Effects of single nucleotide polymorphisms identified at the *μ*-Calpain and Calpastatin gene locus on tenderness of meat from commercial cattle

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DEDICATION

This thesis is dedicated to my beautiful boys Onyx Xavier Tua and Adriel Noel Tua and my husband Alfredo Javier Tua. To them I owe everything.

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Abstract

A total of 102 crossbred cattle from commercial herds with *Bos taurus indicus* influence (CCP) and 22 crossbred *Bos taurus taurus* (Senepol x Charolais) from the UPR herd (UPRCP) were evaluated for meat quality and genotyped for candidate markers in the CAPN1 and CAST genes. The *Bos taurus indicus* influence in the commercial group was detrimental to overall tenderness with a mean of 9.15 kg in Warner Bratzler Shear force (WBS) 24h postmortem. Low free calcium concentrations among the CCP at 24h and 14d post mortem (53.97 ± 19.29 and 64.11 ± 26.91 μM respectively) could have contributed to the high WBS. High ultimate pH values (5.75) in the CCP also suggest that long term stressors, during the handling procedures prior to slaughter, may have influenced this result. Acceptable tenderness (4.6 kg WBS) was achieved in a pasture-fed system used with the locally adapted *Bos taurus taurus* group. A positive correlation between WBS at 24h postmortem and free calcium concentration at 24h (r = 0.52) and 14d (r = 0.33) postmortem was observed in the CCP. Older animals with 8 permanent teeth showed increased tenderness in combination with little change in free calcium concentration from 24h to 14d postmortem (39 μM and 48 μM respectively) and the highest ultimate pH values (6.09). These results in the order animals, contrary to those in the other age groups, could be credited to a sarcopenic phenotype observed as increased muscle wasting due to altered calcium homeostasis, depleted muscle ATP content and activation of the calpain system and other apoptotic pathways (Bartoli et al., 2005; Andersson et al., 2011).

A total of 26 SNP were genotyped from the CAPN1 and CAST genes in both populations, of which 16 were previously identified and 11 were newly found. Low frequencies of the desirable tender meat genotype (less than 0.05%) were observed in the CCP among the commercially available SNP markers 530, 316, 4753 and 5331 in the CAPN1 gene. Only the CAPN1 316 marker produced a -4.72 kg change in WBS 24h when the CC genotype

segregated in the CCP. Low frequencies were also observed among the other SNP evaluated in the CAPN1 gene. Despite this low frequency, differences in WBS of -5.14 kg, -5.30 kg and -7.74 kg were observed when the GG, TT and INS C genotypes found in introns 8 and 9 of the CAPN1 gene segregated in SNPs 430, 573 and 643 respectively. The UPRCP segregated higher frequencies of the tender genotype in almost all SNP from the CAPN1 gene, but significant differences in tenderness were observed in only two of those found in intron 14 of this gene. SNP 209 genotype AA produced an increase of 45.45 in MFI and SNP 351 genotype GG produced a difference of -3.35 kg in WBS. Among the SNP studied in the CAST gene higher frequencies of the tender genotype were observed (greater than 45%) for markers 282, 2959 and 3016 in both populations. A significant difference in tenderness was found only in CAST 2959 (-2.33 kg in WBS) when the AA genotype segregated in the UPRCP. In this investigation two candidate markers for determining tenderness were found in intron 14 of the CAPN1 gene in cattle with mostly *Bos taurus taurus* influence. Polymorphisms in introns 8 and 9 of the CAPN1 gene show greater effect on tenderness in the *Bos taurus indicus* population although their frequencies were greater in the *Bos taurus taurus* population.

Introduction

In Puerto Rico, beef has been gradually losing market share to competing meats and other protein sources. The same is happening throughout the developed world (Moloney et al., 2001). This decline in consumption reflects consumer concerns about diet, health, food safety, animal welfare and environmental sustainability of beef production. In addition, changing consumer lifestyles and the availability of more conveniently prepared foods have affected the demand for beef products (Moloney et al., 2001). All these factors including the low economic value obtained per pound produced have greatly affected local production of beef. In the last four years a 15% decrease, equivalent to 5 million pound of meat have been reported by the USDA Agriculture Statistics of Puerto Rico. Consumers are unimpressed by local non classified beef when compared to the increasing variety of imported meat with quality labels. Beef tenderness is closely related to its overall acceptability (Chambers and Bowers, 1993). Tenderness is a complex characteristic determined by intrinsic factors within the muscle, which can be influenced by genetics, age and the environment (Purchas et al., 2002). Such factors can act during the animal's growth, at pre-slaughter, during postharvest management, and during cooking (Harper, 1999; Fergusson et al., 2001). Beef cattle genetic improvement programs have traditionally focused primarily on live animal growth traits. However, as consumers become more concerned with diet-health issues and the beef industry focuses more on value-based marketing, the emphasis on body composition traits will be more important in the design of breeding programs (Marshall, 1994). The National Beef Tenderness survey conducted at Texas A & M University (1990), documented a relatively high incidence of toughness problems in beef cuts at U.S. retail supermarkets, and emphasized the need to improve beef tenderness (Morgan et al., 1991b). To compete with other sources of food, the beef industry must deliver a good product with reliability and it must be economical for the consumer. Most of the beef consumed in Puerto Rico is imported from the continental United States. Puerto Rico produces only 10.3% of the total beef consumed. Therefore, it is imperative to characterize the locally produced beef and compare its quality with that of the imported beef. This is of added importance in Puerto Rico where beef comes mainly from young bulls instead of steers.

Chapter I: Literature Review

1.1. Introduction

Tenderness has become a very important trait to determine meat quality. Consumers rate meat with 4.6kg or less of Warner Bratzler Shear (WBS) as acceptable meat (Steinberg et al., 2009) and are willing to pay a premium for $4.05/kg of guaranteed tender meat (Koohmaraie and Geeksink., 2006). A lot of research has been directed to this attribute with the expectation of better understanding the mechanisms responsible for greater myofibril fragmentation and sensory palatability (Viljoen et al., 2002; Brulė et al., 2010; Huff-Lonergan et al., 2010; Kemp et al., 2010;). The most studied proteolytic systems in meat tenderization are the cathepsins, calpains and 20S proteosome (Hernan et al., 2006; Koohmaraie and Geeksink, 2006). The calpains have gained much interest due to their ability to alter the Z-disk in postmortem aging of meat (Goll et al., 2003; Dargelos et al., 2008; Huff-Lonergan et al., 2010). To make it profitable for the industry to market beef based on tenderness; consumers must be able to consistently recognize tenderness differences (Wheeler et al., 2004). If consumers could detect variation in beef tenderness, beef breeders would have an incentive to produce cattle of increased profitability for the beef industry as a whole, by supplying the beef demanded by consumers (Marshall, 1994).

1.2. ***Conversion of muscle to meat***

One cannot understand the mechanisms of tenderness without first taking in to consideration the biochemical aspects of the conversion of muscle into meat. This conversion has been postulated to occur in tree steps: the pre-rigor, the rigor and the tenderizing steps (Ouali et al., 2006). The onset of the conversion is depletion of blood supply to the muscle. This depletion stops the renewable supply of glucose from the blood stream, where upon the muscle starts to use the accumulated glycogen in the glycolysis pathway to produce ATP. Pyruvate in the absence of oxygen is converted to lactic acid that accumulates in the muscle and thus reduces the pH value. At this time the sarcoplasmic reticulum losses its membrane stability and leaks calcium into the extracellular space initiating the onset of proteolysis in muscle fibers by the proteolytic enzymes (Silva et al., 1999; Bartoli and Richard., 2005).

1.3 Meat quality

1.3.1 Color

Meat color is of great importance since it's the first thing costumers see when they purchase meat (Page et al., 2001; Viljoen et al., 2002). The consumer discriminates against darker colored meat because of its association with decreased freshness (Viljoen et al., 2002). Meat with a high ultimate pH will present a dark purple color. This is known as "dark cutter", and occurs because there is less space between muscle fibers due to the high water holding capability of this meat and not much light is reflected (Page et al., 2001; Adzitey and Nurul., 2011; Duarte et al., 2011). A light red muscle color is due to a greater reflectance of light because the water holding capability is low. This reduction in water holding capability of the meat is due to a lower than normal ultimate pH value (Kerry et al., 2002).

1.3.2. Ultimate pH

Muscle color is directly correlated with meat tenderness and ultimate pH (Page et. al., 2001) *(Figure 1.3*). As pH declines normally, post mortem denaturalization of the muscle proteins occurs as a result of activation of the calpain system. The pH range for bovine longuissimus muscle with normal postmortem metabolism is 5.40 to 5.59 (Page et. al., 2001). According to USDA grading standards meat with a pH value of 5.87 or greater are classified as "dark cutters" (Page et. al., 2001). A high pH and dark meat color is characteristic of cattle that suffered from stress before slaughter (Viljoen et al., 2002; Adzitey and Nurul, 2011). Thus it is not uncommon for cattle raised in a pasture system to show high pH values and meat with darker color (Duarte et al., 2011), because they are more susceptible to stressors caused by the slaughter procedures (Kerry et al., 2002). The exposure to physical or psychological stress before slaughter is known to reduce the quality and shelf life of meat (Viljoen et al., 2002; Adzitey and Nurul, 2011). Stress causes the "flight or fight" response which activates the release of catecholamine's. The catecholamine's, epinephrine and norepinephrine, deplete muscle glycogen, reducing the amount of energy available to support anaerobic metabolism (Duarte et. al., 2011). Thus, not enough lactic acid is produced to lower the pH value of the carcass, and "dark cutter" meat is produced. The water holding capability of the meat is also affected by high ultimate carcass pH. In normal pH decline the concentration of negative charges is reduced and thus the ability to attract water (Duarte et. al., 2011). In dark cutter this reduction of negative charges fails to occur and results in high pH and increased water holding capability. This is characteristic of Dark Firm and Dry (DFD) meat, which normally is tougher

than normal meat and causes enormous losses in the meat industry due to its low quality (Duarte et al., 2011). There have been reports that DFD meat can show increased tenderness based on WBS and Myofibrillar Fragmentation Index (MFI) values (Maddock et al., 2006; Silva et al., 1999; Viljoen et al., 2002). Greater proteolytic activity takes place with higher than normal pH values, mostly because the optimal pH for μ-calpain activity is 6.5 (Maddock et al., 2006; Huff-Lonergan et al., 2010). The sensory attributes of DFD meat have been evaluated by Viljoen et al., (2002) and no significant difference was found between normal pH and DFD fried steaks.

1.3.3. Breed

Breed exerts a great influence on meat tenderness. Numerous studies have concluded that *Bos taurus indicus* cattle produce tougher meat than *Bos taurus taurus* (Geeksink et al., 2001; Kerry et al., 2002, Koomaraie and Geeksink, 2006). *Bos taurus indicus* cattle are extensively used in the tropics due to their adaptation to harsh environments: heat tolerant, disease resistant and high fertility (Jaturasitha et al., 2009; Curri et al., 2010). Not only do *Bos taurus indicus* cattle have lower levels of intramuscular fat and higher levels of connective tissue but also higher levels of calpastatin activity compared to *Bos taurus taurus* (Kerry et al., 2002; Koomaraie and Geeksink, 2006). Bonilla et al. (2010) reported higher intramuscular fat in Bos taurus taurus cattle (8.29 %) compared to *Bos taurus indicus* and crossbreds (4.31 and 5.33 %, respectively) Mexican cattle, $P < 0.03$. Lower intramuscular fat can affect tenderness by cold shorting (Kerry et al., 2002). Calpastatin, the endogenous calpain inhibitor, can also reduce meat tenderness by inhibiting the enzymatic proteolysis of muscle fibers due to calpain activity. Frylinck et al. (2009) reported no difference in calpastatin activities between *Bos taurus taurus* and *Bos taurus indicus* cattle he did find lowered μ-calpain activity in *Bos taurus indicus*. Therefore a higher calpastatin to calpain ratio is expected in *Bos taurus indicus* than in other crossbred cattle (Frylinck et al., 2009). The activity of the calpains is controlled by calcium concentrations in the muscle and can be manipulated with $CaCl_2$ in postmortem muscle. Morgan et al. (1991a) found increased tenderness in strip loin steaks with injections of CaCl2 solution in mature cow cuts, as well as Pringle et al. (1999) in steaks from Brahman and Angus breeds despite the higher calpastatin activity found in Brahman steaks. Calcium concentration in meat could determine differences in tenderness along with calpastatin to calpain ratio. Geeksink et al. (2001) reported no differences in calcium concentration of muscle among breeds of *Bos taurus indicus vs Bos taurus taurus.* Thus the difference observed between breed types is multifaceted.

1.3.4. Age at Slaughter

Age at slaughter is known to influence meat tenderness (Jurie et al., 2006; Xiong et al., 2007). Younger cattle tend to produce more tender meat than older cattle. As cattle mature, more cross linking of individual collagen molecules makes the connective tissue more rigid and less soluble (Lefaucheur, 2010), thereby directly affecting meat tenderness and generally overall palatability (Kerry et al., 2002; Xiong et al., 2007). Xiong et al. (2007), found that *Longuissimus dorsi* (LD) steaks from mature cows, 10-12 years, presented greater WBS values and a reduced rate of protein degradation compared to those of younger cows, 2-6 years. Age at slaughter has proven to be a generally fast and effective way to determine the potential for an animal to render tender meat but high variability in this regard is still a problem. Mature cows represent a significant source of meat in the United States, 14.7%, (Xiong et al., 2007), and in France two-thirds of the beef consumed is derived from cull cows removed from dairy and beef breeds equally (Jurie et al., 2006). In Puerto Rico beef cattle are mostly grass-fed. Longer growing and finishing periods are required to obtain the desired slaughter weight. Animals fed exclusively on pasture depend on the forage quality and quantity. Pasture-raised cattle commonly produce meat that is lean but also suffers from cold shortening due to lack of sufficient adipose tissue to protect the carcass (Kerry et al., 2002; Duarte et al., 2011). Cold shortening reduces meat tenderness because it produces more cross-linkage by overlapping myofilaments, thus reducing available sites for proteolytic activity (Frylinck et al., 2009; Duarte et. al., 2011). Also darker color of meat is associated with advanced chronological age and greater myoglobin content (Kerry et al., 2002; Duarte et al., 2011) or increased myoglobin oxidation (Maddock et al., 2006; Xiong et al., 2007). Research has shown greater incidence of muscle cell susceptibility to oxidative stress with advance chronological age (Xiong et al., 2007; Dargelos et al., 2008; Brule et al., 2010).

The quest for tender meat has given rise to different methods of tenderization. Among the most used is the postmortem aging of meat and conditioning of muscles by injection of $CaCl_2$. Aging of meat from mature cows for a period of 14 d lowers shear force in all steaks regardless of animal age (Xiong et al., 2007; Pflanzer and Felicio, 2009). On the other hand there have been reports in Australia and South Africa of decreased tenderness with advanced chronological age of cattle (Pflanzer and Felicio, 2009). Further investigation is in order to determine the biological basis of greater myofibrillar fragmentation in older cattle and if increased calpain activity is observed.

1.4. Calpains

The calpains in the cell participate in various physiological functions including cell cycle, apoptosis, cytoskeleton organization and signal transduction (Bartoli and Richard, 2005). In the embryonic development of skeletal muscle fibers myoblast differentiation and fusion generate myotubes (Silva et al., 1999). Myoblast fusion requires destabilization of its cell membrane and depends on the ratios of calpain to calpastatin for membrane protein degradation. When calpastatin, the specific inhibitor of calpains, diminishes and calcium leaks into the cytoplasm the calpains can activate and degrade the membrane to permit fusion (Silva et al., 1999). The calpains activity is regulated during both muscle exercise and muscle atrophy which indicates the importance of the calpains in cytoskeletal modifications needed for muscle plasticity (Bartoli and Richard, 2005). Calpain are fully activated by Ca^{2+}, as seen in crystallographic studies (Dargelos et al., 2008). The calpain molecule (Figure 1.4) has an 80 kDa catalytic sub-unit responsible for the enzyme activity (Sentandreu et al., 2002). This large sub-unit contains four domains: domain I has the autolytic activation site, domain II the activation site and domains III and IV the calcium binding sites. The calcium sensibility *in vitro* is in the range of 5-50 μM and 250-1000 μM for μ-calpain and m-calpain respectively (Bartoli et al., 2005). *In vivo* the calcium requirements for calpain activation lie in the range of 30 to50 μM and 400 to 800 μM for μ-calpain and m-calpain respectively (Dargelos et al., 2008) a little lower than concentrations *in vitro*. In postmortem proteolysis of muscle proteins the calpains, in particular the μ-calpain, play an important role in meat tenderization (Doumit and Koohmaraie, 1999; Casas et al., 2005; Curi et al., 2010). This was confirmed in μ-calpain knockout mice that showed inhibited postmortem proteolysis compared to the control mice (Geeksink et al., 2001) with intact gene activity. Improvement in tenderness due to storage has been associated with the activation of the ubiquitous calpain system (μ and m-calpains), a calcium dependent protease (Morgan et al., 1991a, Ouali et al., 2006 and Xiong at al., 2007). The calpain system is activated by calcium ions that leak from the sarcoplasmic reticulum into the cytosol after the muscle ATP is depleted postmortem. Such increase in calcium ions contributes to meat tenderization (Takahashi and Ji, 2006). At the muscle fiber level, the myofibrillar proteins troponin T, desmin, titin, nebulin and vinculin are partially or completely degraded by these proteases. The calpains need a neutral pH for maximum activity (Calkins and Seideman, 1988; Takahasi and Ji., 2006; Neath et al., 2007), but μ- calpain when exposed to calcium undergoes autolysis. This reduces the calcium requirement for proteolytic activity and because autolysis is an intermolecular process it does not result in a complete loss of proteolytic activity (Dargelos et al., 2008; Veiseth et al., 2001).

This explains the increase in tenderness during aging of the meat. Over 50% of the degraded μ-calpain is tightly bound to myofibrils after 7 days of aging (Boehm et al., 1998). The Z disk is the area where most structural changes take place during aging of meat (Palka, 2002) and the degradation of troponin-T, which is a calpain substrate, is the myofibrillar protein that is reported to undergo the most change during that period (Geesink et al., 2001).

1.5. Calpastatin

Calpastatin is an inhibitor of the calpains and a negative correlation between calpastatin activity and meat tenderness has been reported (Doumit and Koohmaraie, 1999). The activity of calpastatin accounts for a greater proportion of variation in beef tenderness, ~40%, than any other measure (Koohmaraie and Geeksink, 2006). Calpastatin enzyme activity has been associated by Smith et al. (2009) to WBS, ($P < 0.05$). Calpastatin contains four homologous domains that can inhibit calpain (Goll et al., 2003; Koohmaraie and Geeksink, 2006; Kemp et al., 2010) (F*igure 1.5*). Each domain has a different affinity to calpain and the constant of dissociation is at its lowest when the four domains are bound to the calpain molecule, < 3nM, (Dargelos et al., 2008). Three regions within each domain of calpastatin (ABC) are responsible for inhibiting calpain either by binding to the large catalytic sub-unit in domain IV or by binding to the small sub-unit in domain VI (Sentandreu et al., 2002). Just as calpain, calpastatin requires calcium for activation and also undergoes autolysis without compromising its activity (Kemp et al., 2010).

1.6 Muscle Wasting and Sarcopenia

Muscle atrophy can arise from fasting, disuse, aging and several pathological conditions including injury (Bartoli and Richard, 2005). Loss of muscle fibers seen in muscle wasting is due to apoptosis and necrosis as a result of different signaling pathways linked to an atrophic program in cell homeostasis (*Figure 1.2*) (Dargelos et al., 2008). During fasting the calpains initiate myofibrillar degradation and the proteasomes remove the myofibrillar fragments to recycle amino acids (Bartoli and Richard, 2005). Observations by electron microscopy in experimental wasting conditions show disorganization of Z-discs, one of the possible sub-cellular locations of μ-calpain (Bartoli et al., 2005; Andersson., 2011). This disorganization occurs in Z-discs that are associated with substrates of μ-calpain including titin, nebulin, filamin, troponin-T and intermediate filament desmin. In aging, muscles increase in calcium permeability and free radicals initiate muscle wasting. One reason for the release of calcium

ions is believed to be oxidation and cysteine-nitrosylation of the ryanodine receptor 1 (RyR1) (Andersson et al., 2011). The RyR1 is responsible for calcium release from the sarcoplasmic reticulum for muscle contraction (*Figure 1.1*) (Huff-Lonergan et al., 2010). In aged 24 month old rodents Andersson et al. (2011) found oxidized RyR1 receptors, which compromised calcium-holding capability resulting in leaky channels. In another case oxidation of longuissimus thoracic muscle of 2.5-year old Chinese cattle showed greater degradation of the α-actin with higher oxidant concentration when incubated with μ-calpain (Xue et al., 2012). Yet its decrease in degradation without oxidative modification and incubation with μ-calpain shows the difficulty of its oxidation during postmortem aging. This is due to the interaction of actin with myosin heavy chains which mask the oxidation site in myofibers (Xue et al., 2012). Thus oxidation can control the proteolytic activity of enzymes and could be linked to meat tenderness (Mercier et al., 2003). Oxidative stress induced protein degradation is well documented in muscle and has been seen to increase with age (Andersson et al., 2011; Dargelos et al., 2008). Sarcopenia is characterized by accumulation of reactive oxygen species (ROS) that produce mutations in the mtDNA and impair adenosine-5'-triphosphate (ATP) production and calcium homeostasis (Dargelos et al., 2008; Brulé et al., 2010). These conditions mark cells for apoptosis by initiating biochemical pathways including the mitogen-activated protein kinases (MAPK) and Bax pathways (Brulé et al., 2010). Calpains are known to be activated and regulated by calcium concentrations and play an important role in cellular death (Bartoli and Richard, 2005; Dargelos et al., 2008; Brulé et al., 2010).

*1.7. Polymorphisms and Tenderness: C*APN1 and CAST Candidate Genes

The CAPN1 gene is 30KB long, has 22 exon and is found in chromosome 29, BTA29, (*figure 1.4*) while the CAST gene is found in chromosome 7, BTA7, (Casas et al., 2005; White et al., 2005). Genetic markers for improved tenderness and meat quality traits in the CAPN1 and CAST gene have been identified and validated by the US National Beef Cattle Evaluation Consortium (Van Eenennaam et. al., 2007). Two genetic marker panels are commercially available: Gene STAR Tenderness (Genetic Solutions Pty. Ltd., Albion, Australia) and Igenity Tender GENE (Merial Ltd., Atlanta, GA). The Gene STAR Tenderness marker panel involves: (1) CAST 2959 (base 2959 accession # AF159246; Barendse et al, 2002) that is a G/A SNP in the 3' untranslated region of calpastatin; (2) CAPN1 316 which is a G/C SNP in exon 9 of μ-calpain (base 5709 accession # AF252504; Page et al., 2002) and (3) CAPN1 4751 that is a C/T SNP in intron 17 of μ-calpain (base 6545 accession # AF248054; White et al., 2005). Igenity Tender GENE includes the same two SNP in the μ-calpain gene and CAST 282 (base

282 accession number AY008276; Schenkel et al., 2006) which is a G/C SNP in intron 5 of the CAST gene. CAPN1-316 produces a missence mutation; a transversion of a cytosine for a guanine that alters the codon translation of alanine to glycine (White et al., 2005). This molecular marker was significantly associated with sensory panel tenderness score, animals with the CC genotype producing more tender meat than those with CG and GG genotype (Casas et al. 2005). CAPN1-4751 is a transition of cytosine for thymine, which is significantly associated with shear force of meat at 14 d postmortem (White et al., 2005; Casas et al., 2005). The other markers that are not commercially used are CAPN1-4753; a transversion of adenine for cytosine found between exon 7 and 8, and CAPN1-5331; also a transversion of adenine for thymine found between exon 1 and 2. These markers also play a role in tenderness and seem to have mutually similar frequencies (Casas et al, 2005). This could indicate linkage disequilibrium between both markers which could be segregating as a haplotype (Casas et al. 2005). Like the polymorphism at CAPN1-316, marker 530 (base 4558 accession number AF248053; Page et al. 2002), produces a missence mutation; a transition of adenine for a guanine changing a codon translation of valine to isoleucine. Page et al. (2002) analyzed the relationship between genotypes and shear force values for SNPs 316 and 530 revealing a difference between CAPN1 alleles in which the allele combination resulting in a translation of isoleucine at position 530 and glycine at position 316 was associated with decreased meat tenderness relative to the allele encoding valine at position 530 and alanine at position 316. The SNP validated by Casas et al. (2005) and discovered by Barendse (2002) at position 2959 in the CAST gene is a transition from a guanine to adenine at the untranslated region of the gene. Animals that inherited the CC or CT genotype produced tougher meat when compared to those with the TT genotype. Schenkel et al. (2006) reported another SNP at position 282 (Accession Number AY008267), between intron 5 and 6. This SNP corresponds to a transvertion from cytosine to guanine with the CC genotype presenting more tender meat compared with genotypes CG and GG over a 21-d postmortem period. A third SNP located in the CAST gene was found at intron 2 and consists of an insertion/deletion of cytosine. Preliminary data suggest that animals with the cytosine deletion presented more tender beef compared to the other genotypes due to differences in myofibrillar fragmentation index (Dr. Melvin Pagan, personal communication).

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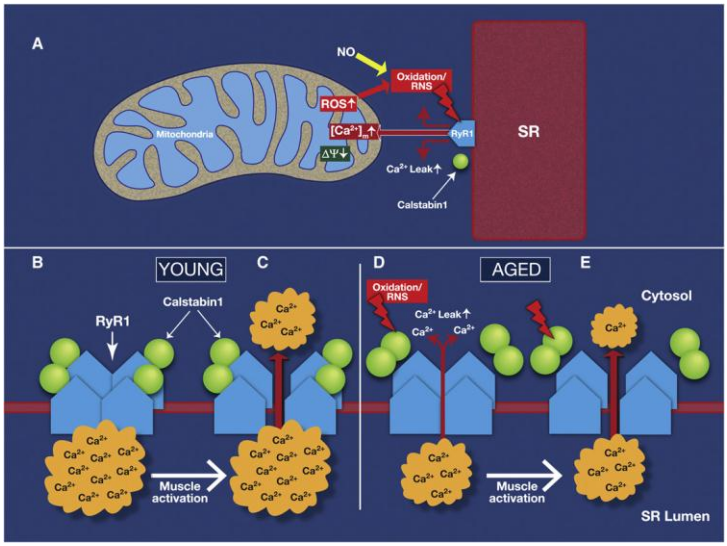
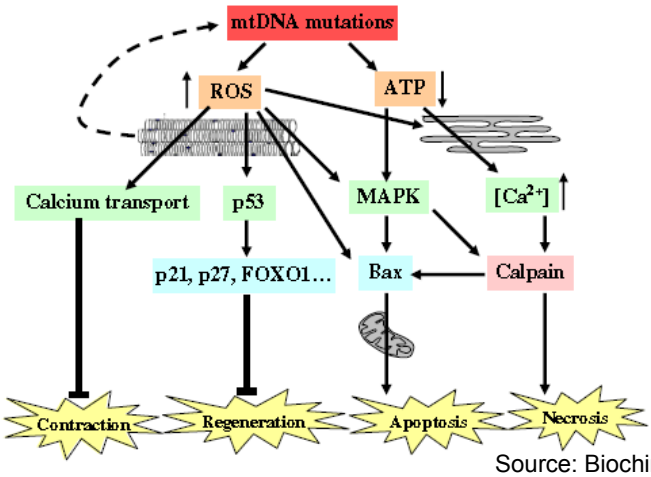


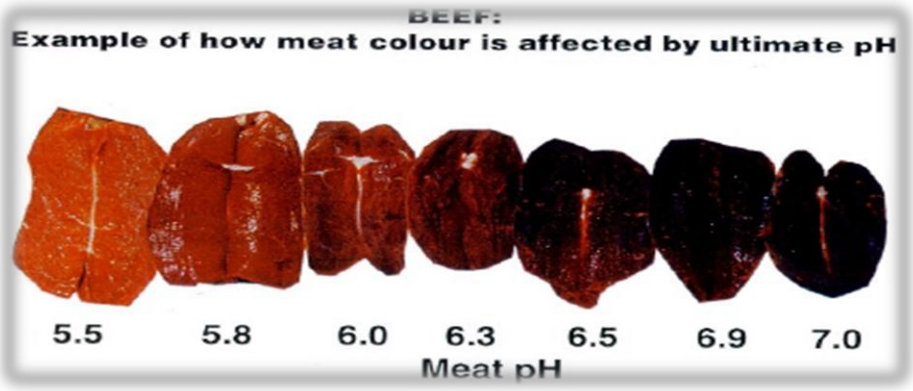
Source: Cell Metabolism (2011) 14: 196–207

Figure 1.1. Effect of ROS on the rynodine receptor and calcium homeostasis. Oxidation of the RyR1 causes calcium to leak from the sarcoplasmic reticulum in older animals skeletal muscles. This change impairs muscle force and contraction due to a decrease in calcium concentration necessary for contraction (Andersson et al., 2011).



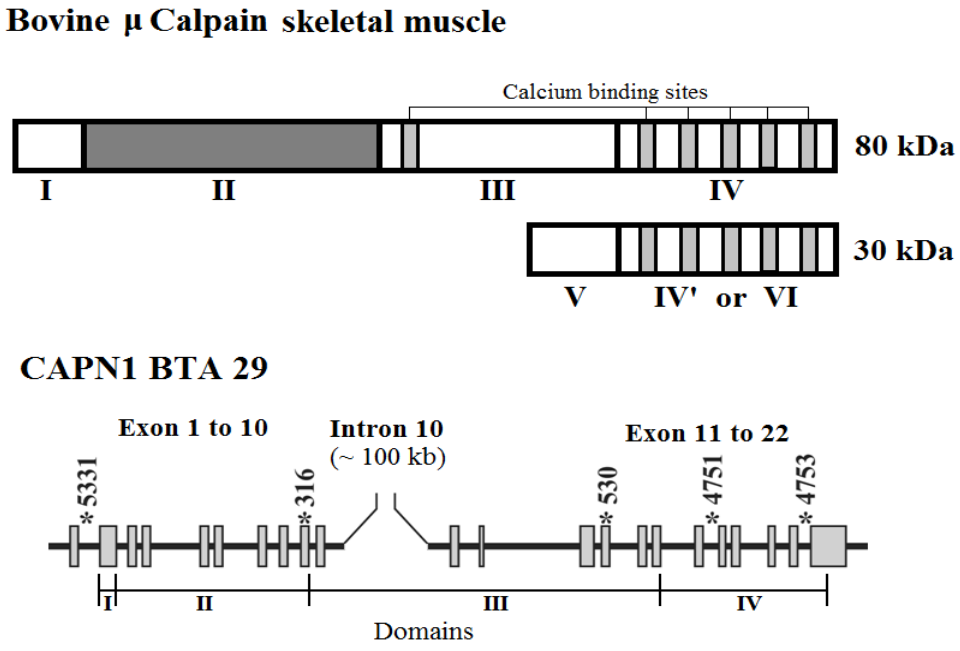
Source: Biochimie (2008) 90: 359-368

Figure 1.2. Sarcopenic Simplified Scheme. The sarcopenic phenotype is seen when accumulation of mtDNA mutation aids in ROS accumulation which in turns reduces cellular ATP content. These changes trigger the activation of the calpains because of the increase in calcium concentration. Other signaling pathways are activated to maintain cellular homeostasis leading to cell death by apoptosis or necrosis (Dargelos et al., 2008).



