobin porphyrin ring to bind ligands. There are three different forms of myoglobin in meat, deoxymyoglobin (purple red), oxymyoglobin (bright red) and metmyoglobin (brownish/greyish red). Their activation is highly dependent on oxygen pressure and state of the iron atom. When the deoxymyoglobin is exposed to oxygen, the iron in its ferrous state (Fe^{2+}) binds an oxygen molecule (O_2). This process occurs instantly and is observed as bright red meat colour in only a few minutes (15-30min) after a cut in fresh meat. The myoglobin ferrous state turns eventually into a ferric state (Fe^{3+}), in this which oxygen can no longer be bound and a water molecule binds instead. This gives the characteristic brown-grey colour (metmyoglobin) (Warriss, 2004). In order to prevent the metmyoglobin formation in the living muscle the enzyme metmyoglobin reductase is present (Hagler *et al.*, 1979). Metmyoglobin reductase is a NADH-dependent enzyme that in the presence of NADH reduces the metmyoglobin level both in living muscles and meat (Young and West, 2001). Similar to oxygen, other molecules can act as ligands of which one is nitric acid (NO) which is utilized as an additive in the production of cured meat to give the characteristic pink colour. Also carbon monoxide (CO) binds to myoglobin which gives the meat a bright and stable red colour, a property that in low concentrations is utilized in some countries when packaging retail cuts (Mancini and Hunt, 2005). Meat colour is also influenced by muscle

micro-structure. Meat with high pH level (>5.7) often appears darker whereas the opposite is observed for meat with low pH (<5.4) which looks paler. This colour effect is due to the level of light scatter in the meat. At high pH levels the meat will be in a state above the iso-electric point of the proteins, which means that the proteins will associate with more water. Thus the space between the myofibrils will decrease and hence, the meat will appear darker. At low pH (or fast pH drop from ~7.1 to ~5.5 within 45 min) the proteins are below their iso-electric point (protein denaturation) which means that less water is bound and the resulting larger spaces between the myofibrils will give the meat a lighter appearance (Abril *et al.*, 2001)

Only a few studies have analysed genetic effects on meat colour and colour stability. A study from 2005 performed on Jersey x Limousin crosses identified QTL peaks on bovine chromosomes 10, 18, 19 and 27 being associated with meat colour. The QTL effect on bovine chromosome 18 may be caused by the *RYR1* (ryanodine receptor) gene that has in pork been suggested to affect pH and WHC and thereby also the colour (the light scattering effect) (Koshkoih *et al.*, 2005). The porcine chromosome 2 has been reported by Andersson-Eklund *et al.* (1996) to have an effect on pH and pigmentation and share segments homologous with the bovine chromosome 19 (Koshkoih *et al.*, 2005).

MATERIALS AND METHODS

A total of 400 potential elite animals from five Swedish beef breed were included in this study, 290 Charolais, 13 Angus, 33 Hereford, 34 Limousin and 30 Simmental. Genotype analysis was performed by Igenity, a division of Merial Animal Health Company and samples were collected in collaboration with Scan AB. The genotype analyses have been voluntary and privately financed. No background investigation or origin verification has been performed on the material, why the given breed in the material not is absolutely.

Samples were collected by the breeders and DNA was extracted from hair follicles. DNA analyses were conducted on selected SNPs that are by Igenity considered to control desirable meat quality characteristics. In the leptin gene two markers in the promoter region (UASMS1 and UASMS2) and one located in the coding region (ExFB2) were analysed for all five breeds. In the *CAPN1* gene three markers (CAPN1-316, CAPN1-530 and CAPN1-4751) were typed. However, due to an alteration in Igenity's SNP panel in year 2005 the analysis were either on marker CAPN1-530 (-2005) or marker CAPN1-4751 (2005-). Additionally, one marker in the *DGAT1* gene (DGAT1 (K232A)) and one in the *CAST* gene (CAST-(C/G)) were analysed. Also the analysis of the *CAST* gene started in year 2005. In total eight SNPs were analysed, but on varying number of individuals. All genotyping results on breeds with fewer than five individuals were excluded from the analysis. Minor differences in the number of analysed individuals within breed mainly result from exclusion of individuals that had ambiguous results for a specific marker.

