Asian-Aust. J. Anim. Sci.
Vol. 26, No. 3 : 443-454 March 2013
http://dx.doi.org/10.5713/ajas.2012.12365

www.ajas.info
pISSN 1011-2367 eISSN 1976-5517

A New Insight into the Role of Calpains in Post-mortem Meat Tenderization in Domestic Animals: A review

Ting Lian, Linjie Wang and Yiping Liu*
College of Animal Science and Technology, Sichuan Agricultural University, Ya'an, Sichuan 625014, China

ABSTRACT: Tenderness is the most important meat quality trait, which is determined by intracellular environment and extracellular matrix. Particularly, specific protein degradation and protein modification can disrupt the architecture and integrity of muscle cells so that improves the meat tenderness. Endogenous proteolytic systems are responsible for modifying proteinases as well as the meat tenderization. Abundant evidence has testified that calpains (CAPNs) including calpain I (CAPN1) and calpastatin (CAST) have the closest relationship with tenderness in livestock. They are involved in a wide range of physiological processes including muscle growth and differentiation, pathological conditions and post-mortem meat aging. Whereas, Calpain3 (CAPN3) has been established as an important activating enzyme specifically expressed in livestock's skeletal muscle, but its role in domestic animals meat tenderization remains controversial. In this review, we summarize the role of CAPN1, calpain II (CAPN2) and CAST in post-mortem meat tenderization, and analyse the relationship between CAPN3 and tenderness in domestic animals. Besides, the possible mechanism affecting post-mortem meat aging and improving meat tenderization, and current possible causes responsible for divergence (whether CAPN3 contributes to animal meat tenderization or not) are inferred. Only the possible mechanism of CAPN3 in meat tenderization has been confirmed, while its exact role still needs to be studied further. (**Key Words:** Calpains, Post-mortem Meat tenderization, Proteolysis System, Domestic Animals)

INTRODUCTION

Nowadays, meat quality has been the focus for livestock industries and also consumers' need. As for most consumers, tenderness is considered as the most important feature for eating quality. Up until now, the primary focus on beef tenderness has been on a longer storage time (at least 14 d) in cooling conditions to obtain the final tenderness. Many studies have shown that the meat tenderization process is complex and could be affected by several different pathways including pre-slaughter and post-slaughter factors and their interaction (Destefanis et al., 2008). Within these factors, it is likely that ultimate tenderness is mainly determined by the extent of proteolysis of key target skeleton proteins within muscle fibres and the alteration of muscle structure (Taylor et al., 1995; Koohmaraie and Geesink, 2006).

CAPNs are a large family of intracellular Ca^{2+}-dependent cysteine proteases. The CAPN system is one of the endogenous proteolysis systems, which plays a major role in meat tenderization. It is consistent with the basic criteria: i) proteases must be endogenous to skeletal muscle cells; ii) they must be able to mimic postmortem changes in myofibrils *in vitro* under optimum conditions; iii) these proteases are available to cytoskeletal protein of myofibrils in tissue (Goll et al., 1983; Koohmaraie, 1996). Numerous studies have shown that the proteolytic CAPNs activity is responsible for meat tenderization (Sentandreu et al., 2002; Koohmaraie and Geesink, 2006). Therefore, the CAPN system is regarded as the main endogenous protease system contributing to postmortem proteolysis and meat tenderization (Koohmaraie and Geesink, 2006; Bernard et al., 2007; Neath et al., 2007).

Even if considerable evidence linking CAPNs (CAPN1 and CAST) to meat tenderization has been established (Koohmaraie et al., 1991), the role of CAPN3 and other members of CAPN family are still controversial. Some evidence supports its involvement, some rejects. CAPN3 is one member of the CAPN family, located in the N2 line by binding to titin (the giant myofibrillar protein) (Sorimachi et al., 1995), where proteolysis has been linked to meat tenderization (Taylor et al., 1995). Thus, its location has encouraged scientists to look for much more functions. In

* Corresponding Author: Yiping Liu. Tel: +86-835-2882509, Fax: +86-835-2886080, E-mail: liuyp578@yahoo.com
Submitted Jun. 28, 2012; Accepted Sept. 15, 2012; Revised Nov. 22, 2012

Copyright © 2013 by Asian-Australasian Journal of Animal Sciences

this review, we mainly concentrate on determining whether CAPN3 functions in postmortem proteolysis and meat tenderization of domestic animals. Meanwhile, the role of CAPN1, CAPN2 and CAST in postmortem meat tenderization is also mentioned.

MEAT TENDERNESS

The conversion of muscle into meat

After slaughter, muscle goes rapidly into rigor mortis and the muscle is tough and tenderness declines. Subsequently, as the aging process occurs there is an improvement in tenderness (Grobbel et al., 2008).

In vivo, energy is obtained through aerobic metabolism. After slaughter, aerobic metabolism declines with the decreasing oxygen supply, instead, anaerobic metabolism accounts for most glycolysis (Mayes, 1993), and a pH value that falls to 5.4 to 5.8. In addition, many investigations have shown a series of intracellular environmental changes occurred during the process that produces a high ionic strength and muscle cells that are unable to keep the reducing conditions (Huff Lonergan et al., 2010). Alteration in pH/ionic strengths may produce conformational changes of the proteolytic enzymes that activate them to autolyze and hydrolyze the protein substrates (Huff-Lonergan and Lonergan, 1999; Melody et al., 2004). In particular, the rate of pH decline is closely related to the extent of proteolysis of myofibrillar proteins by CAPNs, such as the production of PSE pork (Barbut et al., 2008). Furthermore, the decline of temperature and pH parallels the development of rigor, and is partially associated with it (Huff Lonergan et al., 2010). Consequently, these changes have an effect on the rate of tenderization and occasionally on water holding capacity (Melody et al., 2004; Simmons et al., 2008).