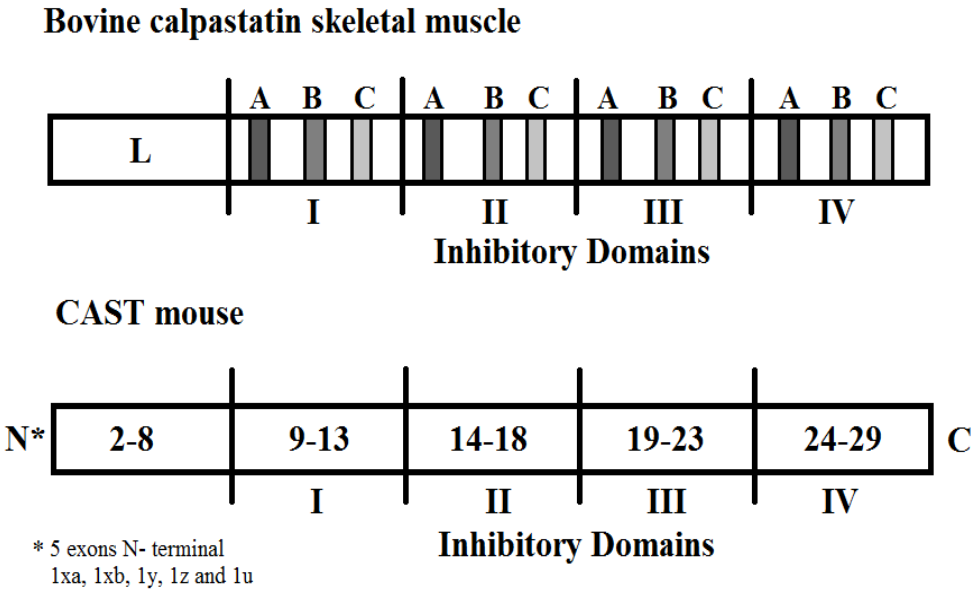
Source: Meat Color Montana State University and Saskatchewan Food Product Innovation Program University of Saskatchewan

Figure 1.3. Effect of ultimate pH on muscle color stability. When the ultimate pH fails to decrease during the conversion of muscle to meat the color stability of the meat is compromised affecting its quality. The normal pH range in beef is 5.4-5.6, pH values higher than 5.9 is considered as dark cutters (Adzitey and Nurul, 2011; Page est al., 2001; Vilijoen et al., 2002).



Source: J Anim Sci (2005) 83:2001-2008; Trends in Food Science & Technology 13 (2002) 400-421

Figure 1.4. Schematics of μ calpain catalytic subunit and single nucleotide polymorphisms found in the CAPN1 gene.



Source: Physiology Review (2003) 83: 731-801; Trends in Food Science & Technology13 (2002) 400-421; Physiol Rev Vol 83 July 2003 www.prv.org

Figure 1.5. Schematics of calpastatin inhibitory domains and single nucleotide polymorphisms found in the CAST gene. Calpastatin homologous domains, I - IV, are known to inhibit calpain activity.

Chapter II. Meat Quality of Commercial Cattle from Puerto Rico

2.1 Abstract

Puerto Rican beef is predominantly pasture-fed and has a strong *Bos* taurus *indicus* influence due to the better adaptation of this type of cattle to tropical environments. A total of 102 commercially produced cattle were evaluated for meat quality. High ultimate pH values were observed (5.75) in this sampling of slaughtered animals. Long term stressors during the handling procedures prior to slaughter could have influenced the high pH values. The *Bos taurus indicus* influence was evident in the overall tenderness mean of 9.15 kg WBS at 24h postmortem. Low free calcium concentrations among the CCP at 24h and 14d post mortem (53.97 ± 19.29 and 64.11 ± 26.91 μM respectively) could have contributed to the high WBS. A positive correlation was observed between WBS at 24h postmortem and free calcium concentration at 24h (r = 0.52) and 14d (r = 0.33) postmortem in the CCP. This positive correlation is attributed to the fact that older animals with 8 permanent teeth showed little increase in free calcium concentration during the 14d aging period (9 μM) and increased tenderness (-5.81 kg WBS). A negative correlation was observed between ultimate pH and WBS (r = -0.74 and r = -0.66) at 24h and 14d of aging respectively. Animals with ultimate pH values higher than 5.86 showed increase tenderness (-5.29 kg) based on WBS. A total of 44% of the animals showing pH values higher than 5.86 had 8 permanent teeth. Muscle wasting caused by a sarcopenic phenotype occurs due to altered calcium homeostasis, depleted muscle ATP content and activation of the calpain system and other apoptotic pathways (Bartoli and Richard., 2005; Andersson et al., 2011), which could have been the mechanism causing the somewhat anomalous results seen in the older cattle of this study. Acceptable tenderness (4.6 kg WBS) could be widely achieved in local pasture-fed systems using adapted *Bos taurus taurus* cattle, as observed in the UPR beef cattle herd composed of Senepol x Charolais crossbreds.

2.2. Introduction

A classification system of bovine meat based on tenderness alone has yet to be established because high variability in this trait is still a common problem. Protein degradation and oxidation as well as the intercellular environment can determine the rate and extent of tenderization (Huff-Lonergan et al., 2010). Younger animals are assumed to produce tender meat compared with more mature animals. This is due to more cross linking of individual collagen molecules making the connective tissue more rigid and less soluble as the animal matures (Lefaucheur, 2010). Muscle color is also an important trait and is found to be directly correlated with meat tenderness and ultimate pH (Page et. al., 2001). A high pH and dark meat color is characteristic of cattle suffering from stress before slaughter. Cattle raised in a pasture system commonly show high pH values and meat with darker color because they are more susceptible to stressors caused by the slaughter procedures (Steinberg et al., 2009). Grass-feed cattle can produce tougher meat not only because of the deficient accumulation of lactic acid due to the depleted energy in the pre-mortem period, but because of longer finishing periods before slaughter. However, greater myofibril fragmentation can be produced in meat from mature cattle by means of an aging period of 14 days (Guillemin et al., 2011).

Improvement in tenderness due to prolonged storage has been associated with activation of the ubiquitous calpain system (μ and m-calpains), a calcium dependent protease (Ouali et al., 2006; Xiong et al., 2007; Kemp et al., 2010). The calpain system is activated by calcium ions that leak from the sarcoplasmic reticulum into the cytosol after the muscle ATP is depleted postmortem (Harper et al., 1999; Kemp et al., 2010). The calcium is used up by the enzymes resulting in lower levels of free calcium as the aging period progresses.

Sarcopenia is an involuntary loss of muscle mass due to activation of the calpain system in aged muscles in-vivo (Andersson et al., 2011). This condition is characterized from a cellular point of view as reactive oxygen species, ROS, accumulation and reduction of cellular ATP

content (Dargelos et al., 2008). The majority of ROS accumulation comes from the production of ATP by aerobic metabolisms known as oxidative phosporylation (OXPHOS) (Wei and Lee, 2002). When the body fails to regulate ROS production by glutathione metabolisms (Thore et al., 2008) high levels of ROS produce large scale deletions, tandem duplications and point mutations of the mtDNA (Wei and Lee, 2002). Aged muscles from animals of advanced chronological age consume high levels of oxygen which in turn accumulates mutations that impair OXPHOS and thus ATP production Wei and Lee, 2002; Thore et al., 2008) by affecting the ATP synthases pump as well as altering calcium homeostasis by affecting the rynodine receptors (RyR1) in skeletal muscles (Andersson et al., 2011). An elevation in the calcium influx due to calcium homeostasis dysregulation can increase calpain activity (Brulé et al., 2010; D'Alessandro et al., 2012). Activating the calpain system after ROS accumulation and reduction of cellular ATP in-vivo will produce myofibril fragmentation due to apoptosis and necrosis in the skeletal muscle (Luciano et al., 2007; Dargelos et al., 2008). Sarcopenia could result from malnutrition, inactivity, hormonal changes and acute chronic inflammation (Brulé et al., 2010). However, this condition has not been previously documented in cattle. The object of this study is to determine potential differences in tenderness between beef cattle harvested at slaughterhouses in Puerto Rico at different chronological ages determined by number of permanent incisors teeth.

2.3. Materials and Methods

2.3.1. Animal Selection

Commercial animals included in this study were randomly selected from the slaughterhouse "Ganadería Santiago" located in Yauco, PR. Slaughter and processing of meat was in compliance with the HACCP model for beef slaughter of the USDA Food Safety and Inspection Service (USDA-HACCP Beef Slaughter., 1999). A licensed inspector from the USDA

monitored every procedure. A total of 102 animals were used in this investigation. Sex and age (determined by the number of permanent incisors 0, 2, 4, 6 and 8) were recorded at slaughter. The first pair of incisors emerges approximately at 23.8 months of age, the second pair at 31.9 months, the third pair at 41.5 months and finally the molars at 52.5 months (Casas et al., 2001; Lawrence et al., 2001). If only one tooth penetrated the gum it was considered as if a pair had emerged. Racial group was recorded but was not included in this analysis because of the high degree of variability among the animals selected. No additional information was obtained other than that collected at the slaughterhouse. Therefore information regarding the animals' growth, nutrition and overall health was not available. Approximately 10 animals per permanent incisor group were investigated for a total of 102 animals of the Commercial Cattle Population (CCP). In addition 22 crossbred Senepol X Charolais animals were evaluated. These bovines were born and raised at the Beef Cattle Research and Teaching Farm of the University of Puerto Rico located in Aguadilla and commonly known as "Finca Montaña". WBS, MFI and a genetic overview of the CAST and CAPN1 gene were the only criteria evaluated in these representatives of the University of Puerto Rico Cattle Population (UPRCP). The CCP were categorized as *Bos taurus indicus* crossbreeds and the UPRCP as *Bos taurus taurus* crossbreeds.

2.3.2. Muscles Samples

The *longissimus dorsi* (LD) muscle was used in this study. Samples (n = 102) were collected 24 hours postmortem to ensure rigor mortus. A total of 1.4 kg of muscle sample was taken from the longissimus directly after deboning of the carcass. Samples were put on ice and transported to the Meat Science Laboratory of UPRM. For this investigation 500 g of each sample was used. The remaining 900 g was used for further analysis, data not included.

Aging

The two aging treatments established were 24 hrs and 14 days postmortem. All samples were weighed, cut into two 250 g pieces and vacuum sealed. One half samples were assigned to the two aging periods. The 24h period sample was stored immediately at -25˚C to stop all enzymatic activity of the protease, while the 14d period sample was aged for 14d at 5˚C and then stored at -25˚C until analyzed. Temperature was monitored throughout the entire aging and storage process to ensure no drastic change in temperature that could compromise the results.

pH and Color Values

The pH was determined before and after freezing. Samples were passed through a food processor to homogenate and 5g ± 0.5g of the homogenate was extracted and placed in a 50mL collection tube with 5mL distilled water. The pH value of each sample was determined in duplicate with a pH-meter (Model No. 1142003 SP70P, VWR International, Batavia, IL). Four pH categories were established, Low (L), Normal (N), High (H) and Dark Cutter (DC), corresponds to pH < 5.40, 5.40 – 5.59, 5.59 – 5.86 and > 5.86 respectively. Color values were determined using a photometer Mini Scan EZ 45/0 LAV (Hunter Lab, Reston, VA). Like pH values, color was determined directly from the muscle samples, following the manufacturer's instructions, before and after freezing. The L* value determines the lightness or darkness of the color. Where 0 indicates black and 100 is white. The a* value indicates the redness of the muscle samples. Higher values of a* indicates a redder color while less values produce a more green color. The b* value corresponds to how much of the color yellow does the sample have. Higher values of b* indicate more yellow and lower values more blue. In meat this value determines the extent of marbling the muscle sample posses (Page et al., 2001).

WBS

Tenderness was measured using a Warner-Bratzler Shear Apparatus (G-R Manufacturing, Manhattan, KS) and following the standardized WBS protocol established by the USDA-ARS U.S. Meat Animal Research Center (Wheeler et al., 1997). Frozen samples were thawed at 4˚C for 24 hours and cooked in an oven broiler. Sample steaks were cooked until reaching an internal temperature of 40˚C, then turned and cooked to 71˚C before removing from the heat. Steaks were cooled to room temperature before coring with a hand-held coring device of 1.27cm diameter. Cores were removed parallel to the longitudinal orientation of the myofibers. A total of six cores were obtained from each steak and care was taken to eliminate all cores with connective tissue. Samples were sheared once in the center to avoid the toughened ends. It must be noted that WBS 24h data were obtained for only 60 carcasses, not including those of females in the C category. The C category included only 7 samples all from male animals. Also WBS on the samples with 14d aging was not determined.

MFI

Myofibril degradation was determined according to procedures of Hopkins et al., (2000; 2004) and Karumendu et al., (2009). A total of 2g per muscle samples were used to determine MFI in duplicate. One gram of sample was suspended in 30mL of MFI buffer (100mM KCL, 7mM KH_2PO_4, 18mM K_2HPO_4, 1mM EDTA and 1mM $NaNO_3$) in a 50 mL collection tube and homogenized at 16,000 RPM with a Polytron 1600E Bench-Top Homogenizer (Thomas scientific, Swedesboro, NJ) for 30 seconds. Samples were filtered through a strainer to a new 50 mL collection tube and rinsed with 5mL of buffer. The samples were centrifuged at 2,000 RPM for 15 minutes at 2˚C. The supernatant was discarded but if a layer of fat was present it was saved. The pellet was re-suspended in 10mL of MFI buffer and centrifuged for an additional 15 minutes at 2,000 RPM. The supernatant was discarded along with the layer of fat.

The pellet was re-suspended a second time in 3mL of MFI buffer. To determine the myofibrillar fragmentation 50µL of the extracted myofibers were diluted in 550µL of MFI buffer and 2.5mL of Biuret reagent was added to taint the myofibers. The absorbency was read at 540nM using a Spectronic Genesys 20 Visible Spectrophotometer (Thermo Electron Scientific Corp., Madison, WI) for determination of concentration for which bovine serum albumin was used to prepare the standards. The samples were then diluted to 0.5mg/mL and the absorbency was measured and multiplied by 200 to obtain the MFI index.

Free Calcium Concentration

Each sample was analyzed in duplicate. A total of 1g of muscle sample was suspended in 50mL of distilled H_2O in a 50mL collection tube. In all samples 1.25mL of Ionic Strength Adjuster (4M KCl) was added for an ionic background strength of 0.1M. Samples were homogenized at 30,000 RPM for 15 seconds using the Polytron 1600E Bench-Top Homogenizer (Thomas scientific, Swedesboro, NJ). The samples were then centrifuged at 12,000 g for 1 hour at 5˚C. The supernatant was used to determine calcium concentration by reading the raw millivolt values using a calcium ion-select electrode, reference electrode (Model No. ELITE 8041 and ELITE 001n, Nico 2000 Limited, Middlese, UK) and a pH-meter (Model No. 1142003 SP70P, VWR International, Batavia, IL). The raw mV was compared to a standard $CaCl_2$ curve at 0.1 and 1 ppm of Ca^{2+} to determine the calcium concentration in µM of each muscle sample. This protocol was adjusted from that used by Hopkins and Thompson (2001).

2.3.3. Statistical Analysis

The analysis of variance was performed using ANOVA Proc Mixed (SAS Inst., Inc., Cary, NC) and the Tukey Kramer test was used for mean separation. Differences among free calcium concentration categories (L = low <5.40, N = normal 5.40 -5.59, H = high 5.59 – 5.86

and DC = > 5.86) were analyzed using contingency tables (Di Rienzo et al., 2007). Hot carcass weight was used as a covariable only in WBS analysis.

The linear models used for this review were:

$$Y_{ijk} = \mu + S_i + A_j + SA_{ij} + e_{ijk}$$

Y_{ijk} = k observations pertaining the sex i of the animal (male or female), the age categories j based on number of permanent teeth (A = 0-2pt, B = 4-6pt and C = 8pt) and interactions ij of sex and age

μ = overall mean

S_i = fixed effect of sex of the animal either male or female

A_j = fixed effect of age categories based on number of permanent teeth, A = 0-2pt, B = 4-6pt and C = 8pt

SA_{ij} = interactions between sex i and age categories j

e_{ijk} = experimental error

$$Y_{ijkl} = \mu + S_i + A_j + P_k + e_{ijkl}$$

Y_{ijkl} = l observations pertaining the sex i of the animal (male or female), the age categories j based on number of permanent teeth (A = 0-2pt, B = 4-6pt and C = 8pt) and pH categories k (L = low <5.40, N = normal 5.40 -5.59, H = high 5.59 – 5.86 and DC = > 5.86)

μ = overall mean

S_i = fixed effect of sex of the animal either male or female

A_j = fixed effect of age categories based on number of permanent teeth, A = 0-2pt, B = 4-6pt and C = 8pt

P_k = fixed effect of pH categories k (L = low <5.40, N = normal 5.40 -5.59, H = high 5.59 – 5.86 and DC = > 5.86)

e_{ijkl} = experimental error

2.4. Results and Discussion

2.4.1. Simple statistics and correlations

Data including the number of carcasses included in each case, mean, standard deviation, maximum and minimum values of the different variables investigated are shown in *Table 2.1.1.* A total of 102 carcasses were studied except in the case of WBS 24h postmortem that included only 60 observations. Since the commercial cattle found at the slaughter house were of mixed breeds with little information available as to the breed proportions, this factor was not evaluated. Frequencies of sex, number of permanent teeth and corresponding relative age categories, ultimate pH and change in [Ca^{2+}] µM during 14d of aging are found in *Table 2.2*.

The mean for pH, at both 24h and 14d of aging was 5.75 ± 0.39. The CCP sampled had ultimate high pH values consistent with stressors during the handling procedures prior to slaughter (Kerry et al., 2002). Depleted glycogen supplies affects the descent in pH as the muscle is converted to meat, producing meat with high ultimate pH. The present result differs from that reported in a survey conducted on 1,062 commercial beef carcasses in the US in which a normal ultimate pH mean of 5.50 was observed (Page et al., 2001). The mean color values found in the local CCP were 31.42, 15.75 and 11.86 at 24h postmortem for L*, a* and b* respectively. These results differed from the corresponding color means reported by Page et al.

(2001), of 39.75, 25.17, and 11.05 in Brahman cattle. The low L* and a* values found in the local CCP indicates that the meat is darker in color and is closer to green than to red. The darker color and the low a* value is also consistent with long term stressors prior to slaughter (Page et al., 2001; Viljoen et al., 2002; Adizitey and Nurul, 2011). The b* value found in the present study is similar to that reported by Page et al. (2002) in Brahman cattle. A darker color of meat in pasture-fed cattle has also been reported by Duarte et al, (2011) and Mercier et al. (2004).

An increase of free [Ca^{2+}] was observed during the 14d aging period (53.97 ± 19.79 to 64.11 ± 26.91 μM respectively). Takahashi and Ji (2006) observed a greater increase in free [Ca^{2+}] of beef from 16 μM at 40min postmortem to a maximum of 210 μM at 4 days postmortem. The relatively low free [Ca^{2+}] found in the CCP was still theoretically adequate to activate the calpain system, 30 – 50 μM (Dargelos et al., 2008), and yet its activity could have been limiting (Geeksink et al., 2001). The low free [Ca^{2+}] found for this CCP could explain its high WBS value (9.15 kg) when compared to the 210μM found by Takahashi and Ji (2006). This meat is considered extremely tough, given its excess of 4.55 kg in WBS, compared to the level required for consumer acceptance with regards to tenderness (4.6kg WBS) (Steinberg et al., 2009). The color and WBS found in CCP could diminish consumer acceptability of Puerto Rican beef and explain the common preferences for imported meat over local commercial meat. The mean of WBS in the UPRM samples was 4.60 kg (*Table 2.1.2*) which is 4.55 kg lower than the WBS of CCP. The CCP has greater *Bos taurus indicus* influence while the UPRCP are Senepol x Charolais crossbreds with more *Bos taurus taurus* influence. This could explain the difference in meat tenderness between the two populations; *Bos taurus indicus* cattle tend to have lower calpain activity and higher leves of calpastatin than *Bos taurus taurus* animals (Geeksink et al., 2001; Kerry et al., 2002; Koohmaraie and Geeksink, 2006; Frylinck et al., 2009). Also Kemp et al. (2010) reported a down regulated gene, DNAJA1 which encodes for a heat shock protein

(Hsp40), in Charolais bulls. This down regulation of Hsp40 reduces anti-apoptotic activity in muscles and thus could increase tenderness by facilitating cell death postmortem. Overall the UPRCP showed acceptable tenderness based on WBS. Animals with 50% or more Senepol influence showed a significant decrease in WBS (-2.10 kg P = 0.02), compared to animals with 50% or more Charolais influence (F*igure 2.2*). The high *Bos taurus indicus* influence present in the CCP greatly affected the overall tenderness of their meat.

Significant correlations between the different variables investigated and their p-values are found in *Table 2.3*. Negative correlations were found between ultimate pH and WBS (r = - 0.74, P = <0.01) and ultimate pH and free [Ca^{2+}] at 24h (r = - 0.63, P = <0.01) and at 14d (r = - 0.55, P = <0.01). Thus as ultimate pH increased, WBS and free [Ca^{2+}] decreased. High ultimate pH values usually coincide with high pH values (Duarte et al., 2011), and yet this was not so in the present CCP data. Silva et al. (1999) also found a significant decrease (P < 0.05) in WBS with higher pH values in meat from 23 bulls between the ages of 8 to 11 months. As WBS increased free [Ca^{2+}] also increased (r = 0.52, P = <0.01) at 24h and (r = 0.39, P < 0.01) 14d postmortem. This finding was not consistent with the report of Geesink et al. (2001) of negative correlations between free [Ca^{2+}] and WBS (P < 0.10) in lamb *longissimus.* Free [Ca^{2+}] is known to increase with postmortem aging in order to activate the calpain system which improves tenderness of meat.

Negative correlations were found between ultimate pH at 24h and L* values at 24h (r = - 0.26, P = <0.01) and at 14d (r = - 0.55, P = <0.01) and ultimate pH at 14d and L* values at 24h (r = - 0.32, P = <0.01) and 14d (r = - 60, P = <0.01). The same was observed between ultimate pH at 14d and b* values with 14d of aging (r = -0.24, P = 0.01) in the CCP meat. With high ultimate pH a decrease in L* values was observed. L* values indicate the lightness/darkness of meat, lower values being attributed to darker meat and higher ones to lighter meat. Muscle tissue color in relation to its lightness or darkness depends on how much free water and

oxygenated myoglobin present (Page et. al., 2001). In meat classified as “dark cutter” the high pH value makes the protein increase its water holding capability. If lactic acid accumulation is insufficient a higher percent of negative charges will be present in the meat (Duarte et. al., 2011). As a result the muscle fibers will attract more water molecules tightly, thus reducing the free water present. The decrease in free water and the swollen myofibers produces less refection of light. The surface of the meat with this condition is seen as dark and dry.

2.4.2. Differences among sex and permanent teeth categories (PTC)

A significant difference was found in WBS between sexes in the CCP ($P < 0.01$). Females showed lower WBS than males by a -3.14kg margin (F*igure 2.2).* The CCP were also genotyped for SNPs found in the CAPN1 gene and a greater segregation of the tender alleles was found in females compared to males. The CAPN1 gene codes for μ-calpain and are not sex oriented. This segregation of tenderness promoting alleles in females explains the differences between sexes observed. These results were also reported by Lundesjö et al. (2012) in Swedish red cattle, where 24 and 34 month old bulls showed higher WBS, 5.67kg, than heifers and cows, 4.11 kg ($P = 0.02$). These differences were lost when a pelvic suspension was used during rigor mortus.

Significant differences were found between the PTC in WBS ($P = 0.01$) (F*igure 2..3)*. Unexpectedly the C category (older animals) showed lower WBS, - 3.34 ± 1.30 kg, then the A and B categories. These results differ from those reported by Duarte et al., (2011) in a study with pasture-fed Nellore bulls, in which younger cattle with 2 – 4 permanent incisors presented acceptable tenderness (4.6 kg WBS) compared to older animals with 6 or 8 permanent incisors (7.35 and 7.59 kg respectively). Xiong et al. (2007) found in crossbred Angus x Simmental cattle a similar WBS (4.6 kg) in animals from 2 to 8 years of age compared to 10 to 12 year old cattle (5.4 kg WBS). The cattle studied by those authors lacked *Bos taurus indicus* influence.

As cattle mature an increase in connective tissue and decrease in collagen solubility take place due to the cross linking of individual collagen molecules (Lefaucheur, 2010). This change greatly affects the overall tenderness of mature cattle but was not observed in the older CCP of the present study. The difference observed in the older cattle could be due to the background toughness, which is the resistance to shearing of the muscle prior to rigor mortus (Koohmaraie et al., 2006). The difference in background toughness is due to the amount of connective tissue as described by Koohmaraie et al. (2006). An altered calcium homeostasis, because of ROS accumulation, can produce apoptosis and muscle wasting in older animals and thus the background toughness does not influence tenderness to the same extent (Dargelos et al., 2007; Brulé et al., 2010; Andersson et al 2011). Apoptosis eliminates damaged or dangerous cells without affecting surrounding cells in living organisms and has been described in postmortem muscle cells and during meat aging (Herrera-Mendez et al., 2006; Luciano et al., 2007). The extent of apoptosis postmortem can increase tenderness as well the occurrence of apoptosis during the life of the animal.

2.4.3 Free Calcium Concentrations

Figure 2.4.1 presents the change observed in free [Ca^{2+}] during the 14d aging of beef samples from the three PTC. No significant difference was observed among the CCP although a numerical trend towards smaller changes was observed in the older animals ($P = 0.26$). The mean increase in [Ca^{2+}] during 14d of aging was 10.14 μM ± 21.10. A significant difference between PTC by sex was observed in free [Ca^{2+}] ($P < 0.01$) at both 24h and 14d of aging (*figure2.4.2)*. The youngest males of in the A PTC (0 – 2 permanent teeth category) showed a significant increase in free [Ca^{2+}] at both 24h and 14d post mortem compared to the female A PTC and the male C PTC (8 permanent teeth category) also at both 24h and 14d post mortem. An increase in [Ca2+] of 12 μM and 22 μM at 24h and 14d postmortem respectively, compared to the mean of the population, was observed in the A PTC. While the oldest males of C PTC

showed significantly lower free [Ca^{2+}] at both 24h and 14d post mortem compared to the male A and B PTC. A decrease of - 15 μM at 24h and - 25 μM at 14d post mortem, compared to the mean of the population, was observed in the male C PTC. In all PTC there was a tendency of increase free calcium concentration during the aging period, except for the males of category C that showed a decrease of – 9.66 μM compared to the mean. During the conversion of muscle to meat, calcium is known to leak from the sarcoplasmic reticulum and activate the calpain system (Silva et al., 1999; Bartoli and Richard, 2005). Takahashi and Ji (2006) reported 240 μM of free [Ca^{2+}] at 5 days postmortem in beef. The low free [Ca^{2+}] found in the CCP meat could explain the high WBS mean and poor tenderness rating. Although the free [Ca^{2+}] found should have been enough to activate the calpain system, as concentrations of 30-50μM have been reported to produce autolysis of μ-calpain (Bartoli and Richard, 2005; Dargelos et al., 2008). WBS was not determined in the 14d aged samples so elucidation of the relationship between the change in free [Ca^{2+}] and tenderness is not attempted in this study and the WBS values in the C PTC are all from males. Increase tenderness and the decrease in free [Ca^{2+}] found in the males of the C PTC suggest greater myofibrillar fragmentation than in the other PTC in the absence of increased calcium influx during the aging period. To the contrary an increase in tenderness is expected with increased calcium concentration and meat from younger animals should be tenderer then that of older animals. In this study the reverse was observed. Meat from the younger animals with increase free [Ca^{2+}] did not show an increase in tenderness. This unexpected result could be due to a higher proteolytic activity of the calpain system prior to slaughter rather than during the rigor phase. The beef production system of Puerto Rico based on grazing is similar to current practice in Brazil (Duarte et al., 2011). In Puerto Rico there has been little adaptation of intensive production technologies, rather the cattle are raised on native grass of variable quality and quantity throughout the year due to fluctuating environmental conditions. It is not uncommon to have periods of involuntary fasting in our commercial cattle. During fasting cellular homeostasis is compromised producing the activation of different cellular

pathways that lead to apoptosis. Calcium is the trigger for apoptotic pathways and also includes the activation of the calpain system (Herrera-Mendez et al., 2006; Luciano et al., 2007), during muscle wasting in order to degrade the myofibers and recycle the amino acids for other physiological processes (Bartoli and Richard, 2005). Andersson et al. (2011) reported disorganization of structural proteins of the Z-Disk consistent with their use as substrates of μ-calpain during experimental wasting conditions. Also accumulation of reactive oxygen species have been reported in older animals, the resulting oxidation can impair the rynodine receptor (RYR1) producing increased liberation of calcium from the sarcoplasmic reticulum (Andersson et al., 2011). This change in calcium homeostasis activates the calpain system and other physiological pathways that lead to apoptosis and muscle wasting, which could explain why there was little calcium release during the aging period in the animals belonging to the C PTC. Beef cow longevity is usually in the range of 8 – 11 years in terms of adequate reproductive performance for cow-calf operations and in crossbred cows this age limit may increase (Parish, 2010). In a study of pasture-fed Hereford cows from Argentina between the ages of 3 to 12 years showed no significant difference in WBS among the age groups but an increase in meat tenderness was observed in the 12-year old cattle (Galli et al., 2008). The mean of WBS reported in this case was 5.26 kg, substantially lower than the 9.15 kg found in the CCP of the present study. This difference can be ascribed to the lower level of production technology and management of the beef cattle found in Puerto Rico.

2.4.4. Color Values

Significant differences were found among means of permanent teeth categories (PTC: A = 0 -2, B = 4 – 6 and C = 8) within each sex in color values of meat at 14 days of aging. Differences among PTC within sex in the L* ($P < 0.01$), a* ($P < 0.01$) and b* ($P = 0.01$) values are seen in T*able 2.4*. The L* values indicate the lightness or darkness of the red color of meat. The highest L* value was observed in the male A PTC (35.76) and differed significantly from the

female B and C PTC. The lowest L* value was observed in the male C PTC (29.06) and differed significantly from the other age categories in males and the A PTC in females. The low value differs appreciably but the high value only slightly from the results of Duarte et al. (2011) in pasture-fed Nellore bulls of four PTC with means of 37.64, 37.63, 37.25 and 38.98 corresponding to 2, 4, 6 and 8 permanent incisors, respectively. In the present study with CCP the L* values of the males tended to decreased with age where Duarte et al. (2011) found them to slightly increased with age. Long term and short term stressors can produce changes in meat color. When short term stressors decreases pH values to rapidly, water holding capability is lost and thus greater light is reflected producing a pale red color characteristic of Pale Soft and Exudative meat. The contrary occurs when long term stressors fail to decrease the pH value and increases water holding capability, thus reflecting less light and producing a darker color of meat characteristic of Dark Firm and Dry meat (Adzitey and Nurul., 2011). The a* values determines the degree of the color red. High values indicate more red and low values more green. Among this value a significant difference was observed only between the male A PTC (16.46) and the male B PTC (14.42) ($P < 0.01$). This a* value exceeded that found by Duarte et al., (2011), 11.04, in Nellore bulls with 2 PT but was lower than that reported by Page et al., (2001), 25.17, in Brahman cattle. Differences in myoglobin content and oxidation can explain the variation in a* values among cattle populations (Kerry et al., 2002; Maddock et al., 2006; Duarte et al., 2011). An increase in myoglobin content intensifies the meat color and is influenced by muscle type, breed and animal age. Thus increase muscle use and age require greater myoglobin content for effective oxygen delivery and storage (Kerry et al., 2002). The b* values which indicate yellowness of the sample and indirectly meat marbling. The male A PTC showed an increase in a* value, significantly different from the other age categories in both male and female groups, excluding the female C PTC. Duarte et al. (2011) reported a mean of 7.38 in a* value which increased with age in pasture fed Nellore Bulls. The b* values can also reflect increased carotene concentration in adipose tissue, which is common in pasture-fed

systems (Jaturasitha et al., 2009; Steinberg et al., 2009; Duarte et al., 20011). Galli et al. (2008) measured the color of fat tissue of pasture-fed Hereford cattle from Argentina and reported increased yellowness with age class from 2 to 12 years.