The Hardy-Weinberg equilibrium principle was tested on all breeds (with results from more than five individuals of each marker) with a Chi square test.

RESULTS

The genotypes in all analysed loci were approximately distributed according to Hardy-Weinberg (H-W) proportions ($P>0.05$), if no other information is given in the text for respective markers.

DGAT

Marker DGAT1 (K232A)

The frequencies of the K232A polymorphism in the *DGAT1* gene are illustrated in Table 10. The results give an indication of a high population frequency of the alanine variant in the studied breeds. Apart from the two breeds that were monomorphic in this study, the genotype frequencies were in agreement with the Hardy-Weinberg equilibrium.

Table 10. Allele frequencies and observed and expected genotype frequencies in the *DGAT1* gene, marker K232A.

Breed	n	Allele		Genotype (Observed /Expected)					
		A ¹	G ²	A/A		A/G		G/G	
Angus	13	0.23	0.77	0.08	0.05	0.31	0.35	0.62	0.59
Charolais	290	0.13	0.87	0.01	0.02	0.24	0.23	0.75	0.76
Hereford	33	0.00	1.00	0.00	---	0.00	---	1.00	---
Limousin	33	0.08	0.92	0.03	0.01	0.09	0.14	0.88	0.85
Simmental	26	0.00	1.00	0.00	---	0.00	---	1.00	---
Total	395			0.01	0.01	0.19	0.19	0.79	0.65

¹The A nucleotide in position 232 gives rise to a lysine

²The G nucleotide in position 232 gives rise to an alanine

Leptin

Marker Ex2FB

Allele frequencies of the leptin marker Ex2FB are listed in Table 11. Frequencies of the three genotypes (CC, CT and TT) in the Charolais breed deviated significantly from H-W proportions ($P<0.025$), with heterozygous (C/T) individuals presented in higher proportions than expected.

Table 11. Allele frequencies and observed and expected genotype frequencies in the leptin gene, marker Ex2FB.

Breed	n	Allele		Genotype (Observed /Expected)					
		C	T	C/C		C/T		T/T	
Angus	13	0.42	0.58	0.23	0.18	0.38	0.49	0.38	0.33
Charolais	288	0.63	0.37	0.37	0.40	0.52	0.47	0.11	0.14
Hereford	33	0.36	0.64	0.15	0.13	0.42	0.46	0.42	0.41
Limousin	34	0.65	0.35	0.35	0.42	0.59	0.75	0.06	0.12
Simmental	28	0.46	0.54	0.18	0.20	0.50	0.46	0.25	0.27
Total	396			0.33	0.36	0.52	0.49	0.15	0.17

Marker UASMS1

The UASMS1 marker alleles were in all analysed breeds found at intermediate frequencies (0.37-0.58 C allele) (Table 12). The three genotypes (CC, CT and TT) were except in the breed Charolais approximately distributed according to Hardy-Weinberg proportions ($P < 0.025$). In the breed Charolais the heterozygote genotype (C/T) had higher frequencies than expected.

Table 12. Allele frequency and observed and expected genotype frequencies in the leptin gene, marker UASMS1.

Breed	n	Allele		Genotype (Observed /Expected)					
		C	T	C/C		C/T		T/T	
Angus	13	0.58	0.42	0.38	0.33	0.38	0.49	0.23	0.18
Charolais	288	0.38	0.62	0.11	0.14	0.53	0.47	0.36	0.39
Hereford	33	0.58	0.42	0.36	0.33	0.42	0.49	0.21	0.18
Limousin	33	0.38	0.62	0.09	0.14	0.58	0.47	0.33	0.38
Simmental	26	0.37	0.63	0.08	0.13	0.58	0.47	0.35	0.40
Total	393			0.14	0.16	0.52	0.47	0.34	0.36

Marker UASMS2

The proposed favourable T allele of the marker UASMS2 occurred at low to intermediate frequencies in all the analysed breeds (0.15-0.36) (Table 13).

Table 13. Allele frequencies and observed and expected genotype frequencies in the leptin gene, marker UASMS2.

Breed	n	Allele		Genotype (Observed /Expected)					
		C	T	C/C		C/T		T/T	
Angus	13	0.77	0.23	0.62	0.59	0.31	0.35	0.08	0.05
Charolais	289	0.85	0.15	0.73	0.73	0.25	0.25	0.02	0.02
Hereford	33	0.64	0.36	0.36	0.41	0.55	0.46	0.09	0.13
Limousin	33	0.70	0.30	0.52	0.48	0.36	0.42	0.12	0.09
Simmental	26	0.69	0.31	0.50	0.48	0.38	0.43	0.12	0.10
Total	394			0.66	0.66	0.30	0.30	0.04	0.04

Calpain

Marker CAPN1-316

The frequencies of the G allele of the marker CAPN1-316 were ranging between 0.69-0.95 in the five analysed Swedish beef breeds (Table 14).

Table 14. Allele frequencies and observed and expected genotype frequencies in the calpain gene, marker 316.

Breed	n	Allele		Genotype (Observed /Expected)					
		C	G	C/C		C/G		G/G	
Angus	13	0.31	0.69	0.15	0.09	0.31	0.42	0.54	0.48
Charolais	288	0.23	0.77	0.06	0.05	0.34	0.36	0.60	0.59
Hereford	33	0.05	0.95	0.00	0.00	0.09	0.09	0.91	0.91
Limousin	33	0.24	0.76	0.03	0.06	0.42	0.37	0.55	0.57
Simmental	26	0.06	0.94	0.00	0.00	0.12	0.11	0.88	0.89
Total	393			0.05	0.05	0.31	0.32	0.64	0.63

Marker CAPN1-530

Angus and Hereford were the two breeds that showed the highest frequencies for the G allele of the marker CAPN1-530, 0.80 and 0.94 respectively (Table 15). The other breeds had according to this study intermediate G allele frequencies (0.41-0.57).

Table 15. Allele frequencies and observed and expected genotype frequencies in the calpain gene, marker 530.

Breed	n	Allele		Genotype (Observed /Expected)					
		A	G	A/A		A/G		G/G	
Angus	5	0.20	0.80	0.00	0.04	0.40	0.32	0.60	0.64
Charolais	80	0.43	0.57	0.15	0.18	0.55	0.49	0.30	0.33
Hereford	32	0.06	0.94	0.00	0.00	0.13	0.12	0.88	0.88
Limousin	14	0.54	0.46	0.29	0.29	0.50	0.50	0.21	0.21
Simmental	17	0.59	0.41	0.41	0.35	0.35	0.48	0.24	0.17
Total	148			0.16	0.17	0.43	0.40	0.42	0.43

Marker CAPN1-4571

The C allele frequencies in marker CAPN1-7451 varied considerably from 0.88 in Aberdeen Angus to 0.28 in Simmental (Table 16).

Table 16. Allele frequencies and observed and expected genotype frequencies in the calpain gene, marker 4751.