2.4.5. Ultimate pH values and free calcium concentration categories

In Figure 2.6 significant differences in ultimate pH values between sexes among the PTC are shown ($P < 0.01$). The males of the C PTC showed a significantly higher ultimate pH, pH 6.10 ($P < 0.01$) compared to the males in the other PTC and to the females in the C PTC. This further explains the low WBS and free [Ca2+] values found in this PTC. With increasing chronological age an increase in muscle oxidation can decrease the muscle ATP content, and thus contribute to a high ultimate pH as not enough energy is available to accumulate lactic acid and reduce the pH value (Xiong et al. 2007; Dargelos et al., 2008; Brulé et al., 2010). The same is observed with long term stressors but stressors are known to increase meat toughness (Duarte et al., 2011) and that was not observed in the C PTC of the CCP studied. To further examine the effects of ultimate pH, four pH categories were established, T*able 2.2 and 2.5*. Significant differences were observed in the color values of meat at 14d of aging among the different pH categories. The dark cutter category ($pH > 5.9$) showed significantly lower L* values compared to the other pH categories ($P < 0.01$). The Low pH category had the lowest a* value (13.31) compared to the other pH categories ($P = 0.01$). With lower a* values a lighter red color is observed in the meat, this is characteristic of Pale Soft and Exudative meat. The Normal pH category showed the highest b* value (13.30), but differed significantly only from the dark cutter pH category ($P = 0.02$). Significant differences were also observed in WBS among the categories of ultimate pH ($P = <0.01$). As pH values increased a tendency toward greater tenderness was observed. A significant difference in WBS was observed in the dark cutter pH category compared to the other pH categories (5.29 kg, $P < 0.01$). Hopkins and Thompson (2001) found evidence to support greater protein degradation due to μ-calpain activity before

the pH falls below 6.2. If ultimate pH stays high maybe μ-calpain activity is favored. The free [Ca^{2+}] of Dark Cutter meat was significantly lower than that of the other pH categories, 32.27 and 39.50 μM at 24h and 14d of aging respectively ($P < 0.01$). The difference in free [Ca^{2+}] release during the 14d aging period was significantly higher in the Normal pH category, 21.87 μM ($P = 0.01$). This increase in free [Ca^{2+}] did not improve tenderness. This could be attributed to a decrease in calpain activity due to higher calpastatin to calpain ratio, which have been reported in *Bos taurus indicus* cattle (Frylinck et al., 2009). The proportional frequency of meat in each pH category is seen in *figure 2.8*. Sixty percent of the population showed pH values above 5.6. This is detrimental to overall meat quality due to the decrease shelf life of high ultimate pH; increases microbial contamination. A re-evaluation of handling procedures prior to slaughter could help decrease the incidence of high ultimate pH in commercial cattle. Thus, not only do commercial cattle from Puerto Rico produce predominantly meat of high toughness but with high ultimate high pH values. Long term stressors can affect the ultimate pH values during the handling of the animals prior to slaughter especially in pasture-fed cattle (Viljoen et al., 2002; Adzitey and Nurul, 2011). Also a high level of *Bos taurus indicus* influence contributes to higher pH values in the beef produced (Duarte et al., 2011). Burdick et al. (2011) found that loading and unloading of *Bos taurus indicus* cattle is more stressful than transportation. Increased tenderness in conjunction with low [Ca^{2+}] and high pH values could be indicative of muscle wasting during the life of the animal. A negative change in free [Ca^{2+}] was observed during the 14d aging period, so all the free [Ca^{2+}] in the muscle was released before the [Ca^{2+}] was determined 24h postmortem. To determine the effect of PTC on calcium change during the 14d aging period three free [Ca^{2+}] change categories (FCC) were establishes, T*able 2.2*, and the percentage frequencies of PTC distribution therein are seen in *Figure 2.8*. A total of 48% of the animals with meat of negative free [Ca^{2+}] change were in the C PTC. Animals that produce meat with less than 10.14μM free [Ca^{2+}] increase were found mostly (45%) in the B PTC and those with higher than 10.14μM free [Ca^{2+}] release belonged mostly to the A PTC. *Figure 2.9*

shows the overall frequency of animals in each free [Ca^{2+}] change category. A total of 52% of the population had a free [Ca^{2+}] increase higher than 10.14 µM. The older animals with negative free [Ca^{2+}] changes during the 14d aging period greatly affect the overall mean of free [Ca^{2+}] change and yet this is not consistent with the tenderness results. The pH value and PTC proved to be better predictors of tenderness than free [Ca^{2+}].

The sarcopenic phenotype involves effects of the environment (Burks and Cohn et al., 2011), nutritional protein deficiencies increases energy requirements in order to degrade muscle fibers (Jaturasitha et al., 2009), and direct effect of genetic mutations, product of ROS accumulation (Dargelos et al., 2008; Brulé et al., 2010) increasing mtDNA mutations and altering cellular functions including OXPHOS (Wei and Lee, 2002; Thore et al., 2008) and calcium homeostasis (Andersson et al., 2011). Pasture-fed cattle grazing tropical low nutritional pastures will compensate quality for quantity and increase its activity and oxygen requirement which accumulates ROS by accelerating OXPHOS. Over-production of ROS affects membrane permeability in mitochondria releasing apoptogenic factors leading cells to apoptosis and thus muscle wasting (Wei and Lee, 2002; Dargelos et al., 2008; Thore et al., 2008; Brulé et al., 2010; Andersson et al., 2011).

2.5. Conclusion

Commercial pasture fed cattle of Puerto Rico produce tough meat with high ultimate pH values. This can be expected to directly affect consumer appreciation and acceptance of retail meat and explains why imported meat is preferred over local meat. However acceptable meat tenderness can be achieved with animal grazing native Puerto Rican pasture as seen in the UPR cattle population. The handling procedures prior to slaughter should be reevaluated and improved in order to reduce the ultimate pH values and thus improve meat quality. Increase tenderness was observed in the older animal category and yet a decrease in meat quality was

also observed. These animals showed high ultimate pH values, low color L* values and low free calcium concentrations. Increased muscle wasting and a sarcopenic phenotype is purposed to explain these unusual findings in older cattle. Further research with older cattle of Puerto Rico could confirm or refute this hypothesis. The present study review of meat quality in commercial cattle of Puerto Rico has the objective of helping to establish a classification system of local commercial beef. Before such a system can be developed and implemented improvement of cattle management at both production (farm) and processing (abattoir) levels should be undertaken.

2.6. References

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Variable	N	Mean	Std Dev	Minimum	Maximum
Hot Carcass Weight kg	102	498.87	144.71	247.00	944.00
pH 24h	102	5.75	0.39	5.20	6.87
pH 14d	102	5.75	0.40	5.21	6.88
L* 24h	102	31.42	4.45	22.61	57.00
a* 24h	102	15.74	2.58	5.59	20.94
b* 24h	102	11.89	2.08	7.09	17.14
L* 14d	102	33.58	4.20	20.66	41.79
a* 14d	102	15.28	2.63	10.37	21.62
b* 14d	102	12.66	1.98	6.80	17.36
WBS kg 24h	60	9.15	3.41	2.32	15.85
[Ca^{2+}] µM 24h	102	53.97	19.79	7.00	111.33
[Ca^{2+}] µM 14d	102	64.11	26.91	6.43	134.23
Dif [Ca^{2+}] µM	102	10.14	21.10	-42.95	79.34

Table 2.1.1. Mean and standard deviation of various meat quality measures for 102 cattle of various ages from the commercial cattle population.

Variable	N	Mean	Std Dev	Minimum	Maximum
MFI	22	40.54	17.23	17.45	84.95
WBS	22	4.60	2.19	1.80	10.52

Table 2.1.2. Mean and standard deviation of myofibrillar fragmentation and Warner Bratzler Shear value for UPR cattle population.

Variable		Frequency
Sex		**N = 102**
F		52
M		50
Permanent Teeth		
0		20
2		18
4		19
6		15
8		30
Permanent Teeth Categories (PTC)		
A	0 - 2	38
B	2 - 6	34
C	8	30
pH Categories (pHC)		
Low	< 5.4	16
Normal	5.4 - 5.6	25
High	5.6 - 5.9	36
Dark Cutter	> 5.9	25
Free [Ca^{2+}] Change Categories (FCC)		
Negative	< 0.00	29
< μ	0.00 - 10.14	20
> μ	< 10.14	53

Table 2.2. Frequency among categories of sex, permanent teeth, ultimate pH and free [Ca^{2+}] change during 14 days of aging

	Hot Carcass Weight	pH 24h	pH 14d	L* 24h	a* 24h	b* 24h	L* 14d	a* 14d	b* 14d	WBS 24h	Ca 24h
pH 24h	-0.16										
	0.10										
pH 14d	**-0.22**	**0.93**									
	0.02	**<0.01**									
L* 24h	0.10	**-0.26**	**-0.32**								
	0.30	**<0.01**	**<0.01**								
a* 24h	-0.11	0.09	0.04	-0.19							
	0.27	0.34	0.72	0.06							
b* 24h	-0.04	-0.03	-0.10	**0.41**	**0.73**						
	0.72	0.77	0.33	**<0.01**	**<0.01**						
L* 14d	0.04	**-0.55**	**-0.60**	**0.55**	-0.06	**0.28**					
	0.67	**<0.01**	**<0.01**	**<0.01**	0.55	**<0.01**					
a*14d	-0.05	0.16	0.11	**0.20**	**0.27**	**0.30**	-0.05				
	0.59	0.12	0.25	**0.04**	**<0.01**	**<0.01**	0.63				
b* 14d	-0.04	-0.18	**-0.24**	**0.48**	**0.29**	**0.56**	**0.52**	**0.67**			
	0.70	0.07	**0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**			
WBS 24h	-0.16	**-0.74**	**-0.66**	0.03	0.03	-0.01	**0.38**	-0.15	0.16		
	0.23	**<0.01**	**<0.01**	0.82	0.83	0.96	**<0.01**	0.26	0.21		
Ca 24h	0.05	**-0.63**	**-0.60**	0.13	0.03	0.08	**0.41**	-0.02	**0.23**	**0.52**	
	0.62	**<0.01**	**<0.01**	0.18	0.73	0.43	**<0.01**	0.84	**0.02**	**<0.01**	
Ca 14d	-0.01	**-0.49**	**-0.55**	0.15	0.16	**0.22**	**0.43**	0.02	**0.33**	**0.39**	**0.63**
	0.99	**<0.01**	**<0.01**	0.12	0.10	**0.03**	**<0.01**	0.82	**<0.01**	**<0.01**	**<0.01**

Table 2.3 Pearson correlation table, the top number represents the correlation coefficient and the bottom number the probability.

PTC	L*	a*	b*
Females			
A	33.41 ± 0.83 [abc]	14.89 ± 0.59	12.05 ± 1.10 [b]
B	30.57 ± 0.94 [cd]	15.96 ± 0.67	12.11 ± 0.76 [b]
C	31.47 ± 0.79 [bcd]	15.71 ± 0.56	12.13 ± 0.65 [ab]
Males			
A	3.76 ± 0.79 [a]	16.46 ± 0.56 [a]	13.71 ± 0.76 [a]
B	33.42 ± 0.79 [ab]	14.42 ± 0.56	1.55 ± 0.58 [b]
C	29.06 ± 1.12 [d]	15.90 ± 0.79 [b]	11.85 ± 0.93 [b]

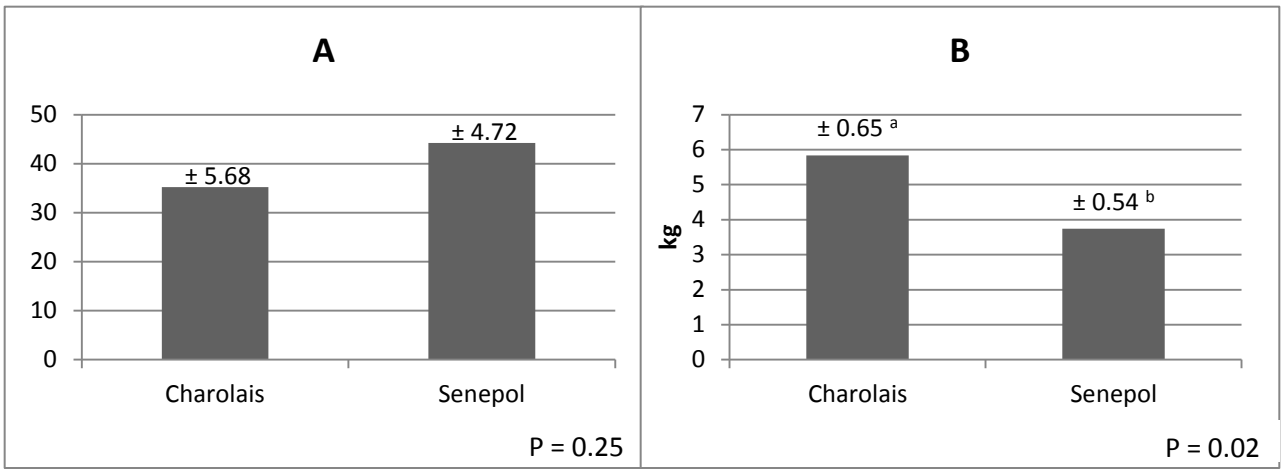
[abc] Means within a column lacking common superscript letter are significantly different (P ≤ 0.01)

Table 2.4. Significant Differences in mean color values for interactions among sex and permanent teeth categories (A = 0 – 2, B = 0 – 6 and C = 8 PTC) at 14 days postmortem.

	Category Ultimate pH				
Variables	**Low**	**Normal**	**High**	**Dark Cutter**	**p value**
Hot Carcass Weight	521.85 ± 29.34	501.36 ± 23.46	512.98 ± 19.63	486.33 ± 23.61	0.77
Color Values 24h					
L*	32.72 ± 1.06	31.29 ± 0.85	31.95 ± 0.71	29.57 ± 0.85	0.09
a*	15.03 ± 0.65	15.06 ± 0.52	16.65 ± 0.43	15.77 ± 0.52	0.07
b*	11.58 ± 0.51	11.37 ± 0.41	12.59 ± 0.34	11.55 ± 0.41	0.08
Color Values 14d					
L*	34.43 ± 0.85 [a]	35.30 ± 0.69 [a]	33.85 ± 0.58 [a]	30.40 ± 0.70 [b]	**<0.01**
a*	13.31 ± 0.63 [b]	15.68 ± 0.51 [a]	15.83 ± 0.43 [a]	15.46 ± 0.52 [a]	**0.01**
b*	11.96 ± 0.46 [ab]	13.30 ± 0.37 [a]	12.99 ± 0.32 [ab]	11.91 ± 0.38 [b]	**0.02**
WBS kg 24h	11.99 ± 1.03 [a]	11.50 ± 0.89 [a]	9.48 ± 0.67 [a]	5.29 ± 0.64 [b]	**<0.01**
[Ca^{2+}] uM 24h	62.55 ± 3.89 [a]	66.24 ± 3.11 [a]	56.44 ± 2.60 [a]	32.27 ± 3.13 [b]	**<0.01**
[Ca^{2+}] uM 14d	72.09 ± 5.51 [a]	80.75 ± 4.46 [a]	64.68 ± 3.77 [a]	39.50 ± 4.53 [b]	**<0.01**
Diff [Ca^{2+}] uM	6.59 ± 5.05 [a]	21.87 ± 4.09 [b]	6.34 ± 3.46 [a]	5.02 ± 4.15 [a]	**0.01**

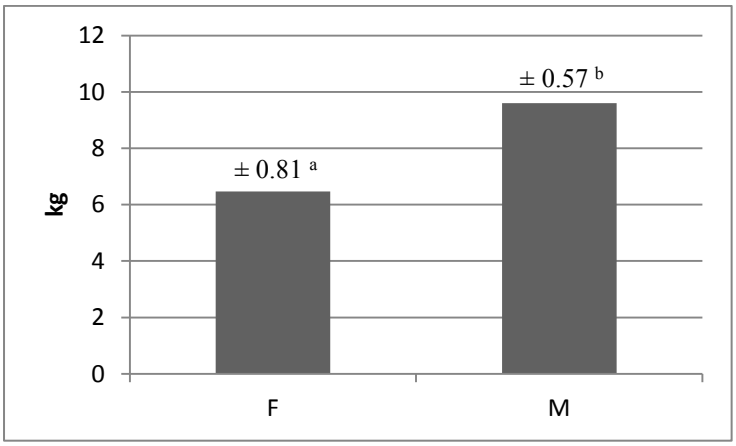
[abc] Means within a row lacking common superscript letter differ significantly

Table 2.5. Effect of ultimate pH categories on means of variables under study



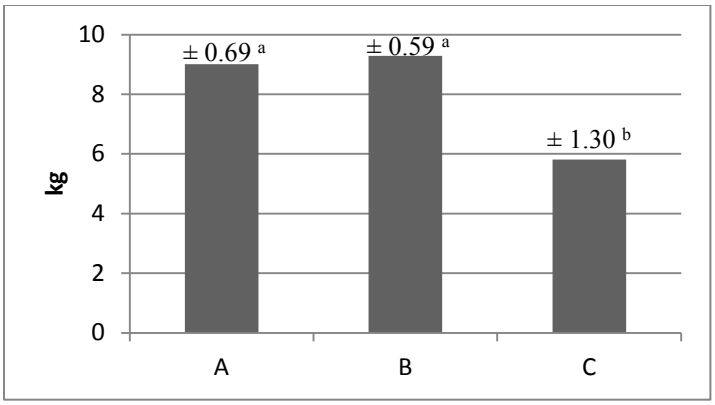
[abc] Means lacking common superscript letter differ significantly (P < 0.05)

Figure 2.1. Means of MFI (A) and WBS kg (B) values by breed in UPR cattle population



[abc] Means lacking common superscript letter differ significantly (P < 0.01)

Figure 2.2. Means of Warner Bratzler Shear values for each sex 24h postmortem.



[abc] Means lacking common superscript letter differ significantly (P = 0.01)

Figure 2.3. Mean WBS 24h postmortem among age categories (A = 0-2, B = 4-6 and C = 8) determined by permanent incisors postmortem.

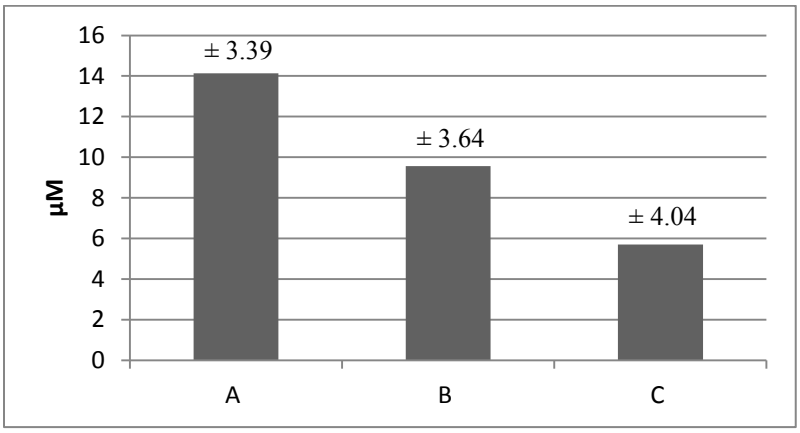
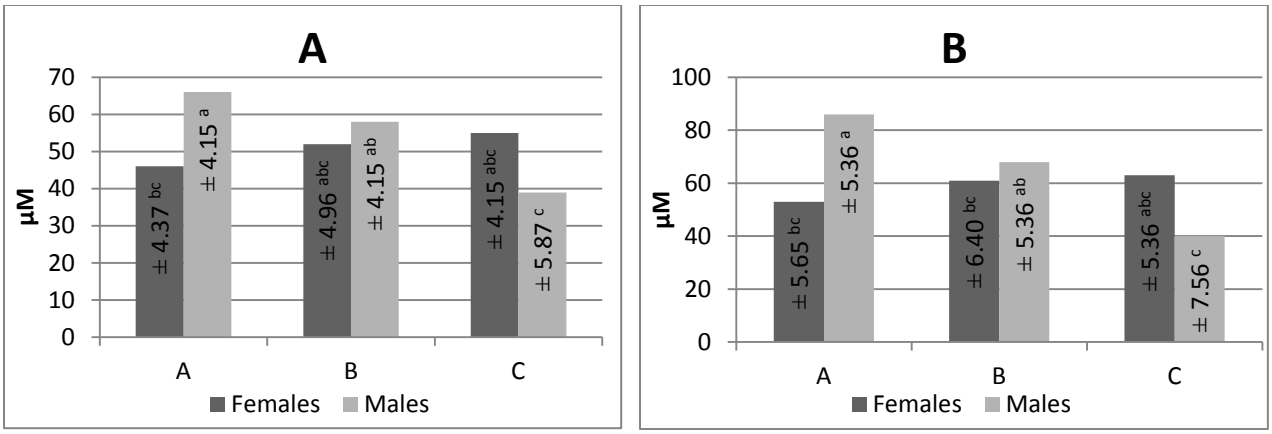
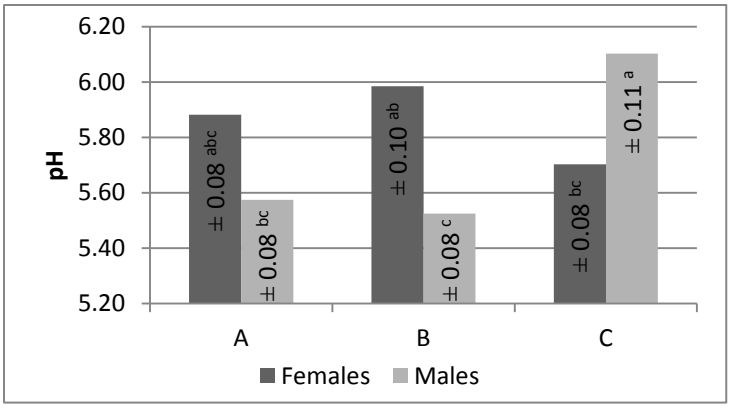


Figure 2.4.1. Changes in free [Ca^{2+}] during 14d of aging in different permanent teeth categories (A = 0 – 2, B= 2 – 6 and C = 8), P = 0.26.



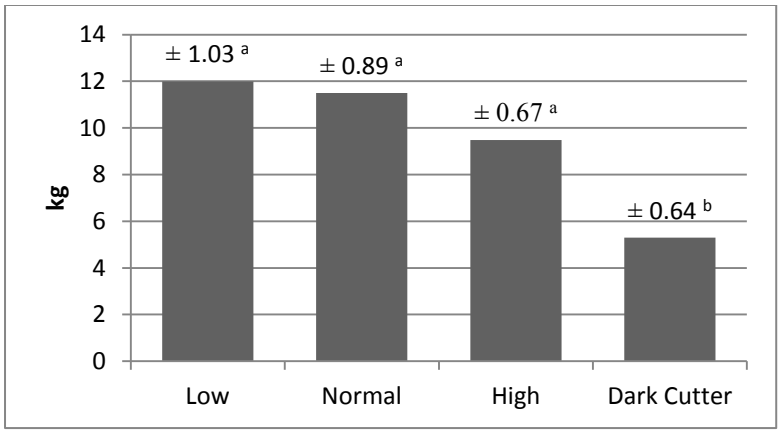
[abc] Means lacking common superscript letter differ significantly (P < 0.01)

Figure 2.4.2. Means of free [Ca^{2+}] for 24h (A) and 14d (B) of aging for interactions among sex and permanent teeth category (A = 0-2, B = 4-6 and C = 8).



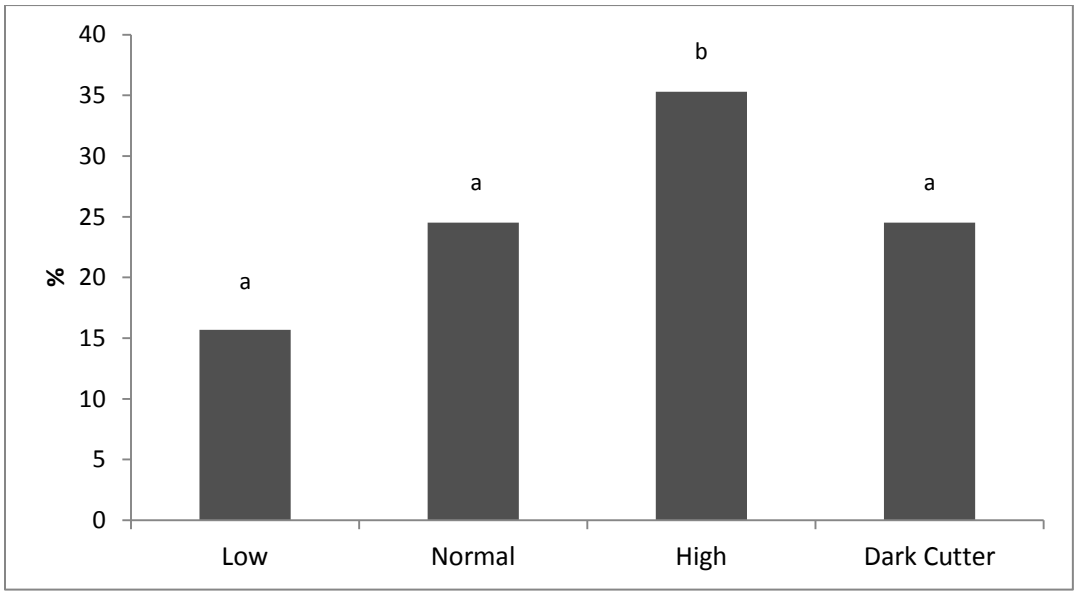
[abc] Means lacking common superscript letter differ significantly (P < 0.01)

Figure 2.5. Mean of ultimate pH for interaction among sex and permanent teeth categories (A = 0-2, B = 4-6 and C = 8).



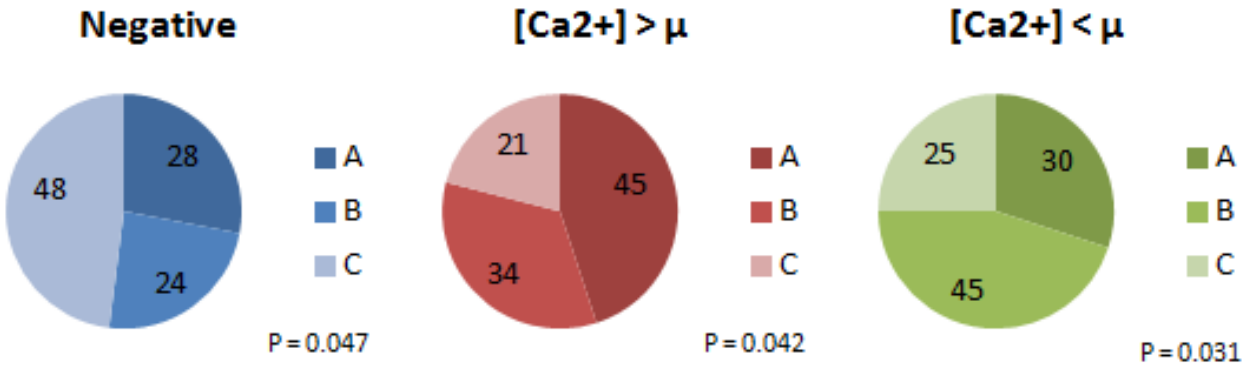
[abc] Means lacking common superscript letter differ significantly (P < 0.01)

Figure 2.6. Mean of WBS at 24h postmortem among ultimate pH categories (Low < 5.4, Normal = 5.4 – 5.6, High = 5.6 – 5.9 and Dark Cutter > 5.9).



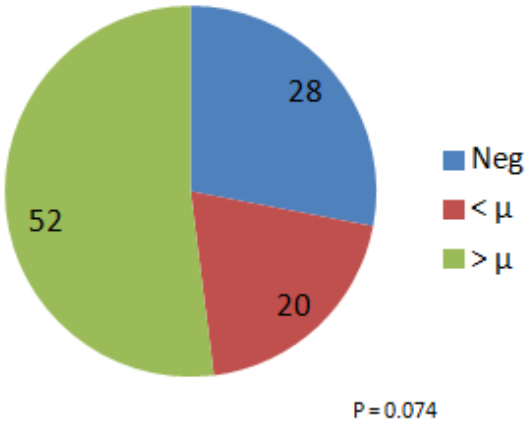
[abc] Means lacking common superscript letter differ significantly (P < 0.01)
p values are from X^2

Figure 2.7. Frequency distribution of ultimate pH categories (Low < 5.4, Normal = 5.4 – 5.6, High = 5.6 – 5.9 and Dark Cutter > 5.9) in commercial cattle population.



p values are from X^2

Figure 2.8. Percent distribution of three age categories within each category of change in [Ca^2+] with 14d of aging.



p values are from X^2

Figure 2.9. Percent distribution sample population among three categories of free [Ca^2+] change with 14d of aging.

Chapter III. Frequencies of Polymorphisms found in the CAPN1 and CAST candidate genes in Commercial Cattle and UPR Cattle Population

3.1 Abstract

The CAPN1 and CAST genes have proven to be highly polymorphic in cattle and have received much attention due to their high correlation with meat tenderness. Commercial application of several polymorphisms including their identification and validation has occurred in cattle of both *Bos taurus taurus* and *Bos taurus indicus* influence. In this study a total of 102 animals of the commercial *Bos taurus indicus* crossbred cattle population (CCP) and 22 Senepol x Charolais crossbred cattle (UPRCP) from the University of Puerto Rico herd were genotyped for 26 polymorphisms in the CAPN1 and CAST genes. Of the 26 polymorphisms 15 had been previously discovered and 11 were newly identified. Low frequencies of the genotype associated with tender meat were observed (< 0.05 %) in the CCP with regards to the commercially available SNP markers 530, 316, 4753 and 5331 in the CAPN1 gene. Only marker 4751 segregated the tenderness genotype AA in higher frequency (26%). The UPRCP segregated higher frequencies of the tenderness genotype, 18% in markers 530 and 4751 and 36% in the marker 316 found in the CAPN1 gene. The higher frequency of the marker 4751 in the CCP showed that this marker segregated in higher proportion in Bos indicus cattle than in Bos taurus, while the opposite was observed with marker 316 from the CAPN1 gene. Among the newly identified SNPs, markers 573, 643 (Aviés et al., 2009) and 652 segregated the tenderness genotype at higher frequencies in the UPRCP (>35 %). Low segregation of the tenderness genotypes of the newly identified polymorphism in the CAPN1 gene was observed in the CCP. Among the polymorphisms studied in the CAST gene, higher frequencies of the tenderness genotype were observed (> 45%) for markers 282, 2959 and 3016 found in the CAST gene. The marker CAST 3016 did not segregate the tenderness genotype in the UPRCP; segregation occurred only in *Bos taurus indicus* cattle. Marker assisted selection for

increased meat tenderness can be done in applied to cattle with Bos indicus influence using markers in the CAST gene or the marker 4751 in the CAPN1 gene.

3.2 Introduction

Establishing the genetic basis of variation in meat tenderness would add to the selection criteria for improving this trait in cattle (Page et al., 2002). Tenderness determined by Warner Bratzler Shear Force (WBS) has an estimated heritability of 0.26 with a range of 0.09 to 0.71 (Splan et al., 1998). The calcium-activated neutral protease (CAPN1) gene encodes for μ-calpain and consists of 22 exons spanning 30 kb (White et al., 2005). This gene mapped to the chromosome BTA29 telomeric end which coincides with the QTL for tenderness (Costello et al., 2007). Five single-nucleotide polymorphisms (SNP) have been reported by White et al. (2005) and are known as markers: (1) 316 (base 5709 accession # AF252504; Page 2002); (2) 530 (base 6545 accession # AF252504; Page 2002); (3) 4751 (base 6545 accession # AF248054; White 2005); (4) 4753 (base 8676 accession number AF248054; Casas et al, 2005); and (5) 5331 (base 327 accession number AF252504; Casas et al, 2005). The calpastatin (CAST) locus also contains multiple SNPs, two of which are located in a non-transcriptional part of the gene, CAST 2959, and CAST 3016, (Accession Number AF159246; Barendse, 2002). This gene has been mapped to a QTL for shear force in chromosome BTA7 (Reardon et al., 2010). The CAST SNP at base 2959 is used commercially in the GeneSTAR Tenderness Panel Test (Pfizer Animal Genetics; Casas et al., 2006). Another CAST SNP used in Ingenity Tender – GENE Test (Merial Limited, Duluth, GA) and is found at base 282 (accession number AY008276; Schenkel, 2006). Both tests described include two SNPs from the CAPN1 gene, labeled CAPN1-316 and CAPN1-4751. An additional, not reported SNP, found 33 bp downstream of CAST exon 2, seems to be correlated with meat tenderness (Dr. Melvin Pagan, personal communication). Even though multiple SNP have been identified on these candidate genes and reported in the scientific literature, special attention has been given only to the SNPs

validated by the USDA and The National Beef Cattle Evaluation Consortium, CAPN1 316 and 4751 and CAST 282 and 2959. Further evaluation of all the polymorphisms published for tenderness in the cattle population of Puerto Rico could shed light on the local meat quality. A thorough genetic evaluation of the published polymorphisms in the cattle of Puerto Rico would provide valuable information for cattle farmers. The objective of this study is to determine the genotypic background of commercially available tenderness markers found in the CAPN1 and CAST gene on cattle raised commercially in Puerto Rico. The CAPN1 and CAST genes are highly polymorphic and are associated with improved tenderness when the favorable allele is present in high frequency in a population. Determination of the commercial markers as well as novel polymorphisms could provide the necessary genotypic knowledge of tenderness to serve as a guide for improving this trait in commercial cattle of Puerto Rico.

3.3. Materials and Methods

3.3.1 Animal Selection

This aspect of the investigation was described in section 2.3.1 (page19).

3.3.2. DNA isolation and SNP Genotyping

Blood samples were obtained at slaughter during exsanguinations of the animals. DNA was extracted as described by Juszcuk-Kubiak et al. (2009). Polymerase chain reaction was performed using a thermocycler (Model No. 950040015, Eppendorf, Hauppauge, NY). The primer sequence used to amplify each SNP is presented in table 3.1. PCR conditions were as previously reported (Barendse et al, 2002; Page et al., 2002; White et al., 2005; Casas et al., 2006 and Pagan et al. unpublished). Sequencing was performed using the MacroGen Sequencing and Genotyping Facility at Seoul, Korea, for the purpose of determined the SNP profile for each candidate gene (CAST, n = 3 SNPs; CAPN1, n = 5 SNPs).

3.3.3. Statistical Analysis

The allelic and genotypic frequencies were determined and analyzed following the Hardy-Weinberg equilibrium (Rodriguez et al., 2009). The differences between allelic frequencies and sex (male and female) or populations (CCP and UPRM) were analyzed using contingency tables (Di Rienzo et al., 2011). The analysis of variance was performed using ANOVA Proc Mixed (SAS Inst., 2000) and the Tukey Kramer test was used for mean separation.

The linear model used in this review was:

$$Y_{ijkl} = \mu + S_i + A_j + G_k + e_{ijkl}$$

Y_{ijkl} = l observations pertaining sex i (male or female), age categories j based on number of permanent teeth (A = 0-2pt, B = 4-6pt, C = 8pt) and genotype k of either CAPN1 or CAST genes.

μ = overall mean

S_i = fixed effect of sex of the animal either male or female

A_j = fixed effect of age categories based on number of permanent teeth, A = 0-2pt, B = 4-6pt and C = 8pt

G_k = fixed effect of genotype of polymorphisms in either CAPN1 or CAST gene

e_{ijkl} = experimental error

3.4. Results and Discussion

3.4.1 Frequencies among commercially used molecular marker in the CAPN1 and CAST genes

A total of nine different polymorphisms associated with increase tenderness and located in the CAPN1 and CAST genes have been commercialized. The calpain system in particular μ-calpain has been extensively studied and directly correlated with increased degradation of the myofibrillar structural proteins. Calpastatin is the endogenous inhibitor of calpain and can directly influence tenderness by decreasing its concentration and activity. Five single nucleotide polymorphisms have been documented in the CAPN1 gene, markers 5331, 316, 530, 4751 and 4753. Four SNP are associated with tenderness in the CAST gene, three of which, markers 282, 2959, and 3016, are commercially used to select for increased tenderness. The higher frequencies of the tender genotype in the CAST gene among crossbred animals facilitates the use of these polymorphisms to promote tenderness (White et al., 2005; Casas et al., 2005; 2006; Curi et al., 2010;). The fourth SNP in the CAST gene is marker UPRM/MSU which is an INS/DEL of C in intron 2 of the gene. This SNP has been evaluated and associated with increase tenderness with the DEL of C, (Dr. Melvin Pagan/ UPRM). Further information of each SNP is given in *Table 3.2,* including location, fragment length evaluated and reference base number. Additional SNP were evaluated in the CCP: (1) 7 in the CAPN1-316 735 base fragment, SNPs 208 C/T, 430 C/G, 438 G/A, 573 C/T and 652 A/C (these SNPs were previously identified by Aviés et al., (2009) bases 80, 302, 310, 445 and 524, respectively, accession number EU386166 – EU386183) and two newly identified, SNP 212 T/G and SNP 643 INS/DEL C (*table 3.3)*; (2) 6 in CAPN1-530 568 base fragment, SNPs 175 C/T, 191 A/C, 209 G/A, 306 C/T (previously identified by Juszucuk-Kubiak et al., 2004), 351 G/A and an INS/DEL of CGAT at SNP 354 (*table 3.4*); and (3) 5 SNP in the CAST-2959 150 base fragment, SNP 75 INS/DEL G, 93 T/C, 94 T/C and 122 A/C (*table 3.5*). Of the seven polymorphisms near the marker

CAPN1 316, SNP 652 is a missence mutation found in exon 10, a transversion of A/C produces a change in the amino acid sequence of Ile to Leu. Two of the SNPs identified near the CAPN1 530 marker were found in exon 14. One is SNP 175 a transition of C/T, this SNP does not change the amino acid sequence (both alleles codify for aspartic acid). The other SNP 191 is a missence mutation and like SNP 652 is a transversion of A/C that changes the amino acid sequence from Ile to Leu. The genotypic sequence of the different genotypes observed in the commercial CAPN1 markers, 5331, 316, 530, 4751 and 4753, are shown in *Figures 3.1, 3.2, 3.10, 3.17 and 3.18,* respectively. Frequencies of commercial polymorphisms found in the CAPN1 gene of CCP, N=102, were compared to those of crossbred Senepol x Charolais animals, N = 22, raised at the Experimental Station of UPR," Finca Montaña", located in Aguadilla (*Table 3.6*). The tender allele for markers in the CAPN1 gene are A, C, C, A and T for CAPN1 530, 316, 4751, 4753 and 5331, respectively (White et al., 2005). All commercial markers in the CAPN1 gene, except CAPN1 4751, showed frequencies of < 0.05 % for the tender genotype in all CCP. Low frequencies of the tender genotype are found in animals with increase *Bos taurus indicus* influence (White et al., 2005; Casas et al., 2005; 2006; Smith et al., 2009; Curi et al., 2010). The tender genotype CC of marker CAPN1 4751 did segregate at a higher frequency (0.26) than the other CAPN1 markers in the CCP. Bonilla et al. (2010) also found this same frequency of 0.26 in the CC genotype of CAPN1 4751 marker in Mexican cattle as well as low frequency (0.03) of the CC genotype of CAPN1 316 marker. White et al. (2005); Van Eenennaam et al. (2007) and Smith et al. (2009) did not report segregation of the tender genotype of markers CAPN1 316 and 4751 in the Brahman population. White et al. (2005) and Bonilla et al. (2010) found marker CAPN1 4751 to be more useful in determining tenderness in a *Bos taurus indicus, Bos taurus taurus* and crossbreed descent population. A significant difference was observed between the genotypes of the CAPN1-4751 SNP in the CCP, which did not follow Hardy-Weinberg equilibrium due to the low frequency of the heterozygous genotype ($P < 0.01$).

Frequencies of the tender allele in all SNP analyzed in the CAPN1 and CAST genes of commercial and UPRM cattle are observed in *Figure 3.28*. The UPRCP had higher frequencies of the tender allele, by 16, 29 and 10 % margins in CAPN1 530, 316 and 4751, respectively. These increased frequencies are due to the Senepol influence. Low frequency of the tender genotype (0.05 %) of marker CAPN1 316 has been previously reported in Charolais cattle (Page et al., 2002; Van Eenennaam et al., 2007). On the other hand the UPRCP did not segregate the tender allele of CAPN1 markers 4753 and 5331 while the CCP segregated frequencies of 12 and 21 %, respectively.

The genotypic sequence of the different genotypes observed in the commercial CAST markers, 2959, 3016, 282 and UPRM, are shown *in Figures 3.19, 3.20, 3.25 and 3.27,* respectively. The tender allele for the different CAST SNP are C, A, A, and Del of C for markers 282, 2959, 3016 and UPRM, respectively (Barendse, 2002; Schenkel et al., 2006; Van Eenennaam et al., 2007). High frequencies of the tender allele were observed in the different CAST markers in both CCP and UPRCP cattle, except for the SNP CAST 3016 in the UPRCP cattle (*Table 3.9*). The T allele for CAST 3016 was fixed in the Senepol x Charolais crossbreeds, this polymorphism is known to produce increase tenderness with the A allele in animals with a high *Bos taurus indicus* influence (Frylinck et al., 2009). This could explain why it does not segregate in the UPRM cattle. Lower frequencies of the tender allele for markers CAST 282 and 2959 were observed by Van Eennennaam et al. (2007) in Brahman cattle (43 and 57) respectively. In the present study allelic frequencies of 69 and 88 % were observed for the tender allele in CAST 282 and 2959, respectively, in CCP and 93 % in marker CAST 2959 in UPRCP. The marker CAST 2959 was associated to WBS, $P < 0.01$, by Gene Star Tenderness (Johnston and Graser, 2010) in both temperate and tropical cattle breeds. The CAST 282 marker was found to be fixed in the heterozygous genotype in the UPRCP. The 50 % allelic

frequency of the tender C allele of CAST 282 was similar to that found by Van Eennennaam et al. (2007) in Charolais cattle (69 %) for the same marker.

3.4.2 Identified markers in the CAPN1 gene

The SNPs evaluated in the 568 base fragments of marker CAPN1 530 showed similar low frequencies of the tender genotype in both CCP and UPRCP. Except for SNP 306 (base 4685 accession number AF248054, Juszczuk-Kubiak et al., 2004) which show a frequency of 27% for the favorable TT genotype in the UPRCP. This SNP was previously identified by Juszczuk-Kubaik et al. (2004) in 141 bulls of seven breeds including Polish Red, Polish Black and White breeds with frequencies of 38% and 62% for the TT and CC genotypes respectively. In most SNP the tender homozygous genotype was absent in both populations, SNP 175, 209, and 351 in the CCP and 191 and 351 in the UPRCP (*Table 3.8*). Genotypic sequences of the SNPs are shown in *Figure 3.10*. A few SNPs did not follow Hardy-Weinberg equilibrium; SNPs 175 and 191 did not due to the high frequency of the heterozygous allele, $P < 0.01$ and $P < 0.01$, respectively, in the CCP; and SNP 209 due to the low frequency of the heterozygous allele, $P = 0.03$, in the UPRCP.

SNPs evaluated in the 735 base fragment of marker CAPN1-316 also showed low segregation of tender genotype in the CCP (*Table 3.6*). Genotypic sequences of the SNPs are shown in *Figure 3.2*. The UPRCP showed an increased in frequencies of the tender allele, 20, 18, 49, 15, 29 and 35 % increase compares to the CCP. SNP 652 did not follow Hardy-Weinberg equilibrium, $P < 0.01$, due to the low frequency of the heterozygous genotype in UPRCP (Table 3.7). SNPs 208, 430, 438, 573 and 652 were previously found in Spanish maternal beef breed including Retinta, Morucha, and Avilenã Negra-Ibérica by Avilés et al., (2009).

CAPN1 316 and polymorphisms found near the marker tended to segregate in three haplotypes in the commercial cattle (N = 68) and five in the UPRCP (N = 11). The SNP 212 was not included in the haplotypes because it did not show linkage with the other SNPs. Two haplotypes, D and E, were observed with the CC CAPN1 316 genotype, but only two animals from the UPRCP population showed segregation in these haplotypes. Further evaluation with a larger number of animals is needed to confirm these haplotypes. Haplotypes A and B segregated with higher frequency in the CCP, 32 and 60 % respectively.

3.4.3. Identified markers in the CAST gene

Four new SNPs were found in the 170 base fragments of marker CAST-2959, *Table 3.9*. Genotypic sequences of the SNPs are shown in *Figure 3.19*. Two of the SNP, 75 and 93, segregated as dominant homozygous genotype in both populations evaluated. The other two SNPs, 94 and 122, lacked the recessive homozygous genotype and segregated as the favored dominant homozygous genotype in both populations. A slight increase in frequency of the dominant homozygous genotype was observed in the UPRCP, 7 and 20 % in SNP 94 and 122, respectably. The SNP 122 also strayed from the Hardy-Weinberg equilibrium the dominant homozygous and heterozygous genotypes segregated with a 50-50 percent chance in the CCP. A microsatellite was found at base 69 in the 523 base fragment of the marker CAST-282, *Figure 3.25. A* repetition of 10 - 12 nucleotides of thymine was observed.

3.4.4. Allelic frequencies of the tender allele among sex

The allelic frequencies of the tender allele among sex with their respective p values are found in *Table 3.11.* A numeric difference was observed among the total frequencies found in both CAPN1 and CAST genes. The females segregated higher frequencies of the tender allele than the males. Significant differences were observed in SNPs 4751 ($P < 0.01$), 573 ($P = 0.04$) and 652 ($P < 0.01$). Tendencies were observed in SNPs 208 ($p = 0.13$), 438 ($P = 0.07$), 573 (P

= 0.06) and 643 (P = 0.13). It seems that the identified SNPs in intron 8 and 9 and exon 10 of the CAPN1 gene show the greatest differences among sex in CCP. When total frequencies among these seven SNPs were determined a 67% of tender alleles segregated in females and only 33% segregated in males (P = <0.01). Interestingly these polymorphisms are not sex oriented. Further evaluation of these polymorphisms and their effect on calpain activity could provide useful information for marker assisted selection.

3.5. Conclusion

The CCP are crossbred descendents mostly of *Bos taurus indicus influence.* A low segregation of the tender genotypes in the CAPN1 gene was previously reported by Van Eenennaam et al. (2007), was observed again in the present study in CCP. The UPRCP population, mainly of *Bos taurus taurus* descent, segregated a slightly higher frequency of the tender alleles found in the CAPN1 gene. Marker assisted selection using the identified SNPs in introns 8 and 9 and exon 10 of the CAPN1 gene can improve tenderness in the CCP. Interestingly these SNPs segregated in higher frequencies in females than in males. In order to increase tenderness in CCP an increase in frequency of the tender genotypes of markers from the CAPN1 gene offers an option. If a breeding program including more UPRCP influence is introduced in the commercial herds of Puerto Rico an increase in the tender allele frequencies could be obtained with time.

3.6. References

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CAPN	Forward (5'-3')	Reverse (3'-5')
5331	GGGCCGAGGAGATACCGTGAA	GCTTCCCGGGTGGCAACT
316	GGGCCAGATGGTGAACCTGA	TTGCGGAACCTCTGGCTCTT
530	GAGCCCAACAAGGAAGGT	AATACAGCCCAATGATGAGG
4751	AAGGGACAGATGTGGACA	GAGGGGTGTTCTCTGAGTGC
4753	TCTGTGGTTTCTGAGGGTGG	GGCATAGAGAGCAGTCAGCC
CAST	**Forward**	**Reverse**
282	GAAGTAAAGCCAAAGGAA	ATTTCTCTGATGGTGGCTGCTCACT
2959	CATTTGGAAAACGATGCCTCAC	TCTACGATTAGCAGCTCAAGAGGAG
UPRM/MSU	AGGACAAAGCAGGGTGTA	TGGTGTTTGAGGCAAATAC

Table 3.1 Primer sequences used in each SNP evaluated

Gen	Marker	N	Fragment bp	Location	SNP	Reference	Base
CAPN1	5331	50	175	intron 1	A/T	AF252504	327
CAPN1	316	67	735	exon 9	C/G	AF252505	5709
CAPN1	530	71	568	exon 14	A/G	AF248053	4558
CAPN1	4751	90	144	intron 17	C/T	AF248054	6545
CAPN1	4753	100	224	intron 21	A/C	AF248054	8676
CAST	2959	89	170	UTR 3‘	G/A	AF159246	2959
CAST	282	101	523	intron 5	C/G	AY008267	282
CAST	UPRM/MSU	95	303	intron 2	INS/DEL C	---	---

Table 3.2 Single Nucleotide Polymorphisms Evaluated

Marker	N	Position AF252505	SNP	Location
208	66	5458	C/T	intron 8
212	64	5462	G/T	intron 8
430	65	5680	C/G	intron 8
438	67	5688	G/A	intron 8
573	70	5823	C/T	intron 9
643	64	5893	INS/DEL C	intron 9
652	64	5902	C/A	exon 10

Table 3.3 Point mutations found in introns 8 – 9 and exon 10 in the CAPN1 gene

Marker	N	Position AF248053	SNP	Location
175	71	4554	C/T	exon 14
191	79	4570	A/C	exon 14
209	92	4588	G/A	intron 14
306	89	4685	C/T	intron 14
351	92	4730	G/A	intron 14
354	48	4733	INS/DEL CGAT	intron 14

Table 3.4 Polymorphisms found in exon and intron 12 in the CAPN1 gene

Marker	N	Position AF159246	SNP
75	87	2969	INS/DEL G
93	93	2986	C/T
94	98	2987	C/T
122	93	3015	A/C
123	93	3016	A/T

Table 3.5 Polymorphisms found in the CAST gen at the UTR 3'region in the CAST gene

Commercial Cattle				UPRM Cattle			
	Allelic	Genotypic			Allelic	Genotypic	
Polymorphism	%	%	Polymorphism	Polymorphism	%	%	Polymorphism
CAPN1 530			*CAPN1 530*	*CAPN1 530*			*CAPN1 530*
G	0.73	0.51	*GG*	*G*	0.60	0.50	*GG*
		0.45	*GA*			0.32	*AG*
A	0.27	0.04	*AA*	*A*	0.40	0.18	*AA*
	P = 0.21				*P = 0.92*		
CAPN1 316			*CAPN1 316*	*CAPN1 316*			*CAPN1 316*
G	0.79	0.64	*GG*	*G*	0.50	0.45	*GG*
		0.30	*GC*			0.18	*GC*
C	0.21	0.01	*CC*	*C*	0.50	0.36	*CC*
	P = 0.40				***P = 0.02***		
CAPN1 4751			*CAPN1 4751*	*CAPN1 4751*			*CAPN1 4751*
T	0.67	0.59	*TT*	*T*	0.57	0.32	*TT*
		0.16	*CT*			0.50	*CT*
C	0.33	0.26	*CC*	*C*	0.43	0.18	*CC*
	P < 0.01				*P = 0.92*		
CAPN1 4753			*CAPN1 4753*	*CAPN1 4753*			*CAPN1 4753*
C	0.89	0.78	*CC*	*C*	0.10	0.10	*CC*
		0.21	*CA*			0.00	*CA*
A	0.12	0.01	*AA*	*A*	0.00	0.00	*AA*
	P = 0.75				*P = --*		
CAPN1 5331			*CAPN1 5331*	*CAPN1 5331*			*CAPN1 5331*
A	79.00	0.60	*AA*	*A*	0.10	0.10	*AA*
		0.38	*AT*			0.00	*AT*
T	21.00	0.02	*TT*	*T*	0.00	0.00	*TT*
	P = 0.30				*P = --*		

p values are from Hardy-Weinberg equilibrium, $p < 0.05$ are not in equilibrium.

Table 3.6 Allelic and Genotypic frequencies of polymorphisms from the CAPN1 gen in Commercial Cattle and UPR Cattle Populations

Commercial Cattle				UPRM Cattle			
	Allelic	Genotypic			Allelic	Genotypic	
Polymorphism	%	%	Polymorphism	Polymorphism	%	%	Polymorphism
SNP 208			*SNP 208*	*SNP 208*			*SNP 208*
C	0.79	0.59	*CC*	*C*	0.59	0.36	*CC*
		0.39	*CT*			0.46	*CT*
T	0.21	0.02	*TT*	*T*	0.41	0.18	*TT*
	P = 0.15				*P = 0.84*		
SNP 212			*SNP 212*	*SNP 212*			*SNP 212*
T	0.92	0.84	*TT*	*T*	0.91	0.82	*TT*
		0.16	*TG*			0.18	*TG*
G	0.08	0.00	*GG*	*G*	0.09	0.00	*GG*
	P = 0.50				*P = 0.74*		
SNP 430			*SNP 430*	*SNP 430*			*SNP 430*
C	0.82	0.66	*CC*	*C*	0.64	0.45	*CC*
		0.31	*CG*			0.36	*CG*
G	0.18	0.03	*GG*	*G*	0.36	0.18	*GG*
	P = 0.86				*P = 0.47*		
SNP 438			*SNP 438*	*SNP 438*			*SNP 438*
G	0.81	0.64	*GG*	*G*	0.68	0.45	*GG*
		0.34	*GA*			0.46	*GA*
A	0.19	0.01	*AA*	*A*	0.32	0.09	*AA*
	P = 0.28				*P = 0.89*		
SNP 573			*SNP 573*	*SNP 573*			*SNP 573*
C	0.83	0.69	*CC*	*C*	0.55	0.46	*CC*
		0.29	*CT*			0.18	*CT*
T	0.17	0.03	*TT*	*T*	0.46	0.36	*TT*
	P = 0.16				***P = 0.04***		
SNP 643			*SNP 643*	*SNP 643*			*SNP 643*
DEL C	0.79	0.59	*DEL*	*DEL C*	0.50	0.36	*DEL*
		0.39	*INS/DEL*			0.27	*INS/DEL*
INS C	0.21	0.02	*INS*	*INS C*	0.50	0.36	*INS*
	P = 0.16				*P = 0.13*		
SNP 652			*SNP 652*	*SNP 652*			*SNP 652*
A	0.76	0.59	*AA*	*A*	0.41	0.36	*AA*
		0.33	*CA*			0.09	*CA*
C	0.24	0.08	*CC*	*C*	0.59	0.55	*CC*
	P = 0.40				***P = 0.01***		

p values are from Hardy-Weinberg equilibrium, $p < 0.05$ are not in equilibrium.

Table 3.7 Allelic and genotypic frequencies of novel SNPs near the CAPN1 316 marker in Commercial Cattle and UPR Cattle Populations

Commercial Cattle				UPRM Cattle			
	Allelic	Genotypic			Allelic	Genotypic	
Polymorphism	%	%	Polymorphism	Polymorphism	%	%	Polymorphism
SNP 175			*SNP 175*	*SNP 175*			*SNP 175*
C	*0.75*	*0.51*	*CC*	*C*	0.86	0.77	*CC*
		0.49	*CT*			0.18	*CT*
T	*0.25*	*0.00*	*TT*	*T*	0.14	0.05	*TT*
	P < 0.01				*P = 0.29*		
SNP 191			*SNP 191*	*SNP 191*			*SNP 191*
A	*0.53*	*0.08*	*AA*	*A*	50.0	0.0	*AA*
		0.90	*AC*			100.0	*AC*
C	*0.47*	*0.03*	*CC*	*C*	50.0	0.0	*CC*
	P =< 0.01				*P = --*		
SNP 209			*SNP 209*	*SNP 209*			*SNP 209*
G	*0.96*	*0.91*	*GG*	*G*	0.91	0.86	*GG*
		0.09	*GA*			0.09	*GA*
A	*0.04*	*0.00*	*AA*	*A*	0.09	0.05	*AA*
	P = 0.66				***P = 0.03***		
SNP 306			*SNP 306*	*SNP 306*			*SNP 306*
C	*0.67*	*0.46*	*CC*	*C*	0.55	0.36	*CC*
		0.42	*CT*			0.36	*CT*
T	*0.33*	*0.12*	*TT*	*T*	0.46	0.27	*TT*
	P = 0.56				*P =.21*		
SNP 351			*SNP 351*	*SNP 351*			*SNP 351*
G	*1.00*	*1.00*	*GG*	*G*	0.96	0.91	*GG*
		0.00	*GA*			0.09	*GA*
A	*0.00*	*0.00*	*AA*	*A*	0.05	0.00	*AA*
	P = --				*P = 0.82*		
SNP 354			*SNP 354*	*SNP 354*			*SNP 354*
INS CGAT	*0.66*	*0.42*	*INS*	*INS CGAT*	0.82	0.68	*INS*
		0.48	*INS/DEL*			0.27	*INS/DEL*
DEL CGAT	*0.34*	*0.10*	*DEL*	*DEL CGAT*	0.18	0.05	*DEL*
	P = 0.84				*P = 0.70*		

p values are from Hardy-Weinberg equilibrium, $p < 0.05$ are not in equilibrium.

Table 3.8 Allelic and genotypic frequencies of novel SNPs near the CAPN1 530 marker in Commercial Cattle and UPR Cattle Populations

Commercial Cattle				UPRM Cattle			
	Allelic	Genotypic			Allelic	Genotypic	
Polymorphism	%	%	Polymorphism	Polymorphism	%	%	Polymorphism
CAST 282			*CAST 282*	*CAST 282*			*CAST 282*
C	0.69	0.46	*CC*	*C*	50.0	0.0	*CC*
		0.47	*CG*			100.0	*CG*
G	0.31	0.08	*GG*	*G*	50.0	0.0	*GG*
	P = 0.40				*P = --*		
CAST 2959			*CAST 2959*	*CAST 2959*			*CAST 2959*
A	0.88	0.78	*AA*	*A*	0.93	0.85	*AA*
		0.21	*AG*			0.15	*AG*
G	0.12	0.01	*GG*	*G*	0.08	0.00	*GG*
	P = 0.81				*P = 0.72*		
CAST 3016			*CAST 3016*	*CAST 3016*			*CAST 3016*
A	0.91	0.84	*AA*	*A*	0.00	0.00	*AA*
		0.14	*AT*			0.00	*AT*
T	0.09	0.02	*TT*	*T*	1.00	1.00	*TT*
	P = 0.13				*P = --*		
CAST SSCP			*CAST SSCP*	*CAST SSCP*			*CAST SSCP*
DEL C	0.86	0.75	*Del*	*DEL C*	0.86	0.73	*Del*
		0.23	*INS/DEL*			0.27	*INS/DEL*
INS C	0.14	0.02	*INS*	*INS C*	0.14	0.00	*INS*
	P = 0.84				*P = 0.46*		

p values are from Hardy-Weinberg equilibrium, $p < 0.05$ are not in equilibrium.

Table 3.9 Allelic and genotypic frequencies of polymorphisms from the CAST marker in Commercial Cattle and UPR Cattle Populations

Commercial Cattle				UPRM Cattle			
Polymorphism	Allelic %	Genotypic %	Polymorphism	Polymorphism	Allelic %	Genotypic %	Polymorphism
SNP 75			*SNP 75*	*SNP 75*			*SNP 75*
DEL G	1.00	1.00	*DEL*	*DEL G*	1.00	1.00	*DEL*
	0.00	0.00	*INS/DEL*			0.00	*INS/DEL*
INS G	0.00	0.00	*INS*	*INS G*	0.00	0.00	*INS*
	P = --				*P = --*		
SNP93			*SNP93*	*SNP93*			*SNP93*
T	*0.99*	0.99	*TT*	*T*	*1.0*	1.0	*TT*
		0.00	*CT*			0.0	*CT*
C	0.01	0.01	*CC*	*C*	0.0	0.0	*CC*
	P < 0.01				*P = --*		
SNP 94			*SNP 94*	*SNP 94*			*SNP 94*
T	0.86	0.72	*TT*	*T*	0.93	0.85	*TT*
		0.28	*TC*			0.15	*TC*
C	0.14	0.00	*CC*	*C*	0.08	0.00	*CC*
	P = 0.12				*P = 0.72*		
SNP 122			*SNP 122*	*SNP 122*			*SNP 122*
A	*0.75*	0.51	*AA*	*A*	0.95	0.90	*AA*
		0.50	*AC*			0.10	*AC*
C	0.25	0.00	*CC*	*C*	0.05	0.00	*CC*
	P < 0.01				*P = 0.81*		

p values are from Hardy-Weinberg equilibrium, $p < 0.05$ are not in equilibrium.

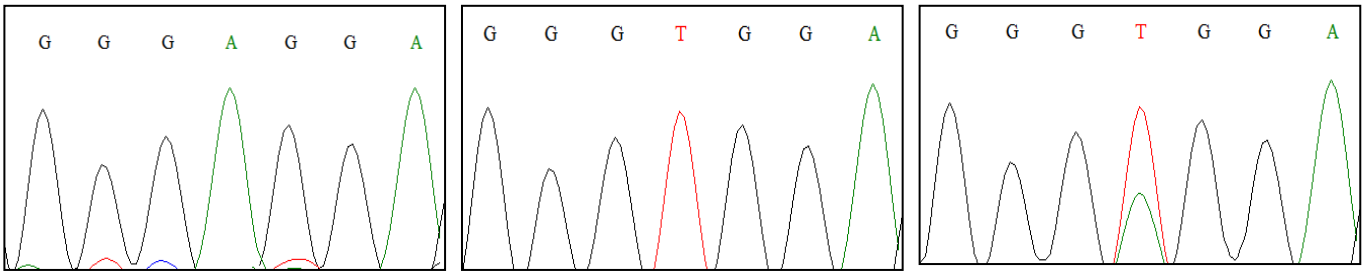
Table 3.10. Allelic and genotypic frequencies of novel SNPs near the CAST 2959 marker in Commercial Cattle and UPR Cattle Populations

		Females		Males		
Marker	**Allele**	**n**	**%**	**n**	**%**	**p value**
CAPN1						
530	A	18	0.47	20	0.53	0.75
316	C	16	0.57	12	0.43	0.45
4751	C	41	0.68	19	0.32	**<0.01**
4753	A	11	0.48	12	0.52	0.83
5331	T	12	0.57	9	0.43	0.51
175	T	14	0.40	21	0.60	0.24
191	C	40	0.53	35	0.47	0.56
209	A	4	0.50	4	0.50	>.99
306	T	29	0.49	30	0.51	0.90
354	DEL	26	0.43	35	0.57	0.25
208	T	18	0.64	10	0.36	0.13
212	G	3	0.38	5	0.63	0.48
430	G	17	0.68	8	0.32	0.07
438	A	16	0.70	7	0.30	0.06
573	T	17	0.71	7	0.29	**0.04**
643	INS	18	0.64	10	0.36	0.13
652	C	33	0.70	14	0.30	**<0.01**
Total		*333*	0.56	*258*	0.44	*0.23*
CAST						
282	C	76	0.55	63	0.45	0.27
2959	A	82	0.52	75	0.48	0.58
3016	A	86	0.51	83	0.49	0.82
UPRM	DEL	85	0.52	79	0.48	0.64
94	T	79	0.49	81	0.51	0.87
122	A	71	0.51	69	0.49	0.87
Total		*479*	0.52	*450*	0.48	*0.69*

p values are from X^2

Table 3.11. Allelic frequencies of tender alleles among sex of polymorphisms in the CAPN1 and CAST genes. Females segregated a higher percent of tender alleles in both genes. Significant differences were found in SNP 4751, 573 and 652 of the CAPN1 gene. Greater total differences were found in the newly identified SNP found in intron 8 and 9 and exon 10 of the CAPN1 gene (SNPs: 208, 212, 430, 438, 573, 643 and 652). Among these seven SNPs, 67% of the tender alleles were segregated in females and 33% in males.

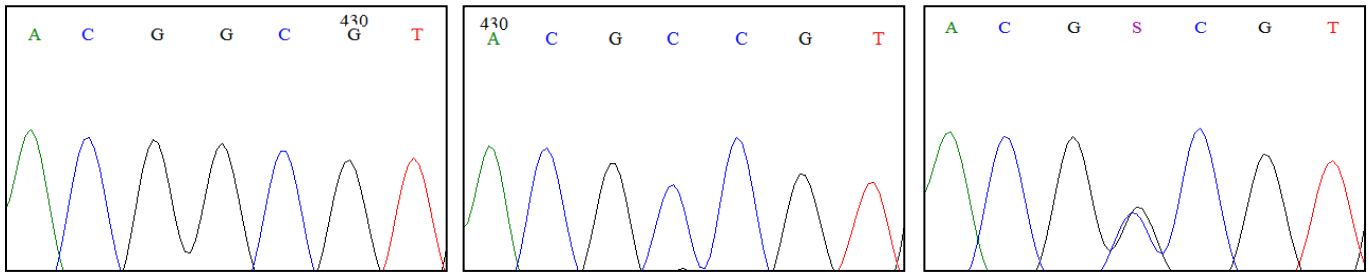
CAPN1 5331



Genotype AA Genotype TT Genotype AT

Figure 3.1. CAPN1-5331 genotypic sequence. A transversion of adenine to thymine found in intron 1of CAPN1 gene (base 327, accession number AF252504)

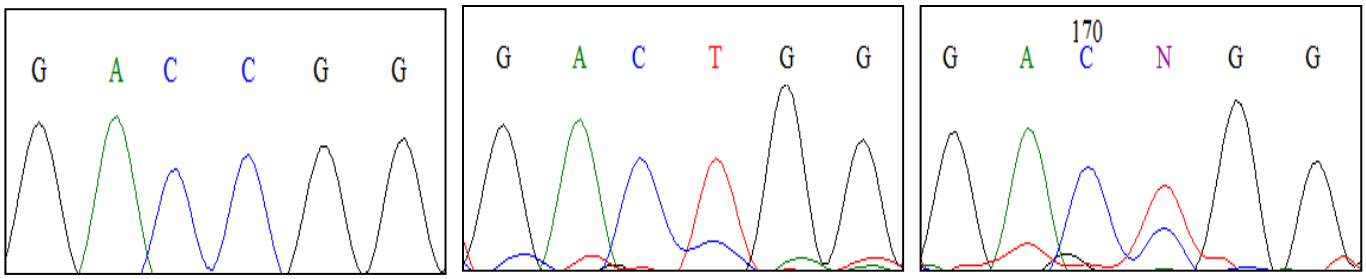
CAPN1 316



Genotype GG Genotype CC Genotype GC

Figure 3.2. CAPN1-316 genotypic sequence. A transversion of guanine to cytosine found in exon 9 of the CAPN1 gene. This missence mutation alters the amino acid sequence from glycine to alanine (base 5709, accession number AF252505).