Breed	n	Allele		Genotype (Observed /Expected)					
		C	T	C/C	C/T	T/T			
Angus	8	0.88	0.12	0.75	0.76	0.25	0.23	0.00	0.01
Charolais	207	0.43	0.57	0.17	0.19	0.52	0.49	0.30	0.32
Limousin	19	0.45	0.55	0.21	0.20	0.47	0.49	0.32	0.31
Simmental	9	0.28	0.72	0.11	0.08	0.33	0.40	0.56	0.52
Total	243			0.19	0.20	0.50	0.48	0.30	0.32

Calpastatin

Marker CAST (G/C)

In a BLAST search (Ensembl, 2007, ID:BLA_gBWGhWC81) based on primer pairs (forward: 5'CCT CGA CTG CGT ACC AAT TCC GAA GTA AAG CCA AAG GAA CA3' and reverse: 5'ATT TCT CTG ATG GTG GCT CAC T3'), from the study by Schenkel *et al.* (2006a) the polymorphism CAST (C/G) was found to be located in the intronic sequence between exon 5 and 6 in the bovine chromosome 7 (base 282 of accession number AY008267). The frequency of the C allele was in the present study ranging between 0.50-0.75 (Table 17). The Limousin breed had a slightly higher frequency of the C allele (0.84).

Table 17. Allele frequencies and observed and expected genotype frequencies in the calpastatin gene, marker CAST (C/G).

Breed	n	Allele		Genotype (Observed /Expected)					
		C	G	C/C	C/T	T/T			
Angus	8	0.75	0.25	0.50	0.56	0.50	0.38	0.00	0.06
Charolais	209	0.69	0.61	0.46	0.48	0.47	0.42	0.07	0.09
Hereford
Limousin	19	0.84	0.16	0.74	0.71	0.21	0.27	0.05	0.03
Simmental	9	0.50	0.50	0.11	0.26	0.78	0.50	0.11	0.26
Total	245			0.47	0.49	0.46	0.41	0.07	0.09

DISCUSSION

In Sweden the selection of beef cattle is mainly based on birth weight and growth rate, only since 2005 has it been possible to include also carcass quality and calving traits. Because the animals that have been genotyped in this study mainly constitute elite animals intended for breeding they can not be considered to represent the average slaughter animal as regards growth rate. However, because meat quality is complicated and expensive to measure and, as a consequence has never been included in the breeding goal, the elite breeding animals in this study are not likely to be superior in meat quality characteristics to the average slaughter animal.

DGAT

Marker DGAT1 (K232A)

Compared to recently performed studies (Kaupe *et al.*, 2004; Ripoli *et al.*, 2006), the Swedish beef breeds have a markedly higher frequency of the suggested “leaner” allele G of the *DGAT1* gene. The breed Aberdeen Angus has in this material the highest frequency for the “fatter” lysine allele (0.23), as compared to a frequency between 0.09-0.13 in previous studies (Kaupe *et al.*, 2004; Ripoli *et al.*, 2006). The high frequencies of the G allele may be a result of “hitch hiking”, i.e. the G allele being in LD with a favourable allele at a linked locus under selection, e.g. for high growth rate. In support for this theory, previous reports on dairy breeds that have primarily been selected for high milk yield, e.g. German Holstein, has somewhat higher frequency of the “leaner” allele G (Table 1). In contrast, the Jersey dairy breed that has been selected for high milk fat levels has a higher frequency of the “fatter” lysine (A) allele (Table 1), which is according to expectations based on the *DGAT1* effect on milk fat concentrations (Kaupe *et al.*, 2004). The enzyme’s function in the milk fat secretion process (Grisart *et al.*, 2001) and the studies on the enzyme in beef cattle (Kong *et al.*, 2007; Thaller *et al.*, 2003) indicate that *DGAT1* is a candidate gene for muscle fat characteristics. The tendency of a fixation of the G allele in some breeds, both in this material and previous studies (Table 1) implies that the locus during recent generations has been subjected to selection or genetic drift. The Hereford breed was monomorphic for the G allele both in the present and in previous studies (Kaupe *et al.*, 2004 and Ripoli *et al.*, 2006). The monomorphism observed for the Simmental breed in our study lacks support in the literature and may rather be an effect of the small sample size.

Leptin

Marker Ex2FB

The Aberdeen Angus breed had a higher frequency of the favourable T allele in the marker Ex2FB than the other breeds. Corresponding results have been published by Nkrumah *et al.* (2005) (T= 0.71), Buchanan *et al.* (2001) (T=0.58) and Schenkel *et al.* (2005) (T=0.55). Results from the quoted literature (Table 2) and the current study (Table 11) show that the leptin gene (marker Ex2FB) segregates in this locus to allow for utilization in marker assisted selection. Results in the literature indicate that selection for this allele may result in animals with a higher carcass fat content but not necessarily a higher IMF content. However, the literature reports are somewhat contradictive why further studies are required before this marker can be included in selection programmes. The high frequency for the heterozygous genotype C/T in the breed Charolais may result from imported animals with different allele frequencies from the Swedish beef population.

Marker UASMS1

The results from the literature study point towards an influence of the marker UASMS1 on e.g. marbling (Nkrumah *et al.* 2004 and Schenkel *et al.* 2005), and according to the same authors the marker ought to be included in future studies on marbling and fat deposition. Somewhat surprisingly, the proposed favourable T allele for improved fat characteristic is both in this study and the literature found at somewhat lower frequencies in the early maturing, and thus often fatter breeds like Aberdeen Angus and Hereford. Thus, before MAS including this polymorphism could be implemented, further studies are warranted on the effect of the marker UASMS1.

Marker UASMS2

The allele frequency analysis in the marker UASMS2 showed a relatively low proportion of the suggested favourable T allele for total backfat. If this effect is confirmed, selection for the T allele may have a strong positive impact on marbling.

Serum concentration of leptin is associated with variation in several fat characteristics (see e.g. Geary *et al.*, 2003). The promoter region of the leptin gene is known to control the transcription rate and thereby have an effect on the level of expressed leptin (Brown, 2007). There are several SNPs located in the promoter region of the leptin gene (Nkrumah *et al.*, 2004) of which some have been strongly associated with meat quality traits in a Canadian study (Schenkel *et al.*, 2005). It would thus be interesting to analyse these SNPs in Swedish beef populations.

The Calpain gene

Many detailed studies have been conducted on this enzyme and its activity in post-mortem proteolysis, and the results have unambiguously shown a significant effect on meat tenderness. Still remains verification of the effect of the different polymorphisms in the Swedish beef populations.

Marker CAPN1-316

The suggested “tougher” G allele that gives rise to the non-polar amino acid change from alanine to glycine has both according to this study (Table 14) and the literature cited (Table 5) (White *et al.*, 2005, Costello *et al.*, 2007 and Page *et al.*, 2002), a higher frequency than the C allele. The polymorphism has been located to domain II in the m- calpain, a domain that has been identified as a proteolysis domain (Smith *et al.*, 2000). Thus, an alteration in this domain can be expected to also alter the activity of the protein and hence perhaps affect the tenderisation process. However, to establish if this SNP should be included as a marker for tenderness, further studies are required.

Marker CAPN1-530

The suggested favourable allele G is in the referred literature occurring more frequently than the alternative A allele. This is true also for the breeds in the present study, where the Aberdeen Angus (0.80) and Hereford (0.94) breeds have the highest G allele frequencies. The limited number of animals of these breed in the present study may underlie the markedly higher allele frequencies for these breeds.

Marker CAPN1-4751

The frequencies of the alleles in the marker CAPN1-4751 varies considerably between breeds (Table 7 and Table 16). The *Bos indicus* breeds have according to the literature a higher frequency of the less tender T allele (White *et al.*, 2005). Somewhat unexpectedly, in the present material the breed Simmental had the highest frequency of this unfavourable T allele. However, this could be a result of “sampling effects” due to the small sample size, i.e. the sample may not be representative for the overall population. In agreement with White *et al.* (2005) (0.86), we found the highest frequency of the supposedly favourable allele C in the Aberdeen Angus breed (0.88).