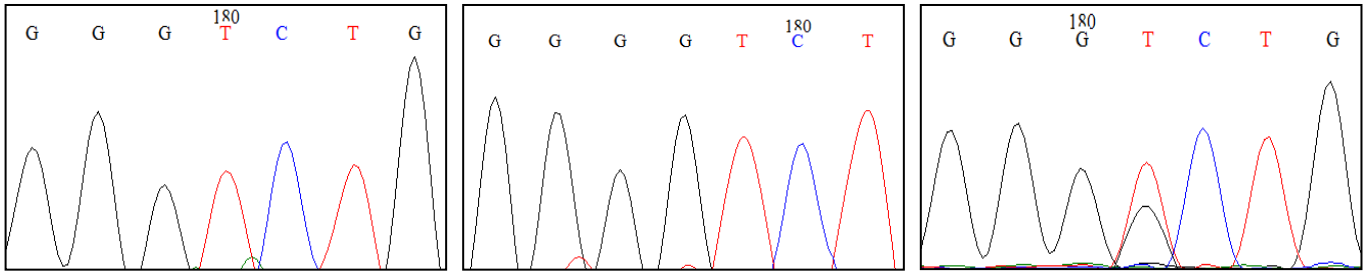
SNP 208



Genotype CC Genotype TT Genotype CT

Figure 3.3 SNP 208 genotypic sequence. A transversion of cytosine to thymine found in intron 8 of the CAPN1 gene (base 5458, accession number AF252505).

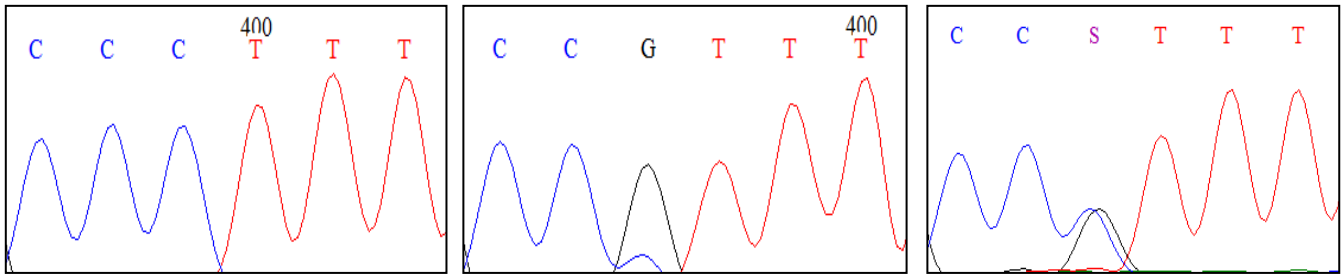
SNP 212



Genotype TT Genotype GG Genotype TG

Figure 3.4 SNP 212 genotypic sequence. A transversion of thymine to guanine found in intron 8 of the CAPN1 gene (base 5462, accession number AF252505).

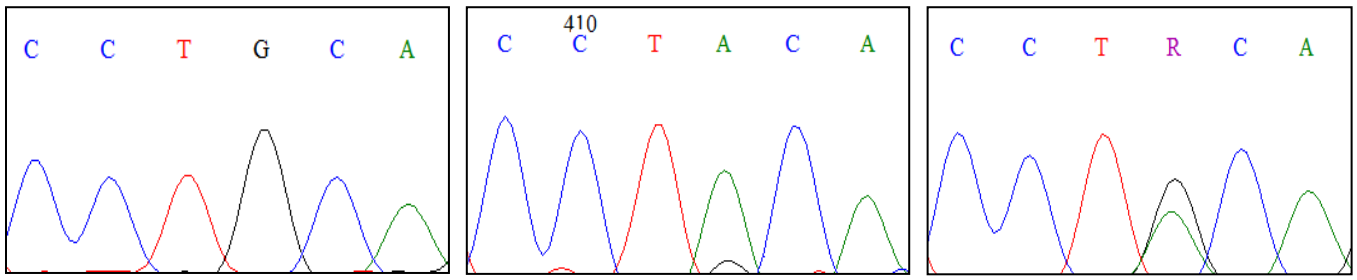
SNP 430



Genotype CC Genotype GG Genotype CG

Figure 3.5. SNP 430 genotypic sequence. A transversion of cytosine to guanine found in intron 8 of the CAPN1 gene (base 5680, accession number AF252505).

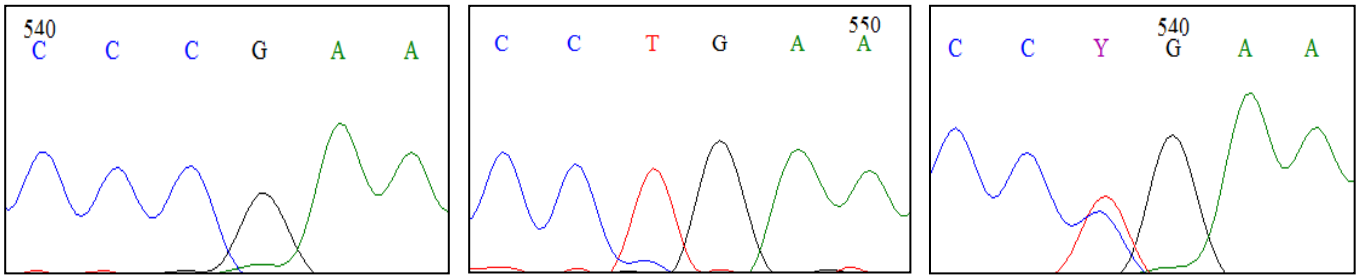
SNP 438



Genotype GG Genotype AA Genotype GA

Figure 3.6. SNP 438 genotypic sequence. A transition of guanine to adenine found in intron 8 of the CAPN1 gene (base 5688, accession number AF252505).

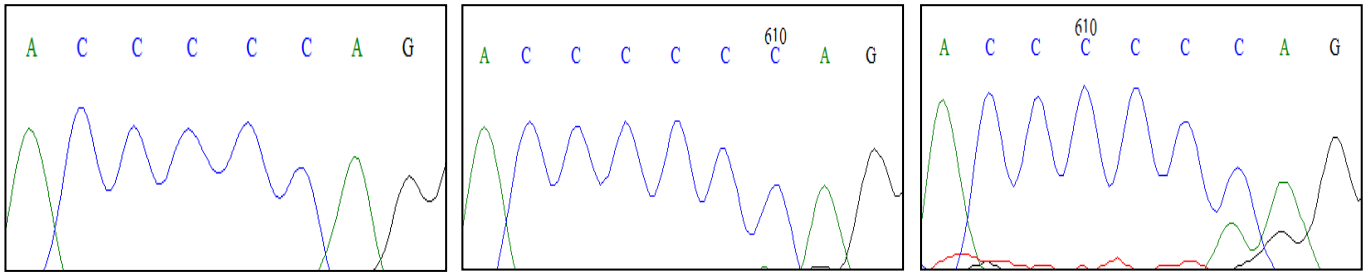
SNP 573



Genotype CC Genotype TT Genotype TC

Figure 3.7. SNP 573 genotypic sequence. A transition of cytosine to thymine found in intron 9 of the CAPN1 gene (base 5823, accession number AF252505).

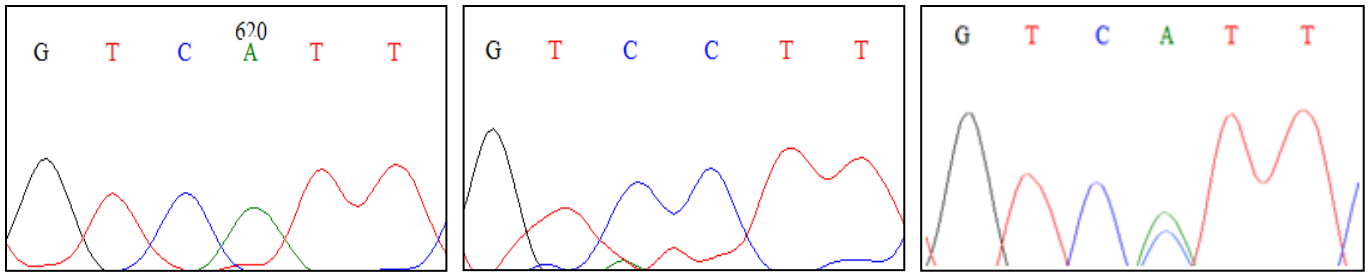
SNP 643



DEL C INS C INS/DEL C

Figure 3.8. SNP 643 genotypic sequence. An INS/DEL of cytosine found in intron 9 of the CAPN1 gene (base 5893, accession number AF252505).

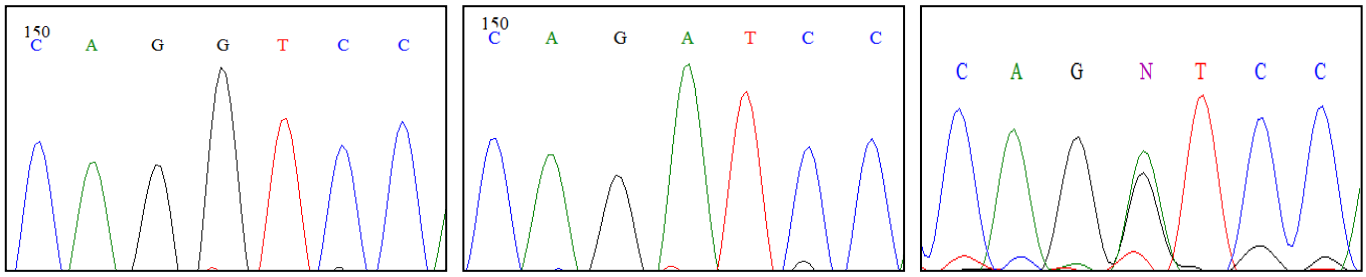
SNP652



Genotype AA Genotype CC Genotype AC

Figure 3.9. SNP 652 genotypic sequence. A transversion of adenine to cytosine found in exon 10 of the CAPN1 gene. This missence mutation alters the amino acid sequence from isoleucine to leucine (base 5902, accession number AF252505).

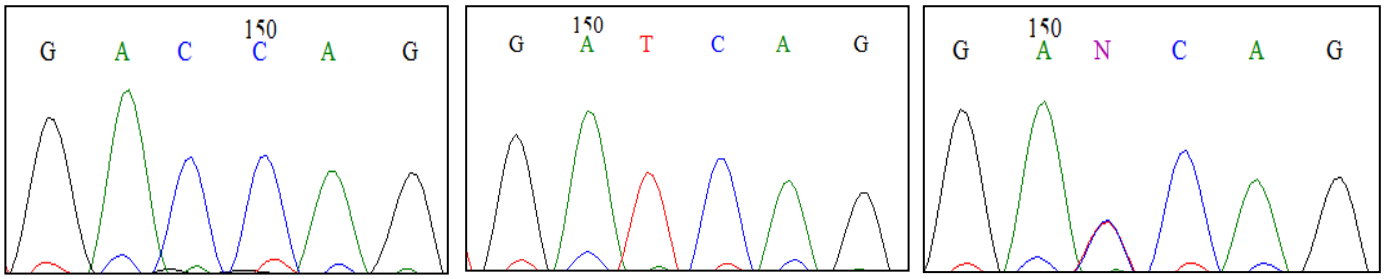
CAPN1 530



Genotype GG Genotype AA Genotype GA

Figure 3.10.CAPN1 530 genotypic sequence**.** A transition of guanine to adenine found in exon 14 of the CAPN1 gene. This missence mutation alters the amino acid sequence from valine to isoleucine (base 4558, accession number AF248053).

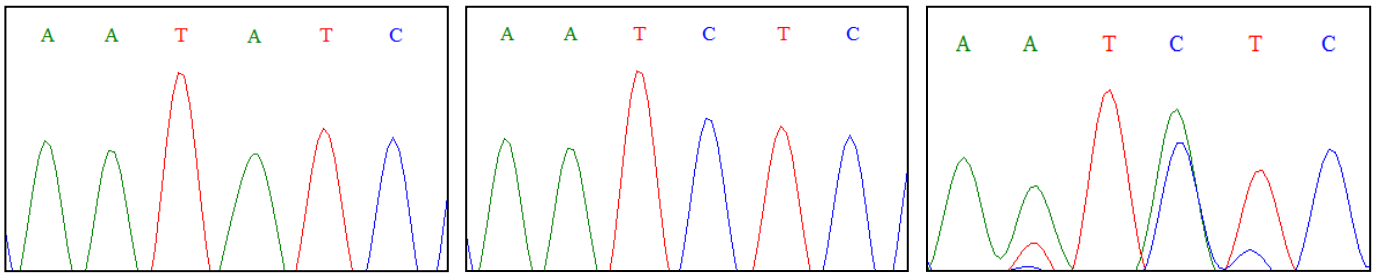
SNP175



Genotype CC Genotype TT Genotype CT

Figure 3.11. SNP 175 genotypic sequence. A transition of cytosine to thymine found in exon 14 of the CAPN1 gene (base4554, accession number AF248053). This is not a misscense mutation, the two alleles codify for Aspartic Acid.

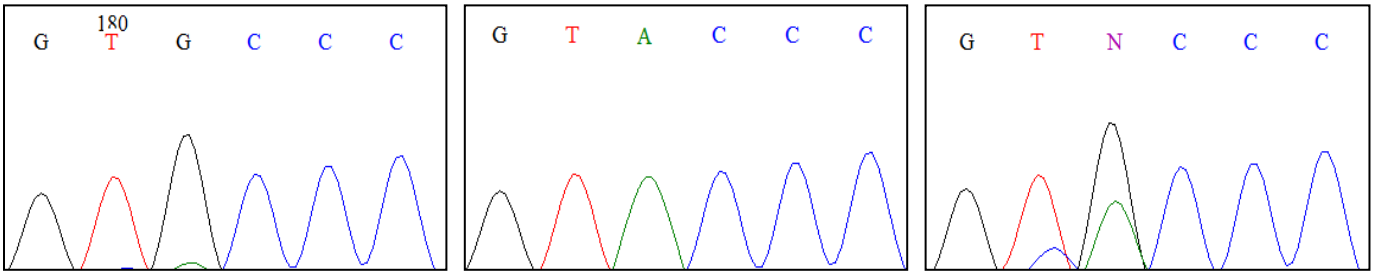
SNP 191



Genotype AA Genotype CC Genotype AC

Figure 3.12. SNP 191 genotypic sequence. A transversion of adenine to cytosine found in exon 14 of the CAPN1 gene (base 4570, accession number AF248053). This misscence mutation changes the amino acid sequence from isoleucine to leucine.

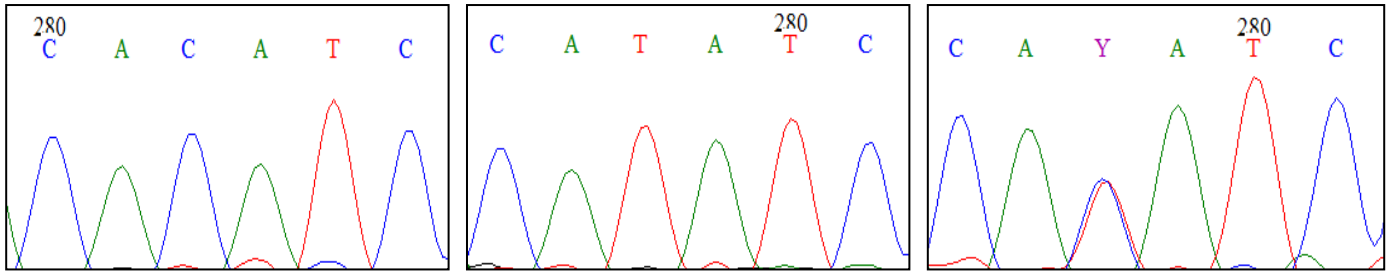
SNP 209



Genotype GG Genotype AA Genotype GA

Figure 3.13. SNP 209 genotypic sequence. A transition of guanine to adenine found in intron 14 of the CAPN1 gene (base 4588, accession number AF248053).

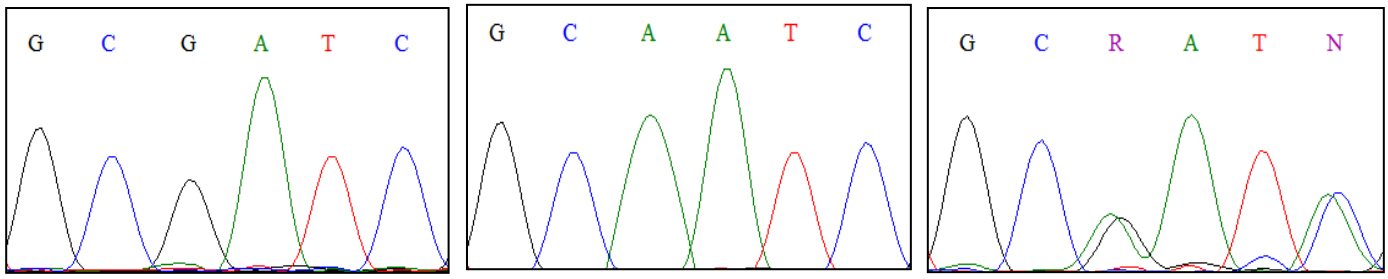
SNP 306



Genotype CC Genotype TT Genotype CT

Figure 3.14. SNP 306 genotypic sequence. A transition of cytosine to thymine found in intron 14 of the CAPN1 gene (base 4685, accession number AF248053).

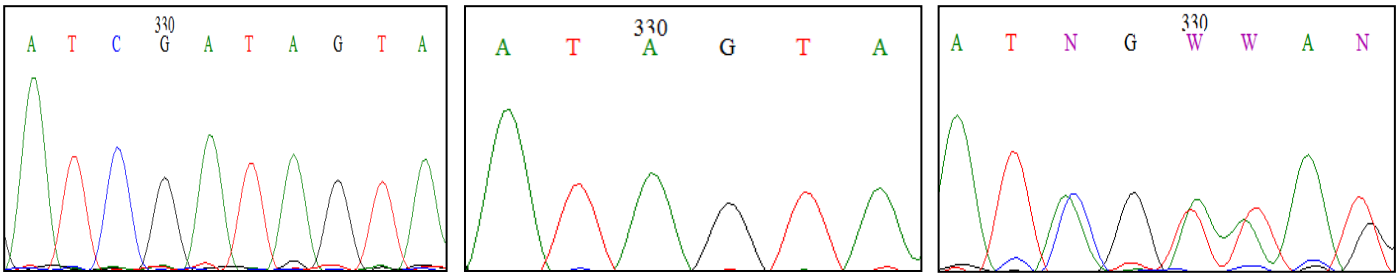
SNP 351



Genotype GG Genotype AA Genotype GA

Figure 3.15. SNP 351 genotypic sequence. A transition of guanine to adenine found in intron 14 of the CAPN1 gene (base 4730, accession number AF248053).

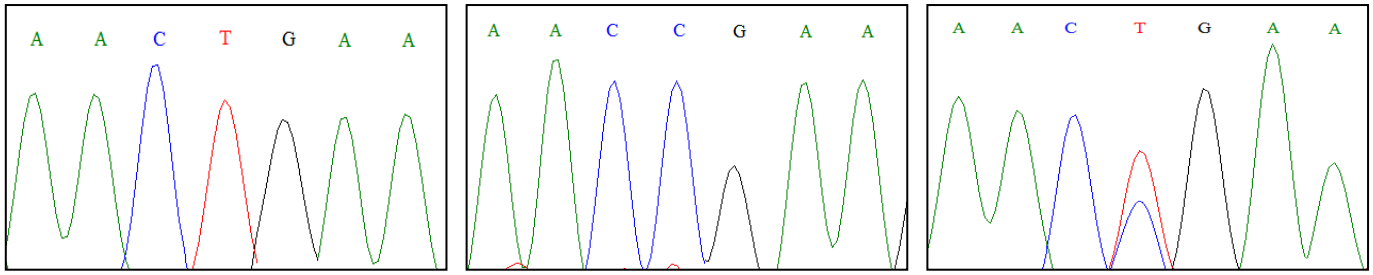
SNP354



INS CGAT DEL CGAT INS/DEL CGAT

Figure 3.16. SNP 354 genotypic sequence. An INS/DEL CGAT found in intron 14 of the CAPN1 gene (base 4733, accession number AF248053).

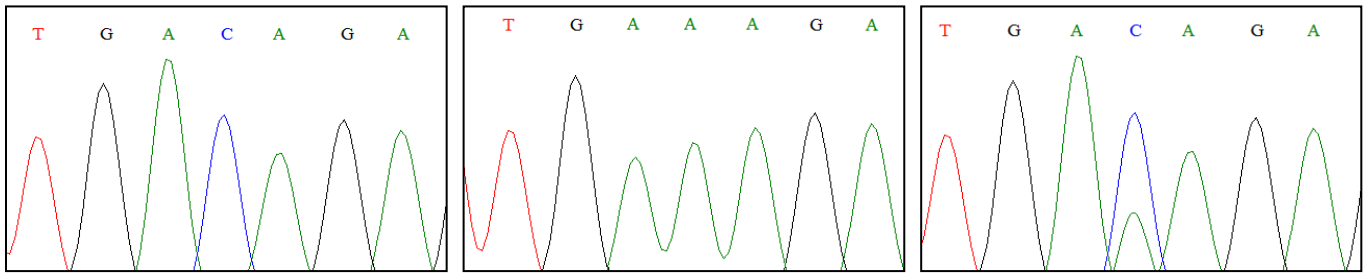
CAPN1 4751



Genotype TT Genotype CC Genotype TC

Figure 3.17. CAPN1 4751 genotypic sequence. A transition of thymine to cytosine found in intron 17 of the CAPN1 gene (base 6545, accession number AF248054).

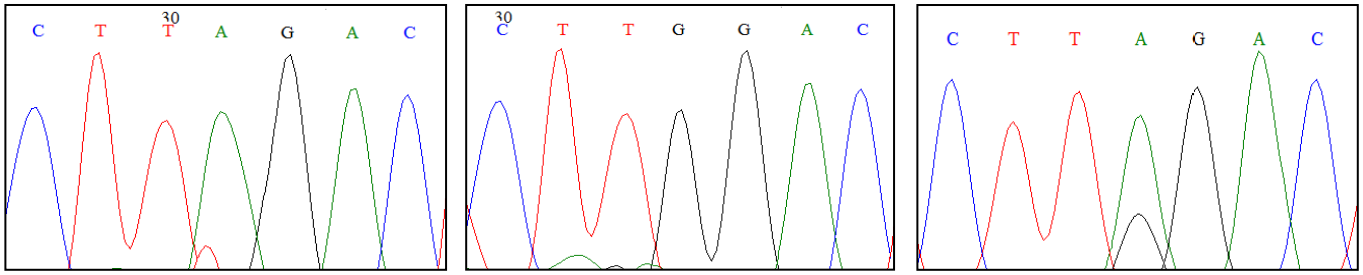
CAPN1 4753



Genotype CC Genotype AA Genotype CA

Figure 3.18. CAPN1 4753 genotypic sequence. A transversion of cytosine to adenine found in intron 21 of the CAPN1 gene (base 8676, accession number AF248054).

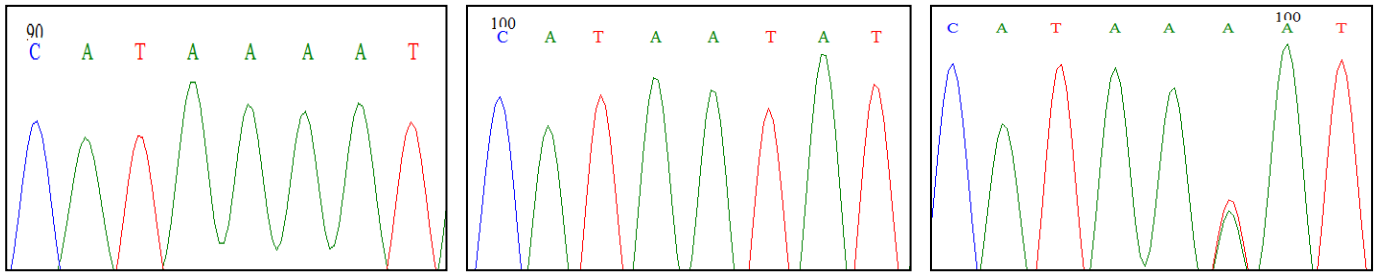
CAST 2959



Genotype AA Genotype GG Genotype AG

Figure 3.19. CAST 2959 genotypic sequence. A transition of adenine to guanine found in the 3" UTR of the CAST gene (base 2959, accession number AF159246).

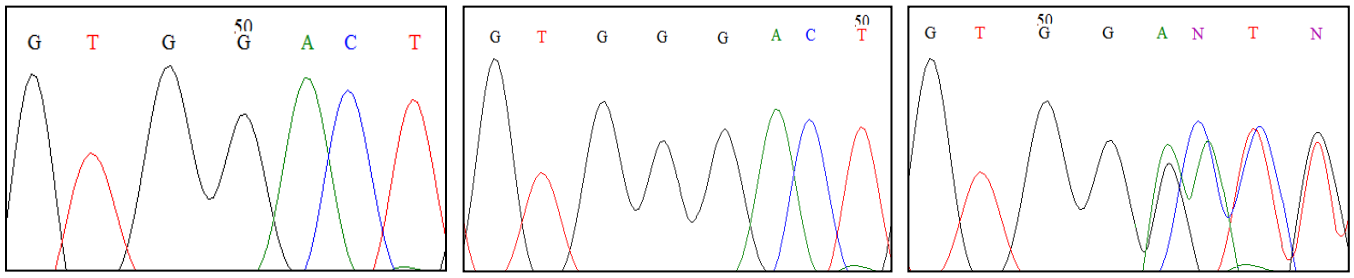
CAST 3016



Genotype AA Genotype TT Genotype AT

Figure 3.20. CAST 3016 genotypic sequence. A transversion of adenine to thymine found in the 3" UTR of the CAST gene (base 3016, accession number AF159246).

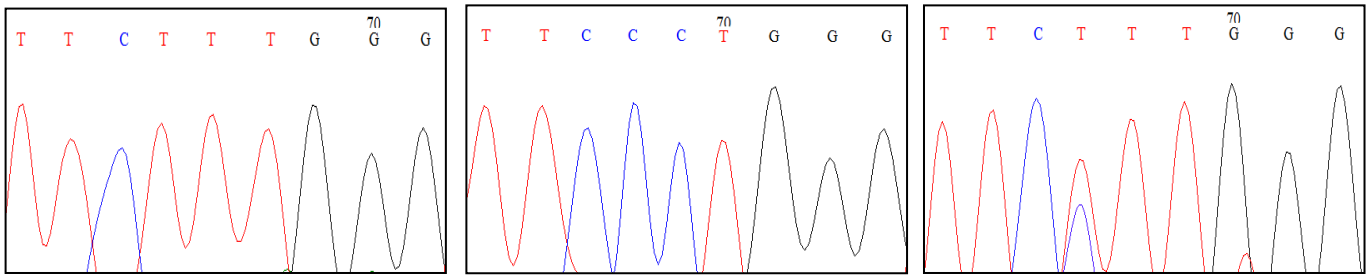
SNP 75



DEL G INS G INS/DEL G

Figure 3.21. SNP 75 genotypic sequence**.** An INS/DEL guanine found in the 3" UTR of the CAST gene (base 2969, accession number AF159246).

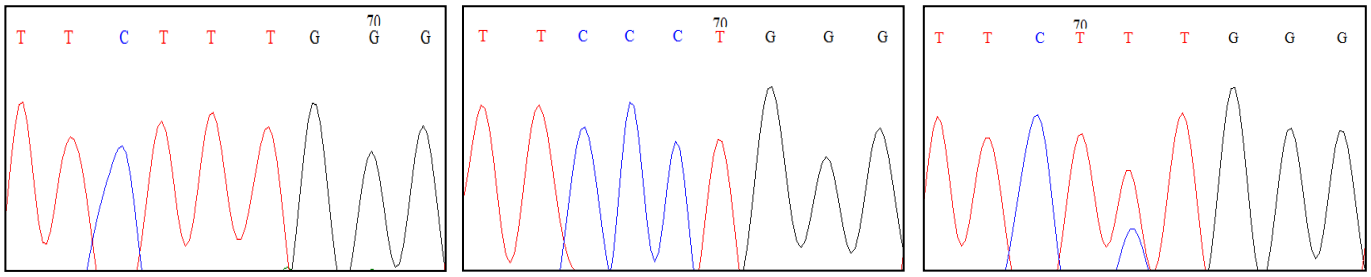
SNP 93



Genotype TT Genotype CC Genotype TC

Figure 3.22. SNP 93 genotypic sequence**.** A transition of thymine to cytosine found in the 3" UTR of the CAST gene (base 2986, accession number AF159246).

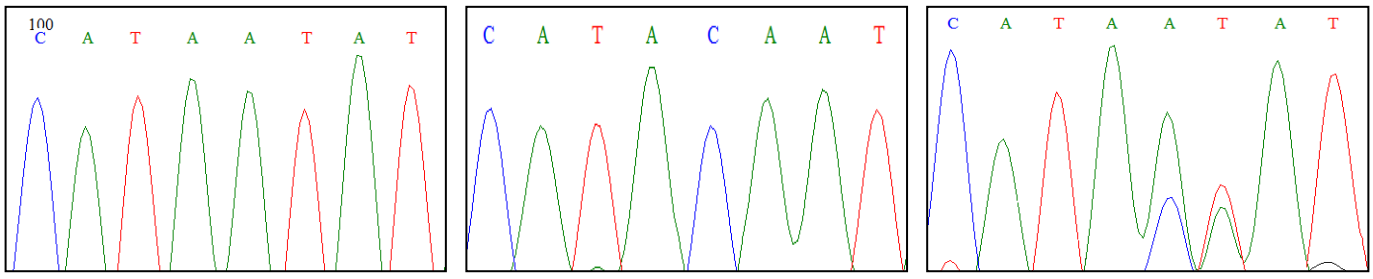
SNP 94



Genotype TT Genotype CC Genotype TC

Figure 3.23. SNP 94 genotypic sequence**.** A transition of thymine to cytosine found in the 3" UTR of the CAST gene (base 2987, accession number AF159246).

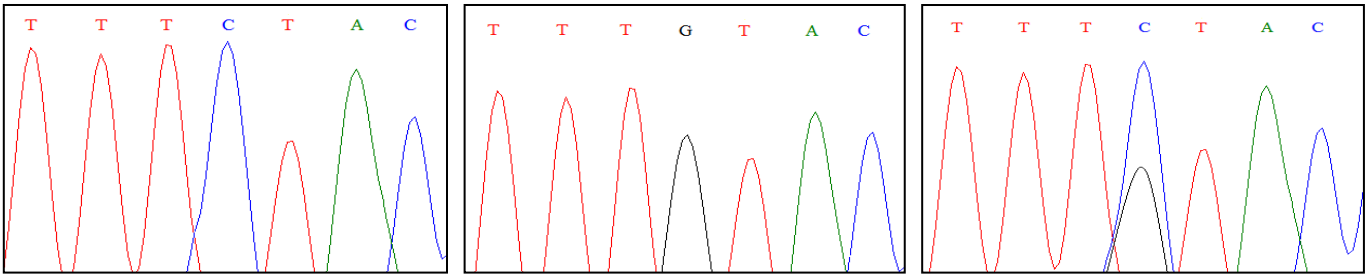
SNP 122



Genotype AA Genotype CC Genotype AC

Figure 3.24. SNP 122 genotypic sequence**.** A transversion of adenine to cytosine found in the 3" UTR of the CAST gene (base 3015, accession number AF159246).

CAST 282



Genotype CC Genotype GG Genotype CG

Figure 3.25. *CAST 282* genotypic sequence**.** A transversion of cytosine to guanine found in intron 5 of the CAST gene (base 282, accession number AY008267).

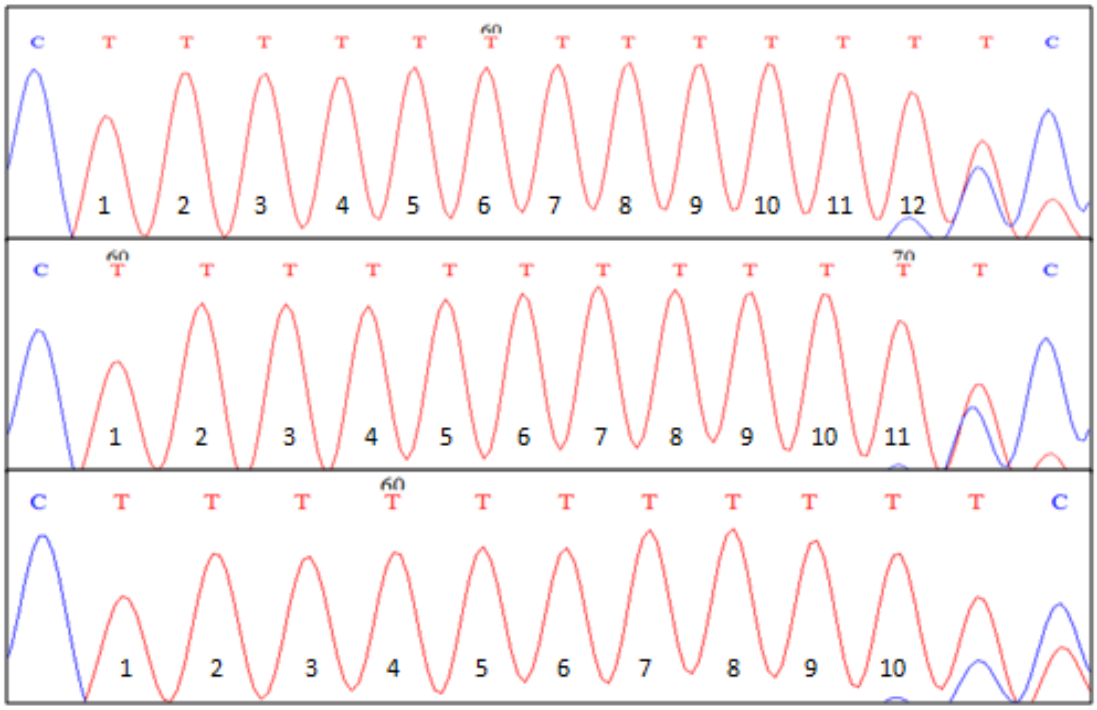


Figure 3.26. Microsatellite found at intro 5 in the Cast gene (base 69 accession number AY008267).

CAST UPRM/MSU

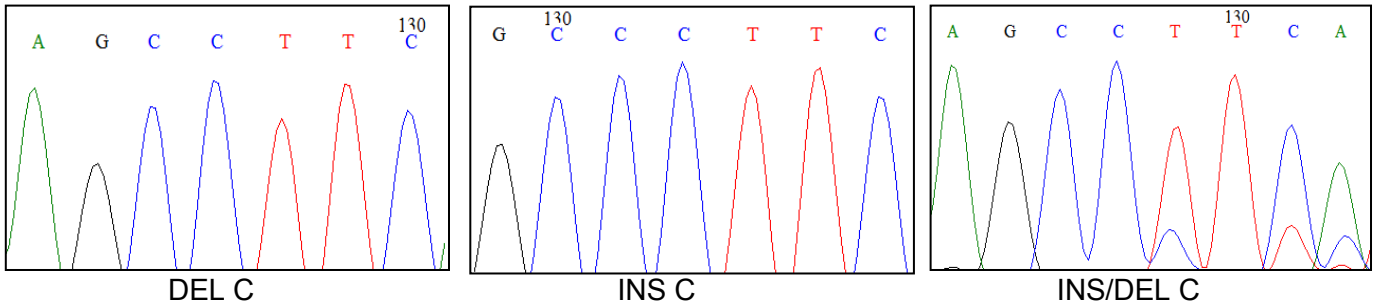
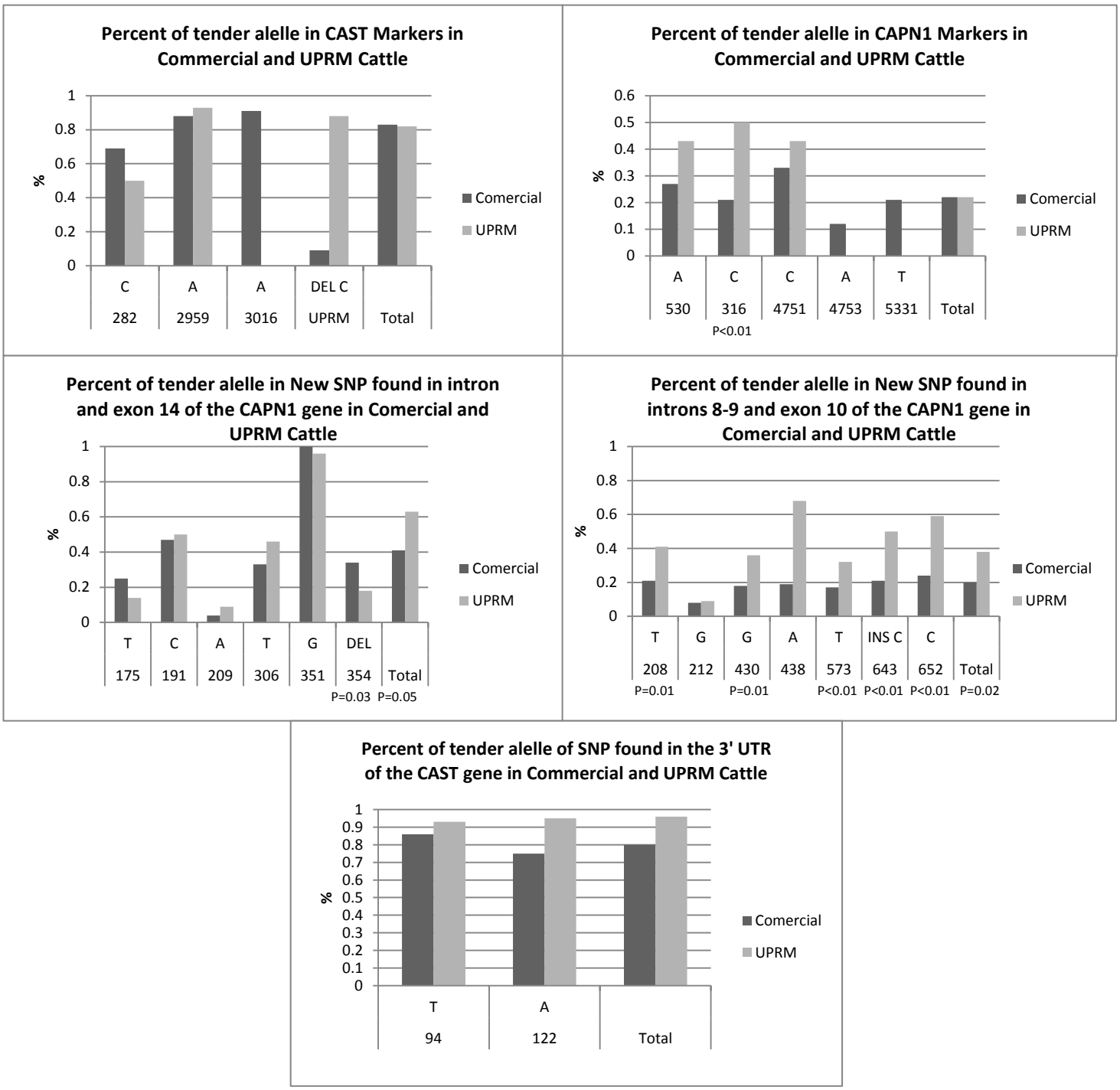


Figure 3.27. CAST UPRM/MSU genotypic sequence**.** An INS/DEL of C found in intron 2 of the CAST gene.



p values are from X^2

Figure 3.28. Differences in allelic frequencies of the tender allele in commercial markers and newly identified SNPs either in the CAPN1 or CAST gene in Commercial Cattle and UPR Cattle Populations.

Chapter IV. Effect of polymorphisms found in candidate genes on meat quality of commercial cattle and UPR cattle populations

4.1. Abstract

A total of 102 crossbred cattle with *Bos taurus indicus* influence and 22 crossbred, with mainly *Bos taurus taurus* influence were genotyped for candidate markers in the CAPN1 and CAST genes. Among the commercial polymorphisms in the CAPN1 gene that have been associated with tenderness, only genotypes with CAPN1 316 and CAST 2959 markers showed significant difference in WBS in the CCP and UPRCP, respectively. Segregation of the CC genotype of the CAPN1 316 marker in the CCP produced a -4.72 ± 0.2 kg change in WBS. When the AA genotype of CAST 2959 segregated in the UPRCP a -2.33 kg change in WBS resulted. Among the newly identified polymorphisms in exon and intron 14 of the CAPN1 gene, two SNP were significantly associated with meat tenderness in the UPRCP. Genotype AA of SNP 209 was associated with an increase of 45.45 ± 2.34 in MFI. While the GG genotype of SNP 351 produced a -3.35 kg change of WBS. Interestingly the greatest changes in tenderness were seen among the new identified SNP (Avilés et al., 2009) found in introns 8 and 9 of the CAPN1 gene in the CCP. The GG genotype of SNP 430 showed a -5.14 ± 0.21 kg change in WBS, and this same genotype produced an increase of 32.03 ± 8.08 μM in free [Ca^{2+}] of meat at 14d of aging. The same was observed when the TT genotype segregated in the SNP 573, resulting in a -5.30 kg change in WBS and 33.76 μM increase in free [Ca^{2+}] at 14d of aging. The greatest change in tenderness was seen with an INS of C in SNP 643, a difference of -7.74 kg in WBS. This INS also resulted in a 47.30 μM greater release of free [Ca^{2+}] at 14d of aging. This study succeeded in identifying two candidate markers for determining tenderness in intron 14 of the CAPN1 gene in cattle with mainly *Bos taurus taurus* influence. Polymorphisms in introns 8 and 9 of the CAPN1 gene show greater implications in *Bos taurus indicus* populations although greater frequencies were observed in the *Bos taurus taurus* population. The INS of C

in SNP 643 showed the greatest correlation with WBS and was identified for the first time in the CCP.

4.2. Introduction

Tenderization of meat is a multifactor process that is influenced by breed, nutrition, pre and post-mortem handling, long and short term stressors, proteolytic activity and genotypic background (Koohmaraie et al., 2006). The effect of the environment on the proteomic integrity of the myofibers produces high variability of tenderness in beef produced. Much emphasis has been given in the use of genetic markers to improve overall tenderness with some success but high variability in tenderness is still a problem (Goll et al., 2003; Luciano et al., 2007). Guillemin et al. (2011) identified three cellular pathways involved in tenderization of meat: apoptosis, heat shock proteins and oxidative stress resistance. When these pathways are taken into consideration a better understanding of the tenderization process is achieved. Integrating knowledge of the biochemical changes that are known to occur in postmortem muscle with differences measured in meat quality among genotypes of point mutations could increase the usefulness of candidate genes in breeding programs to be established in Puerto Rico. It is known that μ-calpain plays a very important role in post-mortem tenderization. Increasing the frequencies of the tender allele of commercially discovered point mutations in the CAST and CAPN1 genes can positively affect the overall tenderness. The commercially available tenderness markers are CAPN1 316 (Page et al., 2002), 4751 (White et al., 2005), CAST 282 (Schenkel et al., 2006) and 2959 (Barendse et al., 2002). Other SNP associated with tenderness have been found in the CAPN1 gene including CAPN1 530 (Page et al., 2002), 306 (Juszczuk-Kubiak et al., 2004), 208, 430, 438, 573, 652 (Avilés et al., 2009), 4753 and 5331 (Casas et al., 2006). These two genes have proven to be highly polymorphic and increasing frequencies of the tender alleles can improve meat quality in any given cattle population. The objects of this study is to investigate commercial tenderness markers as well as novel

polymorphisms found in the CAPN1 and CAST genes in the commercial cattle of Puerto Rico and determine their association with several meat quality traits of greatest importance in meat tenderness. By SNP profiling of CAPN and CAST as candidate genes it may be possible to identify phenotypic traits that constitute a predisposition for tender meat in commercial cattle.

4.3. Materials and Methods

4.3.1. Animal Selection

This aspect of the investigation was described in Section 2.3.1 (page 19).

4.3.2. Muscles Samples, Aging, pH and Color Values, MFI, Free Calcium Concentration

These aspects of the investigation were described in Sections 2.3.2 (pages 20).

4.3.3. DNA isolation and SNP Genotyping

These procedures were described in Section 3.3.2 (page 51).

4.3.4. Statistical Analysis

The differences between genotypic frequencies and group categories sex (males and females) and free calcium categories (L = low <5.40, N = normal 5.40 -5.59, H = high 5.59 – 5.86 and DC = > 5.86) were analyzed using contingency tables (Di Rienzo et al., 2011). The analysis of variance was performed using ANOVA Proc Mixed (SAS Inst., Inc., Cary, NC) and the Tukey Kramer test was used for mean difference. Hot carcass weight was used as a co-variable only in WBS analysis.

The linear models used for this review were:

$$Y_{ijkl} = \mu + S_i + A_j + G_k + e_{ijkl}$$

Y_{ijkl} = l observations pertaining sex i (male or female), age categories j based on number of permanent teeth (A = 0-2pt, B = 4-6pt, C = 8pt) and genotype k of either CAPN1 or CAST genes

μ = overall mean

S_i = fixed effect of sex of the animal either male or female

A_j = fixed effect of age categories based on number of permanent teeth, A = 0-2pt, B = 4-6pt and C = 8pt

G_k = fixed effect of genotype of polymorphisms in either CAPN1 or CAST gene

e_{ijkl} = experimental error

$$Y_{ijklm} = \mu + S_i + A_j + P_k + G_l + e_{ijklm}$$

Y_{ijklm} = m observations pertaining the sex i of the animal (male or female), the age categories j based on number of permanent teeth (A = 0-2pt, B = 4-6pt and C = 8pt), pH categories k (L = low <5.40, N = normal 5.40 -5.59, H = high 5.59 – 5.86 and DC = > 5.86) and genotype k of either CAPN1 or CAST genes

μ = overall mean

S_i = fixed effect of sex of the animal either male or female

A_j = fixed effect of age categories based on number of permanent teeth, A = 0-2pt, B = 4-6pt and C = 8pt

P_k = fixed effect of pH categories k (L = low <5.40, N = normal 5.40 -5.59, H = high 5.59 – 5.86 and DC = > 5.86)

G_l = fixed effect of genotype of polymorphisms in either CAPN1 or CAST gene

e_{ijklm} = experimental error

4.4. Results and Discussion

The use of genetic markers has revolutionized animal breeding aimed at increasing desirable traits in a given population. Nine commercially discovered polymorphisms in the CAPN1 and CAST genes have proven to be useful for increasing tenderness in beef cattle. Eleven SNPs, 5331, 530, 306, 316, 208, 302, 310, 445, 524, 4751 and 4753, have been evaluated in the CAPN1 gene (Jusczuk-Kubiak et al., 2004; White et al., 2005; Corva et al., 2007; Avilés et al., 2009; Curi et al., 2010; Pinto et al., 2010). In the present study a significant difference was observed between the genotypes of the marker CAPN1 530 in ultimate pH value 24h postmortem, P = 0.05 (*Table 4.1*). The AA genotype resulted in higher ultimate pH (6.35, P = 0.05). No reported association of genotypes of the CAPN1 530 marker and ultimate pH exists in the literature. The higher pH values found in the present study are believed to be mostly due to environmental stressors or breed influence. Only three animals of the CCP segregated the AA genotype. Low numbers of animals segregating the AA genotype of CAPN1 530 was also reported by Casas et al. (2005), Corva et al. (2007), Van Eenennaam et al. (2007) and Pinto et al. (2010) in cattle of *Bos taurus indicus* descendent. The animals from the UPRCP, like those of CCP, showed no difference in tenderness based by WBS and MFI between the genotypes of the CAPN1 530 marker (*Table 4.1*). White et al. (2005) observed a tendency, P = 0.08, of the AA genotype to decrease WBS (0.39 ± 0.20 kg). Corva et al. (2007) reported an 11.5% higher WBS value when the GG genotype was present. In the present study this marker showed little association with WBS in both CCP and UPRCP.

4.4.1. CAPN1 316 and 530

The CC genotype of marker CAPN1 316 resulted in decreased shear force values, -4.72 kg, P < 0.01, among animals of the CCP (T*able 4.2*). In the UPRM population no significant

difference was observed in either WBS or MFI (T*able 4.2, Figure 4.1*). Only four animals segregated the CC genotype in both the CCP (n = 72) and UPRCP (n = 11). Curi et al. (2010) and Bonilla et al. (2010) did not observe the CC genotype but found a significant differences (P = 0.05) between the GG and GC genotypes of -0.63 kg in WBS in crossbred cattle. Corva et al. (2007) found a 17% decrease in WBS with the CC genotype and an 11% decrease with the GC genotype. White et al. (2005) found a significant difference in WBS involving the combination of CAPN1 530 and CAPN1 316, GG and CC genotypes. In the CCP the combination of the respective GG and CC genotypes of these two markers a numerical advantage of -4.30 kg in WBS was observed compared to the other genotype combinations of these two markers, P = 0.65 (Figure 4.2). The low frequency of the tender CC genotype in the CCP and high frequency in the UPRCP is consistent with that reported by The National Beef Cattle Evaluation Consortium, NBCEC (Van Eenennaam et al., 2007) in *Bos taurus indicus, Bos taurus taurus* and crossbreds.

4.4.2. CAPN1 4751

No significant differences were observed in the CAPN1 4751 marker in either the CCP (P = 0.30) or UPRCP (P = 0.19) in WBS values (T*able 4.3*). The Gene Star Tenderness Panel (Johnston and Graser et al., 2010) reported significant differences in WBS (P < 0.05) in tropically adapted breeds, especially in Brahman cattle, but not in temperate breeds. In the CCP a numerical difference was observed between the TT and TC genotypes of - 1.7kg WBS in-favor of the TC genotype. Bonilla et al. (2010) also failed to find a significant difference between genotypes of the CAPN1 4751 marker in WBS in Mexican cattle. White et al. (2005), also found no significant difference but a decrease of 0.4kg WBS in meat at 14d of aging when the CT genotype segregated, compared to the un-favored TT genotype in Brahman cattle. While Smith et al. (2009) reported a tendency (P < 0.08) of this marker with WBS in Brahman cattle. Although CAPN1 4751 marker was reported by Frylink et al. (2009) and Pinto et al.

(2010) to be significantly correlated with tenderness in multiple crossbreds, this was not evident in the present study.

4.4.3. CAPN1 4753

The marker CAPN1 4753 was associated with the highest free [Ca^{2+}], 143.30 μM (P = 0.01), at 14d of aging when the AA genotype was present, (*Table 4.4*). A significant increase of 77.78 μM (P = 0.03) in the free [Ca^{2+}] was observed with the 14d aging period with the same genotype in the CCP (*Figure 4.3*). In the UPRM population the CAPN1 4753 was fixed in the CC genotype. WBS data were not obtained for the AA genotype in the CCP so effects on tenderness could not be determined. Increase in tenderness with the AA genotype in marker CAPN1 4753 was reported by White et al. (2005), whereas in other studies no significant difference in WBS was reported for the CAPN1 4753 genotypes (Casa et al., 2005; Pinto et al., 2010). This marker showed a correlation with free [Ca^{2+}] during the aging period of the present study. Marker 4751 did not show significant difference in tenderness in both the CCP and UPRCP which indicate no additive association of the genotypes of this marker with tenderness in the populations evaluated (Table 4.3).

4.4.4. CAPN1 5331

The CAPN1 5331 marker was significantly associated with L* color values at 24h of aging (*Table 4.5*), the TT genotype showed the highest L* value, 38.99 (P = 0.05). These differences might be associated more with environmental factors like short term stressors prior to slaughter than with genetic influence in the CCP. Higher color L* values are associated with stressors during handling of the animals and is consistent with PSE meat (Viljoen et al., 2002; Adzitey and Nurul., 2011). Since only one animal segregated this genotype no additive affect of this genotype with the color L* value could be determined. The UPRCP showed a fixed AA

genotype in the CAPN1 5331 marker. No significant difference was observed in WBS due to CAPN1 5331 marker in the CCP. This result was also observed by Casas et al. (2005).

4.4.5. CAST 282

Marker CAST 282 genotype showed no significant difference for the different variables evaluated (*Table 4.6*). A lack of correlation between this marker and WBS (P = 0.42) was also reported by Reardon et al. (2010) just like the results of the present study (P = 0.11) in the CCP. The heterozygous genotype of CAST 282 showed numeric improvement in tenderness based on WBS, -1.23 kg compared to the CC genotype. In the UPRM population the CAST 282 marker was found to be fixed in the heterozygous genotype. The NBCEC (Van Eenennaam et al., 2007) reported a decrease of 0.19 kg in WBS when the CC genotype of CAST 282 was segregated. Pinto et al. (2010) reported WBS decreases of -0.17, -0.21 and -0.25 kg after 7, 14 and 21 days of aging when the CC genotype of CAST 282 segregated compared to the GG genotype in Nellore cattle. Curi et al. (2010) reported a tendency of the CC genotype to produce an increase in tenderness but no significant association was reported between the CAST 282 genotypes and meat tenderness by WBS and MFI in *Bos taurus taurus* and *Bos taurus indicus* crossbreds. Gill et al. (2009) also reported no significant difference between genotypes in CAST 282 marker in *Bos taurus taurus* cattle. The numeric increase in tenderness found in the GG genotype in the present study was not attributed to the segregation of this genotype and could be due to the effect of other polymorphisms influencing tenderness and not an additive effect of the CAST 282 genotype. Despite the fact that this polymorphism is used in a commercial tenderness panel test, Igenity Tender Gene (Merial Ltd., Atlanta, GA), the CC genotype did not show a significant increase in tenderness like that previously reported.

4.4.6. CAST 2959

No significant effect of the CAST 2959 marker on tenderness was observed in CCP. In the UPRCP an increase in tenderness was found with the AA genotype as indicated by WBS at 24h postmortem (P = 0.02) and a tendency observed by MFI at 14d of aging (P = 0.06) as seen in *Table 4.7*. Differences of -2.33 kg in WBS and 20.63 in MFI were observed when the AA genotype segregated in the UPRCP, while the GG genotype was not observed in this population. The NBCEC reported a decrease of 0.15 kg of WBS when the tender A allele was present (Van Eenennaam et at., 2007). Gene Star Tenderness Panel (Johnston and Graser et al., 2010) reported significant differences between WBS and marker CAST 2959 (P < 0.01) in both temperate and tropical breeds. Casas et al. (2006) also reported an increase in tenderness (-0.48 kg WBS at 14d of aging) when the AA genotype segregated in a cattle population with predominantly *Bos taurus indicus* influence. In the present study the CAST 2959 marker showed greater association with tenderness in *Bos taurus taurus* crossbreds than in *Bos taurus indicus* crossbreds.

4.4.7. CAST 3016

The marker CAST 3016, also known as CAST Brahman, was fixed in the TT genotype in the UPRCP and in the CCP no significant difference in tenderness was observed among genotypes (*Table 4.8*). A fixed segregation was also reported by Frylinck et al. (2009). The genotypes of marker CAST UPRM/MSU showed no significant differences in both CCP and UPRCP (Tables 4.9). The absent of significant differences in WBS observed among the genotypes of these two CAST markers indicate no additive association with tenderness in the population evaluated.

4.4.8. SNPs found in exon and intron 14 in the CAPN1 gene

A total of six additional polymorphisms were evaluated in the 568 base fragment containing the marker CAPN1 530 (*Table 4.10 to 4.14)*. One of these SNP did not segregate

the different genotypes in the commercial cattle population, SNP 351 (a transition of G/A) was fixed in the GG genotype. SNP 175 (transition of C/T) was significantly associated with the color a* values, the CC genotype producing a brighter red color of meat (table 4.10). The SNP 191 (transversion of A/C) presented a numeric decrease in WBS of -2.63 kg when the CC genotype segregated in the CCP, but this difference was not significant (P = 0.34). A numeric increase in free [Ca^{2+}] of 16.87 µM during the 24h to 14d aging period was also observed for the same CC genotype compared to the other AA and AC genotypes (4.41 and 9.98 µM, respectively) and yet no significant difference was observed in the free [Ca^{2+}] at both aging periods. SNP 306 (transition of C/T) previously identified by Juszczuk-Kubiak et al. (2004), was significantly correlated with free [Ca^{2+}]. The TT genotype showed the lowest [Ca^{2+}] at both 24h (40.92 µM, P = 0.03) and 14d (45.84 µM, P = 0.06) postmortem, (*Table 4.13)*. No significant difference was observed in WBS among the SNP 306 genotypes. A tendency of increased tenderness was observed in the INS/DEL of CGAT in SNP 354 (P = 0.09, *Table 4.14)*. The INS and DEL of the four nucleotides produced lower WBS force than the heterozygous genotype (6.62, 5.18 and 8.28kg for INS, DEL and INS/DEL respectively) in the CCP. In the UPRCP the SNP 351 (transition of G/A) was significantly correlated with tenderness, the GG genotype had lower WBS 24h postmortem (- 3.35 kg, P = 0.03) compared to the heterozygous (7.78kg, *Table 4.15*). SNP 209 (transition of G/A) showed greater MFI values at 14d of aging, when the AA genotype was present (45.45, P = 0.03). The tender AA genotype of SNP 209 did not segregate in the CCP and the GG genotype of SNP 351 was fixed in the same population. The SNP 209 is a candidate marker for determining tenderness in cattle with mostly Bos taurus taurus influence.

4.4.9. SNPs found in intron 8-9 and exon 10 in the CAPN1 gene

The marker 316 is a good indicator of increased tenderness but its low frequency segregation in animals with Bos taurus indicus influence limits its potential usefulness. In the

735 base fragment amplified to determine the CAPN1 316 genotype, 7 additional SNP were analyzed. Avilés et al. (2009) previously identified five of these SNPs, 208 C/T, 430 C/G, 438 G/A, 573 C/T and 652 A/C. The additional two SNP, 212 T/G and 643 INS/DEL C, were newly identified in the CCP. Six of these SNP were significantly correlated with free [Ca^{2+}] of meat at 14d of aging, SNPs 208, 430, 438, 573, 643 and 652 in the CCP (*Table 4.16* and *4.18* to *4.22).* SNP 208 (transition of C/T) produced an increase of 40.9 ± 4.2 µM of free [Ca^{2+}] 24h postmortem when the TT genotype was present (P = 0.02, *Table 4.16*) and was significantly different from the CC genotype but not the heterozygous genotype. Notably an increase in free [Ca^{2+}] was not observed during 14d of aging for the TT genotype and did not differ significantly from the other genotypes. However the heterozygous genotype of the same SNP showed a significant increase in free [Ca^{2+}] at 14d postmortem compared to the CC genotype (P = 0.01). In addition a numerical difference was observed in WBS when the TT genotype of SNP 208 segregated, showing an improved tenderness of - 2.2 kg WBS (P = 0.67). Genotypes of SNP 208 also showed significant differences in the color values a* and b* in CCP. The CT genotype produced a significant increase in the color red (a* value increase of 1.49, P = 0.02), and increased marbling (b* 1.05, P = 0.03), at 24h postmortem compared to the CC genotype but not the TT genotype. SNP 430 (transversion of C/G) was significantly correlated with both WBS (*Table 4.18* and *Figure 4.4*), and free [Ca^{2+}] at 14d of aging. The GG genotype produced lower WBS, - 5.14 kg (P = 0.03) compared to the other genotypes and higher free [Ca^{2+}] at 14d of aging (98.53 µM, P = 0.01) compared to the CC genotype. Also a significant difference in the color a* and b* value were also observed in the 430 SNP. The CG genotype showed an increase of 1.44 in a* value compared to the CC genotype (P = 0.02), in the CCP, (*Table 4.18).* This increase results in an increase in the color red meat. When the CC genotype segregated significantly lower b* value was observed (11.50) compared to the CC and CG genotypes of the same SNP (P = < 0.01). This could indicate lower degree of marbling when the CC genotype segregates in the 430 SNP in the CCP. SNP 438 (transition from G/A) also was associated with

the color a* and b* values at 24h postmortem (*Table 4.19*). The AA genotype showed a more intense red color (increase of 2.30, P = 0.03) and greater marbling (more yellow) (increase of 2.44, P < 0.01) compared to the other genotypes in CCP. A significant association with free [Ca^{2+}] at both 24h and 14d postmortem was also observed in the 438 SNP. The AA genotype showed the highest [Ca^{2+}] (95.12 μM) but differed significantly only from the GG genotype but not the heterozygous genotype (P = 0.02). The contrary was observed in the free [Ca^{2+}] at 14d postmortem, the heterozygous GA genotype produced a significant increase of 18 μM compared to the GG genotype but showed no significant difference from the AA genotype of the same SNP (P = 0.01). Another SNP 573 (transition of C/T) showed a significant improvement in tenderness based on WBS when the TT genotype segregated in the CCP (*Table 4.20*). A decrease of – 5.30 kg (P = 0.02) was observed in addition to an increase of free [Ca^{2+}] at 14d postmortem to 98.93 μM (P = 0.01). The difference in free [Ca^{2+}] was significant only between genotypes TT and CC (*Figure 4.5*). Improved color a* and b* values (P =0.03 and P < 0.01 respectively) 24h postmortem were also observed in the SNP 573 when the TT and CT genotypes segregated in the CCP. SNP 643 (INS/DEL of cytosine) and SNP 652 (transversion of A/C) also showed improvement in color a* and b* values 24h postmortem when the INS and INS/DEL segregated in SNP 643 (P = 0.04 and 0.03 for a* and b* respectively) and when the CC genotype segregated in the SNP 652 (CC genotype differed significantly only from the AA genotype, P = 0.04 and 0.02 for a* and b* respectively) (*tables 4.21 and 4.22*). These same two respective SNP increased free [Ca^{2+}] at 14d of aging to 104.59 μM (P < 0.01) in the SNP 643 and 74.91 μM (P = 0.01) in the SNP 652. These differences in free [Ca^{2+}] differed significantly only from the undesirable tough genotype in both SNP but not from the heterozygous genotypes. The INS of C in SNP 643 produced the greatest decrease in WBS, - 7.74 ± 0.24 kg compared to the other polymorphisms evaluated in both the CAPN1 and CAST genes and this was associated with the greatest release of calcium during the 14d aging period, 47.30 ± 4.16 μM, P < 0.01, (*Figure 4.6*).

Significant differences were observed in frequencies of the tender allele of the seven newly identified SNPs in the CAPN1 gene among sex (*Figure 4.8*). Females showed higher segregation of the tender allele in all SNPs except SNP 212 (P = 0.48). SNPs 573 (P = 0.04) and 652 (P = <0.01) showed the greatest difference among gender and SNPs 208 (P = 0.13), 430 (P = 0.07), 438 (P = 0.06) and 643 (0.13) showed a tendency in segregation of the tender allele. No significant differences between sexes were observed in the segregation of the tender allele among the other polymorphisms studied in both CAPN1 and CAST genes. The differences observed among the females and the males in WBS in the CCP (-3.14 kg, P < 0.01) is due to the increase segregation of the tender alleles. These polymorphisms have not been reported to be sex oriented.

No significant difference was observed in the UPRCP between the SNP genotypes in the 735 base fragment containing marker CAPN1 316 (*Table 4.23)* except for an effect of SNP 652 on WBS (-4.16 ± 0.43) when the heterozygous genotype segregated. This scarcity of significant association of the SNPs found in introns 8 and 9 of the CAPN1 gene in the present study in the UPRCP as opposed to the significant difference observed among genotypes in the CCP indicates a greater additive association of the SNPs with tenderness in *Bos taurus indicus* and crossbred populations than in *Bos taurus taurus* population.