The Calpastatin gene

Due to its inhibiting effect on the enzyme calpain, calpastatin is considered to be a candidate gene for meat tenderness. How the calpastatin exerts its effect on tenderness has so far not been established. According to Ertbjerg *et al.* (2006) there is a large variation between breeds as regards the association of calpastatin serum levels and tenderness; only in two out of fifteen beef cattle breeds is the shear force value to a notable degree explained by the calpastatin serum concentration. However, O’Connor *et al.* (1997) associated the higher calpastatin level in *Bos indicus* (compared to *Bos taurus*) with a reduction in tenderness. For upcoming studies it is important to keep in mind that the concentration of calpastatin is complicated to measure and that large numbers of samples are needed for accurate estimations of marker allele effects. Based on the discrepant results in the studies by Ertbjerg *et al.* (2006) and O’Connor *et al.* (1997), one may speculate that the serum concentration of calpastatin in *Bos taurus* breeds is too low to interfere with the tenderisation process, and that the calpastatin effect observed some *Bos taurus* breeds is rather due to the presence of allelic forms of the structural protein. Further studies of genetic differences regarding the calpastatin gene between the two groups of cattle are required to validate this hypothesis.

Marker CAST-(C/G)

The allele frequencies for the breeds in the present study are generally in agreement with the literature where the favourable C allele has a frequency between 0.63-0.73 in the literature and 0.50-0.84 in this study. According to Schenkel *et al.* (2006a), selection for the C allele would increase tenderness but may also lead to diminished cross section on the area of the beef.

The marker CAST (C/G) and the marker in the literature study referred to as CAST (C/T) are both included in commercial DNA tests for tenderness (Igenity TenderGENE™ and GeneSTAR®, respectively). They are, together with one (CAPN1-316) or two polymorphisms (CAPN1-316 and CAPN1-4751) in the calpain gene, confirmed to have an effect on overall tenderness (Quaas *et al.*, 2006). Possibly it is the same effect that is observed for both CAST SNPs; i.e. that none of them are a causative mutation. Additional studies are needed to establish a (haplotype analysis and) potential linkage equilibrium effect.

FUTURE ANALYSIS

With already available genetic tests for marbling and tenderness, e.g. TenderGENE™ and GeneSTAR®, these can be utilized in breeding programs together with existing breeding measures, to avoid selection of individuals with genotype for poor meat quality. However, before MAS can be applied on the Swedish beef population the marker effects have to be evaluated under Swedish condition as genotype×environment interactions may exist for the meat characteristics. Also the population frequencies of the alleles at candidate genes are of importance from a selection perspective. To utilize MAS in a selection program when the favourable alleles are found at low frequencies may lead to substantial changes in the desired trait. On the other hand, this may cause inbreeding, and less room for selection for other economically important traits. Furthermore, there is a need for caution to avoid indirect selection for undesired alleles in loci that are linked to the trait you wish to improve. Although several Swedish cattle breeds are of interest for future studies on meat quality, both beef and milk breeds, a suggestion is to first focus on the purebred beef breeds and determined the allele frequencies for candidate genes of interest as purebreds are a prerequisite for the production of crossbred slaughter animals. Purebred beef breeds constitute the gene pool for production of slaughter beef cattle and are therefore interesting to characterize for their genetic potential to improve meat quality.