4.4.10 Haplotypes of SNPs found in intron 8-9 and exon 10 in the CAPN1 gene

The new SNPs from the CAPN1 316 fragment segregated into three haplotypes in the CCP and five in the UPRCP (*Table 4.24*). In the CCP color differences at 24h postmortem were observed among haplotypes, the B haplotype giving a less intense red color, - 1.28 ± 0.25 a* value (P = 0.05). Also a significantly lower free [Ca^{2+}] at 14d of aging, - 18.74 ± 0.86 μM (P = 0.01), was observed for the B haplotype. No significant difference was observed between haplotypes in tenderness based on WBS in the CCP and yet a tendency was observed in the

UPRCP, - 3.94 ± 0.75 kg (P = 0.10), for the C haplotype. The tender genotype CC for CAPN1 316 marker was not present in the CCP but the C haplotype exerted an effect.

4.4.11 SNPs found in the 3' UTR of the CAST gene

Calpastatin is the endogenous inhibitor of calpain. Polymorphisms found in the CAST gene have been correlated with increase tenderness. Two new polymorphisms were found in the 170 base fragment amplified for CAST 2959 and 3016 (*Table 4.25 to 4.27*). The recessive homozygous genotype was absent in both new CAST SNPs in the CCP and UPRCP. SNP 94 is a transition of T/C and the SNP 122 is a transversion of A/C. No significant difference was observed between genotypes, but a numeric difference in WBS of - 1.35 was seen in the TC heterozygous of SNP 94 in the CCP. The AA genotype of SNP 122, gave a significantly lower WBS (-3.14 kg, P < 0.01) in the CCP (*Figure 7*). The AA genotype of this SNP also resulted in a 0.19 increase in ultimate pH value at both 24h (P = 0.01) and 14d (P = 0.03) of aging. Another SNP found in exon 2 of the CAST gene was previously shown to be related to tenderness in Puerto Rican cattle, CAST UPRM/MSU. In the present study significant differences between CAST UPRM/MSU genotypes in tenderness were not found in the CCP and UPRCP (*Table 4.9*).

4.5. Conclusion

Commercially reported polymorphisms associated with tenderness in the CAPN1 and CAST genes did not show significant effects in the current study, except for the tender CC genotype for CAPN1 316 in the CCP and the tender AA genotype of SNP CAST 2959 in the UPRCP. Candidate polymorphisms found in the CAPN1 and CAST genes showed a higher association with tenderness in both the CCP and UPRCP than the polymorphisms reported by White et al., (2005); Casas et al., (2005); (2006); Corva et al., (2007); Ven Eenennaam et al., (2007) and Pinto et al., (2010). SNPs 430, 573, and 643 found in the CAPN1 gene and SNP

122 found in the CAST gene showed significant differences in WBS at 24h postmortem in the CCP when the allele G, T, Ins C and A segregated for each SNP respectively. In an analogous manner the SNPs 209, 351, 652 found in the CAPN1 gene and SNP 94 found in the CAST gene showed significant difference in WBS in the UPRCP when the allele A, G, C and T segregated for each SNP respectively. The candidate markers near the CAPN1 316 marker showed increased association with tenderness in a population with greater *Bos turus indicus* influence. These markers show greater implications for use in a *Bos taurus indicus* population although their frequencies were higher in the *Bos taurus taurus* population. Since the *Bos taurus taurus* population included in this analysis was relatively small compared to the crossbred population further verification of the usefulness of these markers in *Bos taurus taurus* cattle is in order. The candidate markers near CAPN1 530 marker were more closely associated with tenderness in the population with increased *Bos taurus taurus* influence. To increase tenderness in the CCP a breeding program incorporating more UPRCP influence could increase the frequency of the tender alleles and thus improve overall meat tenderness.

4.6. References

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CAPN1 530 CCP				
Variables	**GG n = 36**	**GA n = 32**	**AA n = 3**	**p value**
Hot Carcass Weight	514.39 ± 21.71	481.77 ± 23.28	475.16 ± 77.23	0.60
pH 24 hour Aging	5.68 ± 0.08 [b]	5.83 ± 0.08 [b]	6.35 ± 0.27 [a]	**0.05**
pH 14 days Aging	5.69 ± 0.08	5.83 ± 0.08	6.36 ± 0.27	0.06
Color Values 24 hour Aging				
L*	30.69 ± 0.82	31.56 ± 0.88	29.63 ± 2.92	0.69
a*	15.77 ± 0.47	15.55 ± 0.51	13.16 ± 1.68	0.34
b*	11.85 ± 0.35	11.73 ± 0.38	9.37 ± 1.25	0.17
Color Values 14 days Aging				
L*	33.03 ± 0.68	32.70 ± 0.73	30.81 ± 2.42	0.68
a*	14.79 ± 0.46	15.57 ± 0.49	12.37 ± 1.64	0.13
b*	12.31 ± 0.35	12.59 ± 0.37	10.29 ± 1.23	0.19
WBS 24 hour Aging kg	7.76 ± 0.86	7.36 ± 0.83	4.47 ± 2.40	0.46
[Calcium] 24 hour Aging uM	58.20 ± 3.70	52.70 ± 3.97	33.12 ± 13.18	0.17
[Calcium] 14 days Aging uM	70.84 ± 4.52	59.63 ± 4.83	37.13 ± 16.09	0.08

[abc] Means within a row lacking common superscript letter differ significantly

CAPN1 530 UPRCP				
Variable	**GG n = 11**	**GA n = 7**	**AA n = 4**	**p value**
MFI	39.22 ± 5.87	41.84 ± 7.68	37.60 ± 10.30	0.92
WBS	4.76 ± 0.62	4.05 ± 0.81	6.24 ± 1.09	0.20

Table 4.1. Means of genotypes of marker CAPN1 530 for different variables in Commercial and UPR Cattle Populations

CAPN1 316 CCP				
Variables	**GG n = 43**	**GC n = 20**	**CC n = 4**	**p value**
Hot Carcass Weight	503.65 ± 16.49	490.59 ± 25.69	535.92 ± 55.99	0.77
pH 24 hour Aging	5.73 ± 0.06	5.66 ± 0.09	5.93 ± 0.19	0.48
pH 14 days Aging	5.73 ± 0.05	5.64 ± 0.09	5.86 ± 0.09	0.50
Color Values 24 hour Aging				
L*	30.42 ± 0.57	31.62 ± 0.88	32.59 ± 1.92	0.32
a*	15.47 ± 0.34	16.92 ± 0.54	16.58 ± 1.17	0.07
b*	11.49 ± 0.30	12.80 ± 0.47	12.28 ± 1.02	0.06
Color Values 14 days Aging				
L*	33.09 ± 0.61	34.73 ± 0.94	35.47 ± 2.06	0.22
a*	14.90 ± 0.38	15.44 ± 0.59	15.33 ± 1.30	0.74
b*	12.37 ± 0.28	13.07 ± 0.43	12.59 ± 0.94	0.40
WBS 24 hour Aging kg	9.00 ± 0.63 [a]	9.40 ± 1.08 [a]	4.48 ± 1.34 [b]	**0.01**
[Calcium] 24 hour Aging uM	51.09 ± 2.86	60.05 ± 4.46	47.95 ± 9.72	0.23
[Calcium] 14 days Aging uM	57.25 ± 3.76	73.66 ± 5.85	61.78 ± 12.75	0.07

[abc] Means within a row lacking common superscript letter differ significantly

CAPN1 316 UPRCP				
Variable	**GG n = 4**	**GC n = 2**	**CC n = 5**	**p value**
MFI	39.73 ± 8.27	15.96 ± 15.25	37.95 ± 12.27	0.36
WBS	5.96 ± 0.80	6.18 ± 1.48	4.58 ± 1.19	0.52

Table 4.2. Means of genotypes of marker CAPN1 316 for different variables in Commercial and UPR Cattle Populations

CAPN1 4751 CCP				
Variables	TT n = 53	TC n = 14	CC n = 23	p value
Hot Carcass Weight	505.18 ± 15.84	490.70 ± 30.26	525.89 ± 24.62	0.65
pH 24 hour Aging	5.74 ± 0.05	5.89 ± 0.10	5.76 ± 0.08	0.41
pH 14 days Aging	5.72 ± 0.05	5.98 ± 0.10	5.72 ± 0.08	0.08
Color Values 24 hour Aging				
L*	30.86 ± 0.59	30.22 ± 1.12	33.24 ± 0.91	0.06
a*	16.20 ± 0.36	15.86 ± 0.69	15.28 ± 0.56	0.40
b*	12.02 ± 0.30	11.68 ± 0.53	12.19 ± 0.43	0.76
Color Values 14 days Aging				
L*	33.21 ± 0.55	32.25 ± 1.04	34.57 ± 0.85	0.21
a*	15.05 ± 0.37	15.18 ± 0.71	16.21 ± 0.58	0.26
b*	12.63 ± 0.27	11.79 ± 0.51	13.36 ± 0.41	0.07
WBS 24 hour Aging kg	8.48 ± 0.61	6.78 ± 1.08	7.57 ± 1.08	0.30
[Calcium] 24 hour Aging uM	54.20 ± 2.68	51.24 ± 5.12	51.44 ± 4.17	0.80
[Calcium] 14 days Aging uM	68.28 ± 3.74	58.83 ± 7.14	61.79 ± 5.81	0.41

CAPN1 4751 UPCP				
Variable	TT n = 7	TC n = 11	CC n = 4	p value
MFI	40.26 ± 7.00	39.14 ± 5.42	40.57 ± 9.19	0.99
WBS	3.66 ± 0.74	5.45 ± 0.57	4.75 ± 0.97	0.19

Table 4.3. Means of genotypes of marker CAPN1 4751 for different variables in Commercial and UPR Cattle Populations

CAPN1 4753 CCP				
Variables	CC n = 78	CA n = 21	AA n = 1	p value
Hot Carcass Weight	505.68 ± 13.17	514.17 ± 24.42	432.79 ± 117.40	0.79
pH 24 hour Aging	5.75 ± 0.04	5.74 ± 0.08	5.32 ± 0.39	0.54
pH 14 days Aging	5.75 ± 0.04	5.75 ± 0.08	5.26 ± 0.39	0.46
Color Values 24 hour Aging				
L*	31.50 ± 0.49	30.31 ± 0.94	31.50 ± 4.33	0.54
a*	15.81 ± 0.30	15.69 ± 0.57	15.69 ± 2.65	0.88
b*	11.91 ± 0.24	11.54 ± 0.46	13.15 ± 2.10	0.64
Color Values 14 days Aging				
L*	33.75 ± 0.43	32.20 ± 0.83	35.56 ± 3.82	0.22
a*	15.34 ± 0.30	15.16 ± 058	11.96 ± 2.67	0.44
b*	12.74 ± 0.22	12.16 ± 0.42	12.51 ± 1.96	0.49
WBS 24 hour Aging kg	8.20 ± 0.55	7.93 ± 0.95	--	0.79
[Calcium] 24 hour Aging uM	54.43 ± 2.26	52.94 ± 4.36	56.61 ± 20.13	0.95
[Calcium] 14 days Aging uM	63.30 ± 2.90 [b]	61.88 ± 5.59 [b]	143.30 ± 25.82 [a]	**0.01**

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.4. Means of genotypes of marker CAPN1 4753 for different variables in Commercial Cattle Population

CAPN1 5331 CCP				
Variables	**AA n = 30**	**AT n = 19**	**TT n = 1**	**p value**
Hot Carcass Weight	502.84 ± 17.62	524.45 ± 22.47	492.13 ± 99.15	0.74
pH 24 hour Aging	5.84 ± 0.07	5.66 ± 0.09	5.08 ± 0.38	0.06
pH 14 days Aging	5.83 ± 0.07	5.66 ± 0.09	5.00 ± 0.38	0.06
Color Values 24 hour Aging				
L*	30.72 ± 0.66 [b]	29.60 ± 0.84 [b]	38.99 ± 3.71 [a]	**0.05**
a*	15.55 ± 0.44	15.96 ± 0.56	15.81 ± 2.46	0.84
b*	11.51 ± 0.38	12.00 ± 0.49	13.94 ± 2.16	0.44
Color Values 14 days Aging				
L*	32.70 ± 0.77	33.66 ± 0.98	30.99 ± 4.31	0.67
a*	15.70 ± 0.44	14.36 ± 0.56	11.79 ± 2.49	0.08
b*	12.79 ± 0.31	11.82 ± 0.40	9.13 ± 1.76	0.06
WBS 24 hour Aging kg	7.96 ± 0.99	8.64 ± 1.11	---	0.57
[Calcium] 24 hour Aging uM	52.20 ± 3.65	58.23 ± 4.65	52.48 ± 20.53	0.59
[Calcium] 14 days Aging uM	62.52 ± 5.03	66.77 ± 6.41	61.02 ± 28.30	0.87

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.5. Means of genotypes of marker CAPN1 5331 for different variables in Commercial Cattle Population

CAST 282 CCP				
Variables	CC n = 46	CG n = 47	GG n = 8	p value
Hot Carcass Weight	510.60 ± 17.18	497.22 ± 17.16	503.85 ± 41.93	0.86
pH 24 hour Aging	5.72 ± 0.06	5.74 ± 0.06	59.02 ± 0.14	0.47
pH 14 days Aging	5.72 ± 0.06	5.74 ± 0.06	5.86 ± 0.14	0.63
Color Values 24 hour Aging				
L*	30.38 ± 0.61	32.62 ± 0.61	30.13 ± 1.49	0.06
a*	16.14 ± 0.39	15.38 ± 0.38	15.87 ± 0.94	0.38
b*	11.94 ± 0.31	11.82 ± 0.31	11.95 ± 0.75	0.96
Color Values 14 days Aging				
L*	32.97 ± 0.56	34.12 ± 0.56	33.29 ± 1.36	0.34
a*	14.77 ± 0.39	15.82 ± 0.39	15.04 ± 0.95	0.16
b*	12.35 ± 0.28	12.98 ± 0.28	12.47 ± 0.70	0.28
WBS 24 hour Aging kg	9.04 ± 0.69	7.81 ± 0.67	6.12 ± 1.30	0.11
[Calcium] 24 hour Aging uM	57.52 ± 2.88	52.49 ± 2.88	43.91 ± 7.03	0.16
[Calcium] 14 days Aging uM	70.43 ± 3.84	59.38 ± 3.83	50.46 ± 9.36	0.06

Table 4.6. Means of genotypes of marker CAST 282 for different variables in Commercial Cattle Population

CAST 2959 CCP				
Variables	**AA n = 69**	**AG n = 19**	**GG n = 1**	**p value**
Hot Carcass Weight	514.57 ± 12.99	470.86 ± 24.96	812.98 ± 109.26	0.01
pH 24 hour Aging	5.73 ± 0.05	5.77 ± 0.09	5.96 ± 0.40	0.78
pH 14 days Aging	5.72 ± 0.05	5.80 ± 0.09	5.95 ± 0.41	0.64
Color Values 24 hour Aging				
L*	32.04 ± 0.52	30.30 ± 1.00	29.27 ± 4.36	0.26
a*	15.80 ± 0.31	14.97 ± 0.60	20.85 ± 2.70	0.09
b*	12.07 ± 0.24	11.17 ± 0.46	13.45 ± 2.00	0.17
Color Values 14 days Aging				
L*	33.77 ± 0.48	32.72 ± 0.92	30.79 ± 4.04	0.48
a*	15.32 ± 0.32	15.59 ± 0.62	10.41 ± 2.70	0.18
b*	12.88 ± 0.24	12.08 ± 0.45	10.38 ± 1.98	0.15
WBS 24 hour Aging kg	8.37 ± 0.57	6.73 ± 0.98	5.70 ± 3.03	0.17
[Calcium] 24 hour Aging uM	53.69 ± 2.41	55.65 ± 4.62	19.92 ± 20.24	0.24
[Calcium] 14 days Aging uM	64.53 ± 2.86	60.09 ± 5.89	20.29 ± 24.03	0.16

CAST 2959 UPRCP				
Variable	**AA n = 17**	**AG n = 3**	**GG n = 0**	**p value**
MFI	42.67 ± 3.95	22.03 ± 9.36	--	0.06
WBS	4.53 ± 0.43 [b]	7.31 ± 1.03 [a]	--	**0.02**

[abc] Means within a row lacking common superscript letter differ significantly

Table 4. 7. Means of genotypes of marker CAST 2959 for different variables in Commercial and UPR Cattle Populations

CAST 3016 CCP				
Variables	**AA n = 78**	**AT n = 13**	**TT n = 2**	**p value**
Hot Carcass Weight	506.48 ± 13.30	502.46 ± 32.95	457.97 ± 82.77	0.84
pH 24 hour Aging	5.76 ± 0.05	5.82 ± 0.11	5.36± 0.28	0.33
pH 14 days Aging	5.76 ± 0.05	5.81 ± 0.11	5.38 ± 0.28	0.37
Color Values 24 hour Aging				
L*	31.42 ± 0.50	31.11 ± 1.24	30.63 ± 3.12	0.84
a*	15.62 ± 0.30	16.04 ± 0.75	14.19 ± 1.88	0.64
b*	11.78 ± 0.23	12.41 ± 0.58	9.84 ± 1.46	0.23
Color Values 14 days Aging				
L*	33.27 ± 0.44	34.57 ± 1.10	34.36 ± 2.77	0.54
a*	15.39 ± 0.30	15.36 ± 0.75	13.01 ± 1.89	0.46
b*	12.66 ± 0.22	12.86 ± 0.56	11.28 ± 1.40	0.57
WBS 24 hour Aging kg	7.89 ± 0.62	7.74 ± 1.16	10.53 ± 2.41	0.53
[Calcium] 24 hour Aging uM	53.05 ± 2.34	52.78 ± 5.80	62.91 ± 14.56	0.80
[Calcium] 14 days Aging uM	61.61 ± 2.83	57.34 ± 7.00	80.27 ± 17.59	0.47

Table 4.8. Means of genotypes of marker CAST 3016 for different variables in Commercial Cattle Population

CAST UPRM/MSU CCP				
Variables	DEL n = 71	INS/DEL n = 22	INS n = 2	p value
Hot Carcass Weight	510.44 ± 14.17	494.96 ± 24.05	415.55 ± 84.80	0.50
pH 24 hour Aging	5.73 ± 0.05	5.80 ± 0.09	5.98 ± 0.30	0.61
pH 14 days Aging	5.72 ± 0.05	5.82 ± 0.09	6.04 ± 0.30	0.38
Color Values 24 hour Aging				
L*	31.24 ± 0.53	31.61 ± 0.94	29.75 ± 3.20	0.84
a*	16.15 ± 0.31	15.18 ± 0.55	12.90 ± 1.86	0.09
b*	12.09 ± 0.25	11.52 ± 0.44	9.73 ± 1.50	0.19
Color Values 14 days Aging				
L*	33.22 ± 0.46	34.03 ± 0.82	31.34 ± 2.76	0.52
a*	15.34 ± 0.33	15.02 ± 0.57	17.06 ± 1.95	0.59
b*	12.55 ± 0.24	12.72 ± 0.42	12.77 ± 1.42	0.93
WBS 24 hour Aging kg	8.31 ± 0.60	6.95 ± 1.08	5.67 ± 3.52	0.38
[Calcium] 24 hour Aging uM	54.79 ± 2.40	53.20 ± 4.24	49.54 ± 14.34	0.90
[Calcium] 14 days Aging uM	66.85 ± 3.14	57.07 ± 5.56	48.62 ± 18.82	0.23

CAST SSCP UPRCP				
Variable	DEL C n = 16	INS/DEL C n = 6	INS n = 0	p value
MFI	41.75 ± 4.54	34.97 ± 7.14	--	0.44
WBS	4.76 ± 0.53	4.86 ± 0.84	--	0.93

Table 4.9. Means of genotypes of marker CAST UPRM/MSU for different variables in Commercial and UPR Cattle Populations.

SNP 175 CCP				
Variables	**CC n = 36**	**CT n = 35**	**TT n = 0**	**p value**
Hot Carcass Weight	504.58 ± 21.64	492.19 ± 21.86	--	0.70
pH 24 hour Aging	5.82 ± 0.08	5.72 ± 0.08	**--**	0.40
pH 14 days Aging	5.81 ± 0.08	5.74 ± 0.08	**--**	0.52
Color Values 24 hour Aging				
L*	30.47 ± 0.81	31.63 ± 0.82	**--**	0.33
a*	16.29 ± 0.45 [a]	14.96 ± 0.45 [b]	**--**	**0.05**
b*	11.95 ± 0.36	11.47 ± 0.36	**--**	0.37
Color Values 14 days Aging				
L*	32.52 ± 0.68	33.10 ± 0.68	**--**	0.57
a*	15.06 ± 0.47	15.05 ± 0.48	**--**	0.99
b*	12.32 ± 0.35	12.43 ± 0.35	**--**	0.83
WBS 24 hour Aging kg	7.04 ± 0.78	7.73 ± 0.88	**--**	0.54
[Calcium] 24 hour Aging uM	55.82 ± 3.77	53.94 ± 3.81	**--**	0.74
[Calcium] 14 days Aging uM	70.31 ± 4.56	58.89 ± 4.61	**--**	0.09

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.10. Means of genotypes of SNP 175, found in exon 14 of the CAPN1 gene (transition of cytosine to thymine; base 4554 accession number AF252505), for different variables in Commercial Cattle Population. This SNP is not a misscence mutation, both alleles codify for aspartic acid.

SNP 191 CCP				
Variables	AA n = 6	AC n = 71	CC n = 2	p value
Hot Carcass Weight	477.72 ± 52.51	500.61 ± 14.91	503.67 ± 89.86	0.91
pH 24 hour Aging	5.81 ± 0.19	5.76 ± 0.05	5.99 ± 0.32	0.76
pH 14 days Aging	5.85 ± 0.19	5.77 ± 0.05	5.90 ± 0.32	0.86
Color Values 24 hour Aging				
L*	32.20 ± 1.93	31.03 ± 0.55	32.98 ± 3.30	0.74
a*	15.31 ± 1.12	15.63 ± 0.32	15.07 ± 1.92	0.93
b*	12.06 ± 0.86	11.64 ± 0.25	12.52 ± 1.48	0.78
Color Values 14 days Aging				
L*	32.96 ± 1.66	32.96 ± 0.47	33.05 ± 2.84	1.00
a*	15.26 ± 1.18	15.19 ± 0.34	15.22 ± 2.02	1.00
b*	13.10 ± 0.87	12.44 ± 0.25	12.99 ± 1.49	0.73
WBS 24 hour Aging kg	9.43 ± 1.59	7.43 ± 0.58	5.80 ± 2.23	0.34
[Calcium] 24 hour Aging uM	53.50 ± 8.97	54.76 ± 2.55	45.62 ± 15.35	0.84
[Calcium] 14 days Aging uM	57.91 ± 11.13	64.74 ± 3.16	62.49 ± 19.04	0.84

Table 4.11. Means of genotypes of SNP 191, found in exon 14 of the CAPN1 gene (transversion of adenine to cytosine; base 4570 accession number AF252505), for different variables in Commercial Cattle Population. This missence mutation changes the amino acid sequence from isoleucine to leucine.

SNP 209 CCP				
Variables	**GG n = 84**	**GA n = 8**	**AA n = 0**	**p value**
Hot Carcass Weight	496.30 ± 12.54	541.13 ± 41.29	--	0.30
pH 24 hour Aging	5.78 ± 0.04	5.56 ± 0.15	--	0.15
pH 14 days Aging	5.78 ± 0.04	5.54 ± 0.14	--	0.12
Color Values 24 hour Aging				
L*	31.17 ± 0.48	31.75 ± 1.59	--	0.72
a*	15.77 ± 0.28	14.81 ± 0.93	--	0.33
b*	11.84 ± 0.23	11.13 ± 0.75	--	0.36
Color Values 14 days Aging				
L*	33.04 ± 0.41	34.53 ± 1.34	--	0.29
a*	15.22 ± 0.29	15.44 ± 0.97	--	0.83
b*	12.47 ± 0.21	12.98 ± 0.71	--	0.49
WBS 24 hour Aging kg	7.58 ± 0.54	8.93 ± 1.32	--	0.31
[Calcium] 24 hour Aging uM	53.88 ± 2.19	56.35 ± 7.20	--	0.74
[Calcium] 14 days Aging uM	63.67 ± 2.80	55.18 ± 9.21	--	0.38

Table 4.12. Means of genotypes of SNP 209, found in intron 14 of the CAPN1 gene (transition of guanine to adenine; base 4588 accession number AF252505), for different variables in Commercial Cattle Population.

SNP 306 CCP				
Variables	CC n = 41	CT n = 37	TT n = 11	p value
Hot Carcass Weight	509.89 ± 18.49	504.07 ± 19.73	464.07 ± 36.79	0.55
pH 24 hour Aging	5.69 ± 0.06	5.79 ± 0.07	5.97 ± 0.13	0.15
pH 14 days Aging	5.69 ± 0.06	5.78 ± 0.07	5.96 ± 0.13	0.16
Color Values 24 hour Aging				
L*	31.21 ± 0.70	31.01 ± 0.75	31.29 ± 1.40	0.98
a*	15.65 ± 0.42	15.85 ± 0.44	15.41 ± 0.83	0.88
b*	11.73 ± 0.33	11.93 ± 0.35	11.41 ± 0.66	0.77
Color Values 14 days Aging				
L*	33.52 ± 0.59	32.62 ± 0.63	32.70 ± 1.17	0.57
a*	15.38 ± 0.43	14.95 ± 0.46	15.77 ± 0.86	0.65
b*	12.80 ± 0.31	12.10 ± 0.33	12.70 ± 0.62	0.31
WBS 24 hour Aging kg	7.47 ± 0.88	7.96 ± 0.74	6.34 ± 1.19	0.51
[Calcium] 24 hour Aging uM	59.39 ± 3.12 [a]	52.68 ± 3.33 [a]	40.92 ± 6.21 [b]	**0.03**
[Calcium] 14 days Aging uM	68.23 ± 4.13 [a]	64.09 ± 4.41 [a]	45.84 ± 8.23 [b]	**0.06**

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.13. Means of genotypes of SNP 306, found in intron 14 of the CAPN1 gene (transition of cytosine to thymine; base 4685 accession number AF252505), for different variables in Commercial Cattle Population.

SNP 354 CCP				
Variables	INS n = 38	INS/DEL n = 43	DEL n = 9	p value
Hot Carcass Weight	500.08 ± 19.19	505.30 ± 18.20	500.08 ± 39.20	0.98
pH 24 hour Aging	5.82 ± 0.07	5.72 ± 0.06	5.75 ± 0.14	0.56
pH 14 days Aging	5.81 ± 0.07	5.72 ± 0.06	5.71 ± 0.14	0.57
Color Values 24 hour Aging				
L*	32.24 ± 0.73	30.86 ± 0.69	29.58 ± 1.49	0.19
a*	15.31 ± 0.43	16.25 ± 0.40	15.56 ± 0.87	0.29
b*	11.82 ± 0.35	12.04 ± 0.33	11.56 ± 0.71	0.79
Color Values 14 days Aging				
L*	33.36 ± 0.63	33.09 ± 0.60	33.23 ± 1.29	0.96
a*	15.57 ± 0.44	14.94 ± 0.42	15.95 ± 0.90	0.45
b*	12.69 ± 0.33	12.40 ± 0.31	12.91 ± 0.68	0.72
WBS 24 hour Aging kg	6.62 ± 0.73	8.28 ± 1.84	5.18 ± 0.71	0.09
[Calcium] 24 hour Aging uM	55.59 ± 3.36	52.02 ± 3.19	58.67 v 6.86	0.59
[Calcium] 14 days Aging uM	58.53 ± 4.39	67.48 ± 4.16	66.87 ± 8.97	0.32

Table 4.14. Means of genotypes of SNP 354, found in intron 14 of the CAPN1 gene (INS/DEL CGAT; base 4733 accession number AF252505), for different variables in Commercial Cattle Population.

SNP 175 UPRCP				
Variable	CC n = 17	CT n = 4	TT n = 1	p value
MFI	38.14 ± 5.10	43.69 ± 10.27	44.50 ± 18.55	0.89
WBS	5.21 ± 0.56	4.05 ± 1.13	2.34 ± 2.05	0.41

SNP 209 UPRCP				
Variable	GG n = 19	GA n = 2	AA n = 1	p value
MFI	38.42 ± 3.45[b]	34.40 ± 10.51[b]	82.19 ± 15.22[a]	**0.03**
WBS	5.02 ± 0.45	3.27 ± 1.38	3.21 ± 2.00	0.37

[abc] Means within a row lacking common superscript letter differ significantly

SNP 306 UPRCP				
Variable	CC n = 8	CT n = 8	TT n = 6	p value
MFI	40.51 ± 7.28	33.72 ± 6.60	47.15 ± 8.85	0.37
WBS	5.40 ± 0.83	3.83 ± 0.75	5.08 ± 1.01	0.31

SNP 351 UPRCP				
Variable	GG n = 20	GA n = 2	AA n = 0	p value
MFI	40.85 ± 4.04	30.74 ± 12.83	--	0.47
WBS	4.42 ± 0.41[b]	7.78 ± 1.32[a]	--	**0.03**

[abc] Means within a row lacking common superscript letter differ significantly

SNP 354 UPRCP				
Variable	INS CGAT n = 15	INS/DEL CGAT n = 6	DEL CGAT n = 1	p value
MFI	40.48 ± 6.80	37.93 ± 9.99	42.15 ± 19.16	0.97
WBS	4.32 ± 0.74	5.92 ± 1.09	3.23 ± 2.09	0.35

Table 4.15. Means of genotypes of SNPs identified in the 568 base fragment amplified for CAPN1 530 for different variables in UPR cattle population.

SNP 208 CCP				
Variables	CC n = 39	CT n = 26	TT n = 1	p value
Hot Carcass Weight	505.42 ± 17.38	491.23 ± 21.94	441.13 ± 111.62	0.76
pH 24 hour Aging	5.72 ± 0.06	5.69 ± 0.08	5.64 ± 0.40	0.95
pH 14 days Aging	5.73 ± 0.06	5.66 ± 0.08	5.63 ± 0.38	0.77
Color Values 24 hour Aging				
L*	30.63 ± 0.59	30.99 ± 0.74	34.03 ± 3.77	0.64
a*	15.35 ± 0.35 [b]	16.84 ± 0.44 [a]	18.57 ± 2.23 [ab]	**0.02**
b*	11.49 ± 0.30 [b]	12.54 ± 0.37 [a]	14.78 ± 1.90 [ab]	**0.03**
Color Values 14 days Aging				
L*	33.15 ± 0.63	34.22 ± 0.80	38.80 ± 4.07	0.25
a*	14.72 ± 0.39	15.57 ± 0.49	13.01 ± 2.52	0.31
b*	12.28 ± 0.30	13.08 ± 0.35	12.92 ± 1.78	0.20
WBS 24 hour Aging kg	8.89 ± 0.57	9.33 ± 0.89	6.91 ± 2.36	0.67
[Calcium] 24 hour Aging uM	50.60 ± 2.85 [b]	59.00 ± 3.60 [ab]	95.70 ± 18.31 [a]	**0.02**
[Calcium] 14 days Aging uM	56.14 ± 3.66 [b]	73.27 ± 4.63 [a]	93.95 ± 23.54 [ab]	**0.01**

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.16. Means of genotypes of SNP 208, found in intron 8 of the CAPN1 gene (transversion of cytosine to thymine; base 5458 accession number AF252505), for different variables in Commercial Cattle Population.

SNP 212 CCP				
Variables	**TT n = 54**	**TG n = 10**	**GG n = 0**	**p value**
Hot Carcass Weight	506.29 ± 15.13	460.72 ± 34.94	--	0.24
pH 24 hour Aging	5.71 ± 0.05	5.72 ± 0.12	--	0.91
pH 14 days Aging	5.70 ± 0.05	5.73 ± 0.12	--	0.82
Color Values 24 hour Aging				
L*	30.90 ± 0.51	30.02 ± 1.19	--	0.50
a*	16.03 ± 0.32	15.98 ± 0.75	--	0.95
b*	11.95 ± 0.27	11.97 ± 0.64	--	0.98
Color Values 14 days Aging				
L*	33.49 ± 0.56	33.93 ± 1.30	--	0.76
a*	15.13 ± 0.35	14.87 ± 0.80	--	0.78
b*	12.59 ± 0.25	12.57 ± 0.58	--	0.97
WBS 24 hour Aging kg	8.93 ± 0.52	8.52 ± 1.00	--	0.70
[Calcium] 24 hour Aging uM	54.35 ± 2.67	55.90 ± 6.17	--	0.82
[Calcium] 14 days Aging uM	62.85 ± 3.48	65.92 ± 8.03	--	0.73

Table 4.17. Means of genotypes of SNP 212, found in intron 8 of the CAPN1 gene (transversion of thymine to guanine; base 5462 accession number AF252505), for different variables in Commercial Cattle Population.