Preferably, an association study including meat quality characteristics should be done on pure bred animals. However, due to the small number of Swedish purebred cattle sent to slaughter crossbred animals have to be used. Another significant complication is the small population size of beef herds in Sweden which means that samples need to be collected at several farms and at different occasions. Sampling will also require close collaboration with beef producers to acquire information on pedigree, production traits and expected slaughter day, and the abattoirs to obtain tissue samples from the correct animal at the right time. The basic conditions required to accurately evaluate meat quality traits vary with the analysed trait. Final meat marbling grades are largely influence by breed and feeding, why it is of major importance that impacts from these parameters can be minimized or eliminated. In contrast, tenderness (shear force) is predominantly determined by slaughter procedures, whereas factors like feeding and breed play comparatively minor roles. Factors associated to the slaughter that may affect the outcome of the tenderization process are the level of stress that an animal experience and the cooling of the carcass. Tenderness is also dependent on time and method of suspension. Muscle samples from carcasses that have been pelvic suspended (see, Abbreviations) or aged for prolonged time are therefore not comparable to corresponding samples from carcasses treated according to customary procedures, i.e. they do not represent the average retail cuts available at the supermarkets. In Sweden the largest beef processor has in its assortment “gourmet meat”. This meat originates mainly from beef carcasses that have been aged longer than regular meat. These carcasses, which constitute a considerable proportion of beef carcasses, can unfortunately not be utilized in our planned tenderness analysis. Although samples from the same farm, but at different occasions would minimize the variation due different feeding and abattoir, the material must be sufficiently large to motivate the money and time spent on the sampling procedure. Therefore large beef producing farms are preferred. To avoid the impact of gender and age of the animal on the results, the material should preferably origin from a single class of slaughter animals, e.g. young bulls slaughtered around 13-19 months would be suitable in this perspective. “Young bull” represent the largest beef slaughter class in Sweden and thus reflects the meat that the consumer generally finds in the shop. Furthermore, young bulls show a large variation in meat quality why genetic differences would be easier to detect. The absence of *ad locum* registrations at the abattoir makes it difficult to evaluate the effect of abattoir on tenderness.

However, measures of the sarcomere length may be included for comparing background toughness between individuals. This is important in order to exclude extreme individuals from the subsequent analysis that may have been differently treated at slaughter.

Although, the meat samples analysed should preferably reflect the variety of beef retail cuts available to the consumer, analysis are often only conducted on the *M. longissimus dorsi*. The main reason is that it is the only muscle that is large and homogenous enough to be utilized for a multitude of measurements. In Sweden the aging time for a fresh meat cut is about seven to ten days. The shear force analysis on the samples should therefore be done using the same time span. However, to obtain meat quality measurements that are comparable with international standards other aging times should have to be applied, where the most widespread intervals for tenderness measurements are 2, 7, 14 and 21 days *post mortem* and often measured in *M. longissimus*.

How would the application of genetic markers for improved meat quality affect the Swedish beef production? Prediction of genetic potential of individual animals for genetic improvement of meat quality is a means to achieve a high beef quality, why genotype information may be utilized to increase the accuracy of selection of breeding animals. Payment to the beef producers based on marker genotype of the slaughter animal is another application for genetic markers that has been suggested, as traits like marbling and final tenderness are difficult to accurately measure on the carcass. The range of applications for genetic analysis may rapidly expand with progressively faster and less expensive analysis methods. Similar to the situation for Danish beef breeders, the meat quality of a carcass is not reflected in the payment to the Swedish beef producer why the significance of improving meat quality is difficult to estimate. Nor can the consumer discriminate between beef retail cuts of good or poor eating quality. However, strong brands and labels exist where quality is premiated and where improvement would be of comparably higher significance to the producer. The utilization of molecular markers has therefore its largest potential in the establishment of market brands that guarantee high meat quality where tests for genetic markers may add valuable information to the carcass parameters on which the payment to the farmer preferably should be based. Supplementary to meat quality improvement, the use of markers can become important for verification of product origin. The traceability of products is important for the customers' confidence in livestock production. It would also be helpful in locating disease outbreaks, e.g. the bovine spongiform encephalopathy, BSE (Jeon *et al.*, 2006). Genetic progress as regards meat characteristics may be feasible, but requires research based on careful monitoring and accurate evaluation.

The majority of the polymorphisms included in the literature review would be of interest to also include in a subsequent study where these SNPs' effect on meat quality in the Swedish beef population will be evaluated. Before including these polymorphisms in selection programmes it is important to establish that a certain meat quality parameter is not negatively correlated to economically important production parameters. Finally, the prospects of implementation of candidate genes in the genetic improvement of beef quality depends on if retailers and consumers are willing to pay a higher price for higher quality, and how the beef farmers will profit from that.

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