SNP 430				
Variables	**CC n = 43**	**CG n = 20**	**GG n = 2**	**p value**
Hot Carcass Weight	503.63 ± 16.77	490.60 ± 26.12	531.56 ± 80.03	0.86
pH 24 hour Aging	5.72 ± 5.72	5.66 ± 5.66	5.73 ± 5.73	0.83
pH 14 days Aging	5.73 ± 5.73	5.64 ± 5.64	5.64 ± 5.64	0.65
Color Values 24 hour Aging				
L*	30.44 ± 0.56	31.62 ± 0.87	35.91 ± 2.66	0.08
a*	15.48 ± 0.33 [b]	16.92 ± 0.52 [a]	18.25 ± 1.60 [ab]	**0.02**
b*	11.50 ± 0.28 [b]	12.80 ± 0.43 [a]	15.01 ± 1.32 [a]	**<0.01**
Color Values 14 days Aging				
L*	33.10 ± 0.60	34.72 ± 0.94	38.61 ± 2.88	0.08
a*	14.91 ± 0.39	15.44 ± 0.60	15.54 ± 1.84	0.72
b*	12.38 ± 0.27	13.07 ± 0.42	14.61 ± 1.29	0.11
WBS 24 hour Aging kg	9.09 ± 0.58 [a]	9.51 ± 1.02 [a]	4.16 ± 1.68 [b]	**0.03**
[Calcium] 24 hour Aging uM	51.20 ± 2.77	60.01 ± 4.31	70.97 ± 13.20	0.10
[Calcium] 14 days Aging uM	57.42 ± 3.54 [b]	73.59 ± 5.52 [a]	98.53 ± 16.91 [a]	**0.01**

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.18. Means of genotypes of SNP 430, found in intron 8 of the CAPN1 gene (transversion of cytosine to guanine; base 5680 accession number AF252505), for different variables in Commercial Cattle Population.

SNP 438				
Variables	GG n = 43	GA n = 23	AA n = 1	p value
Hot Carcass Weight	503.43 ± 16.77	498.48 ± 25.21	440.41 ± 113.11	0.85
pH 24 hour Aging	5.72 ± 0.06	5.66 ± 0.09	5.65 ± 0.38	0.86
pH 14 days Aging	5.73 ± 0.05	5.64 ± 0.08	5.65 ± 0.37	0.65
Color Values 24 hour Aging				
L*	30.43 ± 0.57	32.00 ± 0.85	33.68 ± 3.83	0.24
a*	15.48 ± 0.33 [b]	16.98 ± 0.50 [b]	18.53 ± 2.26 [a]	**0.03**
b*	11.50 ± 0.28 [b]	12.94 ± 0.42 [b]	14.67 ± 1.90 [a]	**0.01**
Color Values 14 days Aging				
L*	33.10 ± 0.61	34.95 ± 0.91	38.56 ± 4.11	0.12
a*	14.90 ± 0.38	15.61 ± 0.57	12.94 ± 2.58	0.44
b*	12.37 ± 0.27	13.26 ± 0.41	12.85 ± 1.85	0.20
WBS 24 hour Aging kg	9.13 ± 0.67	8.16 ± 1.07	6.89 ± 2.77	0.52
[Calcium] 24 hour Aging uM	51.23 ± 2.70 [b]	59.18 ± 4.06 [ab]	95.12 ± 18.20 [a]	**0.02**
[Calcium] 14 days Aging uM	57.38 ± 3.59 [b]	75.38 ± 5.39 [a]	92.76 ± 24.19 [ab]	**0.01**

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.19. Means of genotypes of SNP 438, found in intron 8 of the CAPN1 gene (transition of guanine to adenine; base 5688 accession number AF252505), for different variables in Commercial Cattle Population.

SNP 573 CCP				
Variables	CC n = 48	CT n = 20	TT n = 2	p value
Hot Carcass Weight	504.59 ± 15.68	491.41 ± 25.57	525.92 ± 78.53	0.87
pH 24 hour Aging	5.71 ± 0.05	5.65 ± 0.09	5.73 ± 0.26	0.87
pH 14 days Aging	5.71 ± 0.05	5.63 ± 0.08	5.63 ± 0.26	0.70
Color Values 24 hour Aging				
L*	30.53 ± 0.51	31.65 ± 0.84	35.97 ± 2.58	0.08
a*	15.45 ± 0.33 [b]	16.92 ± 0.54 [a]	18.19 ± 1.66 [a]	**0.03**
b*	11.43 ± 0.27 [b]	12.77 ± 0.44 [a]	15.00 ± 1.35 [a]	**<0.01**
Color Values 14 days Aging				
L*	33.07 ± 0.56	34.70 ± 0.91	38.73 ± 2.79	0.06
a*	15.02 ± 0.36	15.51 ± 0.59	15.39 ± 1.81	0.78
b*	12.39 ± 0.25	13.08 ± 0.41	14.57 ± 1.26	0.10
WBS 24 hour Aging kg	9.08 ± 0.56 [a]	9.60 ± 1.01 [a]	4.03 ± 1.70 [b]	**0.02**
[Calcium] 24 hour Aging uM	52.29 ± 2.62	60.45 ± 4.28	70.89 ± 13.13	0.12
[Calcium] 14 days Aging uM	58.39 ± 3.31 [a]	73.94 ± 5.40 [b]	98.93 ± 16.57 [b]	**0.01**

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.20. Means of genotypes of SNP 573, found in intron 9 of the CAPN1 gene (transition of cytosine to thymine; base 5823 accession number AF252505), for different variables in Commercial Cattle Population.

SNP 643				
Variables	DEL n = 38	INS/DEL n = 25	INS n = 1	p value
Hot Carcass Weight	507.22 ± 17.79	488.14 ± 22.50	618.55 ± 111.79	0.47
pH 24 hour Aging	5.73 ± 0.06	5.67 ± 0.08	5.80 ± 0.38	0.79
pH 14 days Aging	5.74 ± 0.06	5.64 ± 0.07	5.62 ± 0.37	0.53
Color Values 24 hour Aging				
L*	30.59 ± 0.60	31.11 ± 0.76	38.11 ± 3.78	0.14
a*	15.36 ± 0.36 [a]	16.77 ± 0.45 [b]	18.06 ± 2.24 [b]	**0.04**
b*	11.51 ± 0.30 [a]	12.48 ± 0.38 [b]	15.40 ± 1.91 [b]	**0.03**
Color Values 14 days Aging				
L*	33.16 ± 0.65	34.25 ± 0.83	38.73 ± 4.11	0.27
a*	14.71 ± 0.40	15.64 ± 0.51	18.05 ± 2.53	0.18
b*	12.29 ± 0.28 [a]	13.05 ± 0.36 [b]	16.32 ± 1.79 [b]	**0.03**
WBS 24 hour Aging kg	8.86 ± 0.58 [a]	9.35 ± 0.90 [a]	1.36 ± 2.45 [b]	**0.01**
[Calcium] 24 hour Aging uM	49.95 ± 2.85	59.89 ± 3.60	47.91 ± 17.90	0.10
[Calcium] 14 days Aging uM	55.16 ± 3.71 [a]	73.42 ± 4.69 [b]	104.59 ±23.29 [b]	**<0.01**

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.21. Means of genotypes of SNP 643, found in intron 9 of the CAPN1 gene (INS/DEL of cytosine; base 5893 accession number AF252505), for different variables in Commercial Cattle Population.

SNP 652				
Variables	**AA n = 38**	**AC n = 5**	**CC n = 21**	**p value**
Hot Carcass Weight	507.37 ± 17.96	473.37 ± 50.16	499.32 ± 25.37	0.81
pH 24 hour Aging	5.73 ± 0.06	5.68 ± 0.17	5.67 ± 0.09	0.84
pH 14 days Aging	5.74 ± 0.06	5.62 ± 0.16	5.64 ± 0.08	0.53
Color Values 24 hour Aging				
L*	30.60 ± 0.61	29.12 ± 1.69	32.04 ± 0.86	0.23
a*	15.36 ± 0.36 [b]	16.37 ± 1.00 [ab]	16.95 ± 0.50 [a]	**0.04**
b*	11.52 ± 0.30 [b]	11.37 ± 0.84 [ab]	12.95 ± 0.43 [a]	**0.02**
Color Values 14 days Aging				
L*	33.17 ± 0.65	32.57 ± 1.83	34.96 ± 0.92	0.25
a*	14.71 ± 0.40	16.38 ± 1.13	15.56 ± 0.57	0.23
b*	12.29 ± 0.29	13.01 ± 0.82	13.25 ± 0.41	0.15
WBS 24 hour Aging kg	9.14 ± 0.68	9.82 ± 2.03	8.22 ± 1.10	0.67
[Calcium] 24 hour Aging uM	49.93 ± 2.86	61.09 ± 7.98	58.90 ± 4.04	0.12
[Calcium] 14 days Aging uM	55.17 ± 3.76 [b]	74.21 ± 10.50 [a]	74.91 ± 5.31 [a]	**0.01**

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.22. Means of genotypes of SNP 652, found in exon 10 of the CAPN1 gene (transversion of adenine to cytosine; base 5902 accession number AF252505), for different variables in Commercial Cattle Population. This missence mutation changes the amino acid sequence from leucine to isoleucine.

SNP 208 UPRCP				
Variable	CC n = 4	CT n = 5	TT n = 2	p value
MFI	38.7 ± 10.07	31.6 ± 10.07	43.3 ± 16.10	0.80
WBS	6.7 ± 0.76	5.0 ± 0.76	4.0 ± 1.22	0.20

SNP 212 UPRCP				
Variable	TT n = 9	TG n = 2	GG n = 0	p value
MFI	38.19 ± 6.53	23.20 ± 14.61	--	0.35
WBS	5.54 ± 0.63	6.38 ± 1.40	--	0.35

SNP 430 UPRCP				
Variable	CC n = 5	CG n = 4	GG n = 2	p value
MFI	39.73 ± 9.04	26.00 ± 14.58	39.86 ± 14.58	0.63
WBS	5.96 ± 0.85	5.59 ± 1.37	4.16 ± 1.37	0.58

SNP 438 UPRCP				
Variable	GG n = 5	GA n = 5	AA n = 1	p value
MFI	39.73 ± 9.19	28.74 ± 12.87	39.98 ± 22.13	0.76
WBS	5.96 ± 0.76	5.56 ± 1.06	2.91 ± 1.82	0.34

SNP 573 UPRCP				
Variable	CC n = 5	CT n = 2	TT n = 4	p value
MFI	39.73 ± 8.27	15.96 ± 15.25	37.95 ± 12.27	0.36
WBS	5.96 ± 0.80	6.18 ± 1.48	4.58 ± 1.19	0.52

SNP 643 UPRCP				
Variable	DEL C n = 4	INS/DEL C n = 3	INS C n = 4	p value
MFI	38.68 ± 9.70	26.95 ± 11.48	42.91 ± 12.27	0.59
WBS	6.73 ± 0.78	5.03 ± 0.92	4.38 ± 0.98	0.22

SNP 652 UPRCP				
Variable	AA n = 4	AC n = 1	CC n = 6	p value
MFI	38.68 ± 10.21	45.03 ± 22.82	29.56 ± 13.18	0.84
WBS	6.73 ± 0.63 [a]	2.15 ± 1.41 [b]	5.88 ± 0.81 [a]	**0.05**

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.23. Means of genotypes of SNPs identified in the 735 base fragment amplified for CAPN1 316 for different variables in UPR Cattle Population.

CAPN1 316 Haplotype CPP				
Variables	A n = 20	B n =38	C n = 5	p value
Hot Carcass Weight	491.88 ± 26.27	507.29 ± 17.93	475.51 ± 50.13	0.77
pH 24 hour Aging	5.66 ± 0.09	5.73 ± 0.06	5.68 ± 0.17	0.81
pH 14 days Aging	5.64 ± 0.09	5.74 ± 0.06	5.62 ± 0.17	0.55
Color Values 24 hour Aging				
L*	31.67 ± 0.88	30.60 ± 0.60	29.23 ± 1.67	0.40
a*	16.89 ± 0.53 [a]	15.36 ± 0.36 [b]	16.39 ± 1.00 [a]	**0.05**
b*	12.80 ± 0.44	11.52 ± 0.30	11.41 ± 0.84	0.06
Color Values 14 days Aging				
L*	34.74 ± 0.96	33.16 ± 0.65	32.63 ± 1.83	0.36
a*	15.40 ± 0.59	14.71 ± 0.40	16.42 ± 1.13	0.27
b*	13.05 ± 0.42	12.29 ± 0.29	13.06 ± 0.80	0.26
WBS 24 hour Aging kg	9.34 ± 1.03	8.91 ± 0.61	9.52 ± 1.78	0.88
[Calcium] 24 hour Aging uM	59.59 ± 4.21	49.94 ± 2.87	60.89 ± 8.03	0.11
[Calcium] 14 days Aging uM	73.03 ± 5.48 [a]	55.15 ± 3.74 [b]	74.75 ± 10.45 [a]	**0.01**

[abc] Means within a row lacking common superscript letter differ significantly

CAPN1 316 Haplotype UPRCP						
Variable	A n = 2	B n = 4	C n = 1	D n = 2	E n = 2	p value
MFI	14.90 ± 18.40	38.67 ± 10.62	45.03 ± 23.76	38.80 ± 18.40	34.97 ± 18.40	0.77
WBS	6.95 ± 1.02	6.72 ± 0.52	2.15 ± 1.32	4.92 ± 1.02	5.76 ± 1.02	0.10

Table 4.24. Means for different variables in Haplotypes found in the SNPs identified in introns 8-9 and exon 10 of the CAPN1 gene in Commercial Cattle and UPR Cattle Populations.

SNP 94 CCP				
Variables	**TT n = 67**	**TC n = 26**	**CC n = 0**	**p value**
Hot Carcass Weight	500.36 ± 14.15	512.00 ± 22.70	--	0.66
pH 24 hour Aging	5.74 ± 0.05	5.73 ± 0.08	--	0.75
pH 14 days Aging	5.73 ± 0.05	5.80 ± 0.08	--	0.39
Color Values 24 hour Aging				
L*	32.04 ± 0.52	30.34 ± 0.84	--	0.09
a*	15.83 ± 0.32	15.40 ± 0.5	--	0.47
b*	12.07 ± 0.24	11.45 ± 0.39	--	0.18
Color Values 14 days Aging				
L*	33.71 ± 0.48	33.11 ± 0.76	--	0.51
a*	15.28 ± 0.32	15.61 ± 0.52	--	0.59
b*	12.75 ± 0.24	12.62 ± 0.38	--	0.78
WBS 24 hour Aging kg	8.20 ± 0.55	6.84 ± 0.94	--	0.17
[Calcium] 24 hour Aging uM	52.77 ± 2.45	55.20 ± 3.93	--	0.60
[Calcium] 14 days Aging uM	64.43 ± 2.94	54.66 ± 4.72	--	0.08

Table 4.25. Means of genotypes of SNP 94, found in the 3"UTR of the CAST gene (transition of thymine to cytosine; base 2987 accession number AF2159246), for different variables in Commercial Cattle Population.

SNP 122 CCP				
Variables	**AA n = 47**	**AC n = 46**	**CC n = 0**	**p value**
Hot Carcass Weight	497.20 ± 16.87	513.02 ± 17.33	--	0.52
pH 24 hour Aging	5.86 ± 0.06 [a]	5.65 ± 0.06 [b]	--	**0.01**
pH 14 days Aging	5.84 ± 0.06 [a]	5.66 ± 0.06 [b]	--	**0.03**
Color Values 24 hour Aging				
L*	31.42 ± 0.64	31.59 ± 0.66	--	0.85
a*	15.89 ± 0.38	15.39 ± 0.39	--	0.37
b*	11.97 ± 0.30	11.68 ± 0.31	--	0.51
Color Values 14 days Aging				
L*	32.74 ± 0.56	34.27 ± 0.57	--	0.06
a*	15.64 ± 0.39	15.01 ± 0.40	--	0.26
b*	12.57 ± 0.29	12.75 ± 0.29	--	0.67
WBS 24 hour Aging kg	6.58 ± 0.61 [b]	9.72 ± 0.70 [a]	--	**<0.01**
[Calcium] 24 hour Aging uM	50.20 ± 2.94	56.39 ± 3.02	--	0.15
[Calcium] 14 days Aging uM	59.01 ± 3.60	63.88 ± 3.70	--	0.35

[abc] Means within a row lacking common superscript letter differ significantly

Table 4.26. Means of genotypes of SNP 122, found in the 3"UTR of the CAST gene (transversion of adenine to cytosine; base 3015 accession number AF2159246), for different variables in Commercial Cattle Population.

SNP 94 UPRCP				
Variable	**TT**	**TC**	**CC**	**p value**
MFI	42.67 ± 3.95	22.03 ± 9.36	--	0.06
WBS	4.53 ± 0.44 [b]	7.31 ± 1.03 [a]	--	**0.02**

[abc] Means within a row lacking common superscript letter differ significantly

SNP 122 UPRCP				
Variable	**AA**	**AC**	**CC**	**p value**
MFI	39.34 ± 9.10	41.78 ± 11.62	--	0.86
WBS	4.89 ± 0.90	5.24 ± 1.53	--	0.82

Table 4.27. Means of genotypes of SNPs identified in the 170 base fragment amplified for CAST 2959 for different variables in UPR Cattle Population.

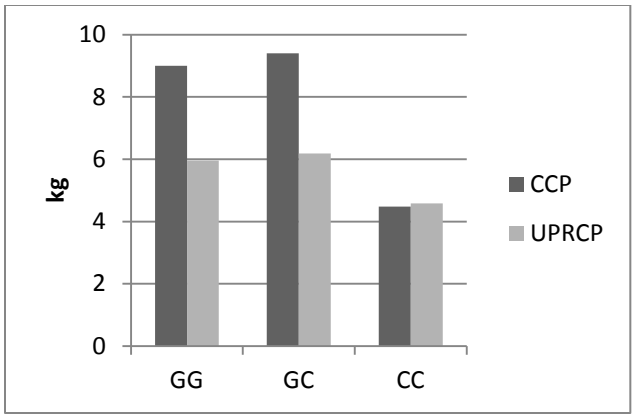

Figure 4. 1. Mean of WBS value for marker CAPN1 316 genotypes in CCP and UPRCP (P = 0.01 and P = 0.52 for CCP and UPRCP respectively).

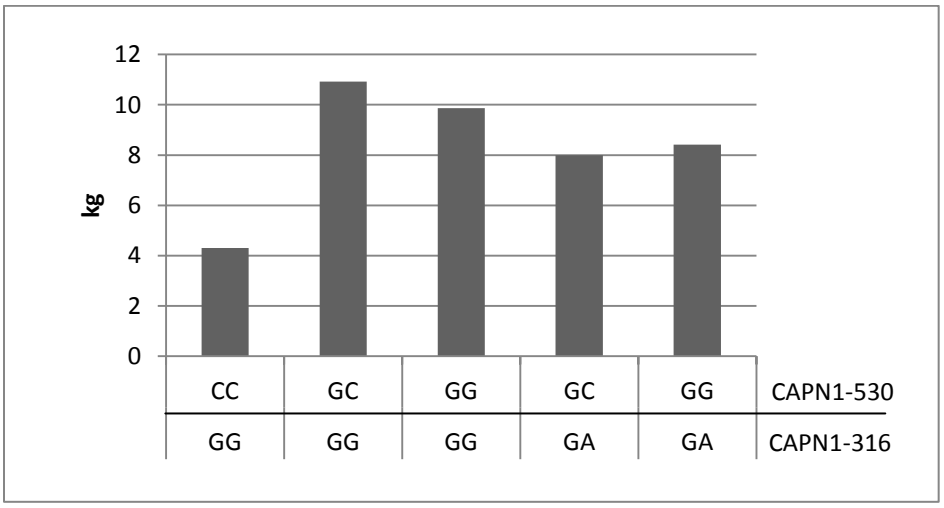

Figure 4.2. Effects of different combinations of markers CAPN1 530 and CAPN1 316 genotypes on WBS at 24h postmortem in Commercial Cattle Population (P = 0.65).

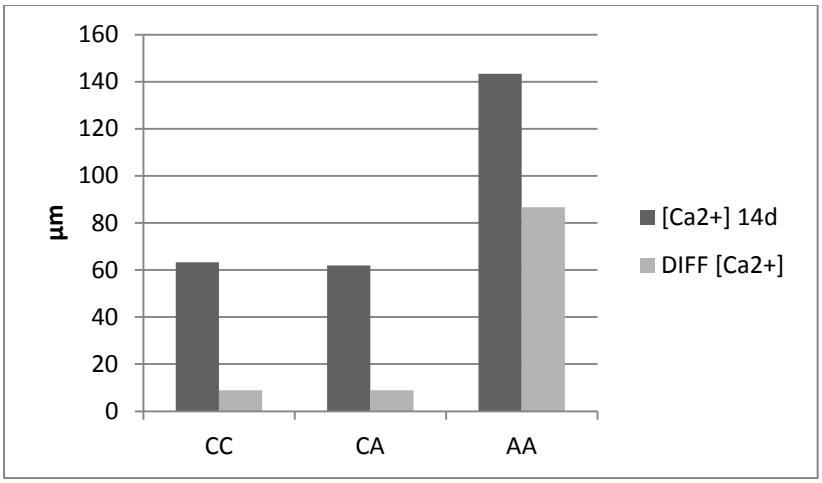

Figure 4.3. Effects of different genotypes of CAPN1 4753 marker on free [Ca2+] at 14d of aging and the increase in calcium during the aging period in Commercial Cattle Population. (P = 0.01 for free [Ca2+] at 14d of ageing and P = <0.01 for the difference in free [Ca2+] during the 14d aging period).

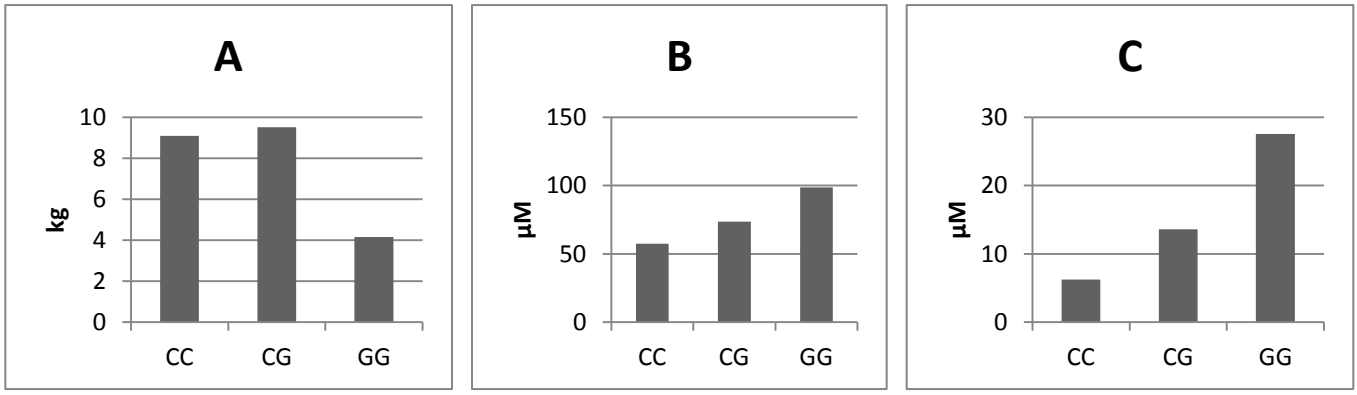

Figure 4.4. Differences among genotypes of SNP 430 in WBS (A), free [Ca2+] at 14d of aging (B) and the increased observed in free [Ca2+] during the aging period (B) in the Commercial Cattle Population (P = 0.03, P = 0.01 and P = 0.10 for WBS, free [Ca2+] at 14d of aging and the increase in free [Ca2+] during the aging period respectively).

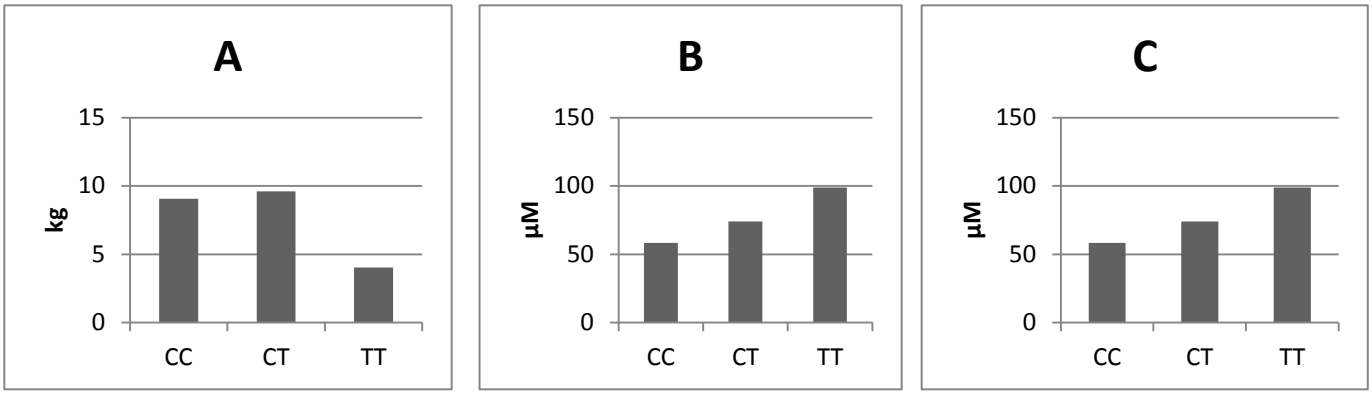

Figure 4.5. Differences among genotypes of SNP 573 in WBS (A), free [Ca2+] at 14d of aging (B) and the increased observed in free [Ca2+] during the aging period (C) in the Commercial Cattle Population (P = 0.02, P = 0.78 and P = 0.01 for WBS, free [Ca2+] at 14d of aging and the increase in free [Ca2+] during the aging period respectively).

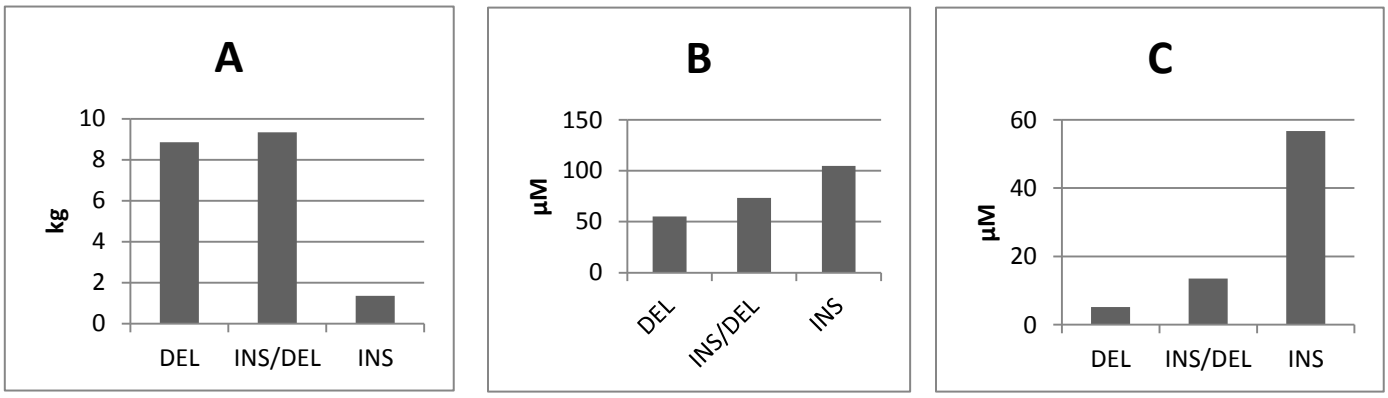

Figure 4.6. Differences among genotypes of SNP 643 in WBS (A), free [Ca2+] at 14d of aging (B) and the increased observed in free [Ca2+] during the aging period (C) in the Commercial Cattle Population (P = 0.01, P = <0.01 and P = <0.01 for WBS, free [Ca2+] at 14d of aging and the increase in free [Ca2+] during the aging period respectively).

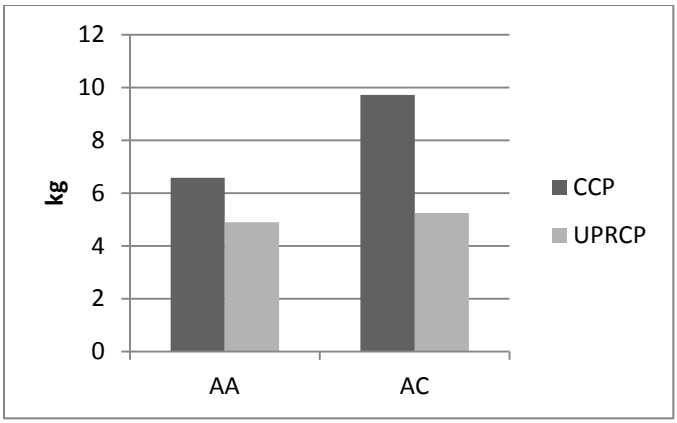

Figure 4.7. Differences between genotypes of SNP 122 genotype WBS of CCP and UPRCP, P = < 0.01 for the Commercial Cattle Population and P = 0.82 for the UPR Cattle Population.

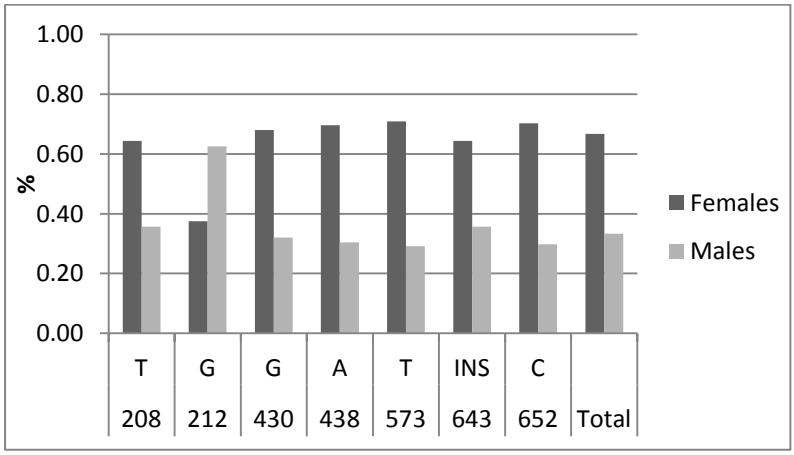

Figure 4.8. Frequencies distribution of tender alleles of identified SNPs found in CAPN1 gene among sex. SNPs 208 (P = 0.13), 212 (P = 0.48) and 430 (P = 0.07) are found in intron 8, SNPs 438 (P = 0.06), 573 (P = 0.04) and 643 (P = 0.13) are found in intron 9 and the last SNP 652 (P = <0.01) is found in exon 10.

General Conclusions

This research had the objective of measuring the quality of meat from commercial cattle of Puerto Rico in order to contribute to creating a classification system of commercial meat. Before this goal can be achieved improved efficiency at both production and processing levels will be necessary to reduce the incidence of tough meat with high ultimate pH values, as seen in this study. However, beef of acceptable tenderness could be produced relying on native pasture as seen in the UPR cattle population. This population of adapted *Bos taurus* cattle segregates higher frequencies of tender alleles located in the CAPN1 gene, than those observed in the commercial cattle population. If a breeding program included a greater genetic contribution from the UPR population in the commercial herds could increase the tender allele frequencies and with time improve overall tenderness. Increase tenderness was also observed in the older animals of the commercial population and yet this was associated with decreased meat quality. Increased muscle wasting and a sarcopenic phenotype in older cattle is purposed to explain these observations. Further evaluation of farm management and pasture quantity and quality could help to explain why this muscle wasting occurs. Also evaluation of calpain and calpastatin activities in these animals could confirm or refute the sarcopenic phenotype explanation. The CAPN1 and CAST genes are highly polymorphic and newly identified point mutations in introns 8 and 9 show interesting correlations with tenderness as indicated by WBS in the commercial cattle population. If genetic selection based on meat tenderness where to be established in our commercial cattle herds more emphasis should be placed on these newly identified markers over the commercially available markers that showed little correlation with tenderness in this same population. Knowledge derived from studies such as this one could move the industry one step closer to improving overall meat quality and consumers acceptability of locally produced beef.