The factors affected meat tenderness

Meat tenderness is considered as the most important parameter determining meat quality (Moeller et al., 2010). Generally, the phenotype of tenderness is the result of genetic-environmental interaction (Peaston and Whitelaw, 2006). Changes on-farm, pre-slaughter, post-slaughter processing factors and other man-made factors such as electric stimulus account for the environmental contribution. Factors originated from the muscle also contribute to meat tenderness, such as intermuscular variation, sensitivity of muscle structural proteins to proteolysis, the distribution of muscular connective tissue and sarcomere length (Koohmaraie et al., 1988; Seideman et al., 1989; Wheeler and Koohmaraie, 1999). A range of studies have shown that postmortem factors, particularly temperature and sarcomere length could determine pre-rigor and proteolysis and affect the conversion of muscle to meat (Taylor et al., 1995; Koohmaraie et al., 1996; Roberts et al., 1996; Gollasch and Nelson, 1997; Hwang et al., 2003; Koohmaraie and Geesink, 2006). For muscle itself, meat tenderness is determined by the structure of myofibres, integrity of muscle cells, the intracellular protease activity and the extracellular matrix (Mccormick, 2009). The ultimate tenderness depends upon the interplay between intra- and extra factors such as the movement of water from myofibrils to extracellular space. Much research has indicated that three endogenous proteolytic systems participated in the postmortem proteolysis and meat tenderization: the system of CAPNs, cathepsins and proteasomes (mainly multicatalytic proteinase complex (MPC), called 20S proteasome) (Dutaud et al., 2006; Koohmaraie and Geesink, 2006; Bernard et al., 2007; Kemp et al., 2010). Here, we focus on the effect of the endogenous protease activity especially the CAPN system on meat tenderization.

ROLE OF CAPN1, CAPN2, CAPN3 AND CAST IN MEAT TENDERIZATION

The CAPN system

Introduction of CAPN system: Discovered in 1964 (Guroff, 1964), CAPNs are a large family of intracellular Ca^{2+}-dependent cysteine neutral proteinases (EC 3.4.22.17; Clan CA, family C02), which are found in almost all eukaryotes and a few bacteria but not in archaebacteria (Croall and Demartino, 1991; Goll et al., 2003). To date, 14 isoforms have been identified, which are expressed in ubiquitous and tissue-specific forms (Goll et al., 2003). In skeletal muscle, the CAPN family contains at least three proteases: CAPN1 (μ-calpain), CAPN2 (m-calpain) and CAPN3 (p94), as well as CAST-being a CAPN inhibitor (Goll et al., 2003; Koohmaraie and Geesink, 2006; Moudilou et al., 2010), and they are encoded by *CAPN1*, *CAPN2*, *CAPN3* and *CAST* genes (Moudilou et al., 2010), respectively.

Structure: The structure of CAPNs is highly homologous. Both CAPN1 and CAPN2 consist of a discrepant 80-kDa subunit (Imajoh et al., 1988; Ohno et al., 1990) and an identical 28-kDa subunit encoded by CAPN4 (Emori et al., 1986; Ohno et al., 1990). In the large subunit, four domains are distinguished (domains I-IV). Domains V and VI are classified in the small subunit (Goll et al., 2003). Domain I modulates the proteolytic activity by removing it or not. Domain II is catalytic with a Cys residue. Domain III, a Ca^{2+}-binding domain is linked to the catalytic domain II, which may regulate the activity of CAPN by participating in critical electrostatic interactions and the binding of phospholipids (Hosfield et al., 1999; Strobl et al., 2000; Tompa et al., 2001). Domain IV contains four sets of sequences that predict EF-hand Ca^{2+}-binding sites. Domain

V is enriched by glycine residues with hydrophobicity (Eisenberg et al., 1982) and is mainly involved in autolysis. Domain VI is similar to IV and consists of five Ca^{2+}-binding areas (Ohno et al., 1986; Blanchard et al., 1997; Lin et al., 1997). Meanwhile, CAPN1 and CAPN2 are activated *in vitro* at micromolar and millimolar calcium concentrations, respectively. However, CAPN3 is a 94-kDa protein whose structure is homologous to CAPN1 (54%) and CAPN2 (51%), and shares the similar properties of Ca^{2+}-dependent activation and maximal activity at neutral pH (Goll et al., 2003). It also has specific characteristics (Sorimachi et al., 1995). i) *CAPN3* is mainly expressed in skeletal muscle (Sorimachi et al., 1989), yet, during development, it also occurs in lens, liver, brain and cardiac muscle (Poussard et al., 1996; Fougerousse et al., 1998; Ma et al., 1998; Konig et al., 2003). ii) CAPN3 is likely to function as a homodimer due to its lacking a small subunit (28 kDa) (Blanchard et al., 1996; Blanchard et al., 1997; Kinbara et al., 1998; Ravulapalli et al., 2005; Ravulapalli et al., 2009). At the same time, CAPN3 has some unique domains distinguishing it from the ubiquitous CAPNs, including its NH2-terminal domain I (contains 20 to 30 additional amino acids) and two "insertion sequences" which contain 62 and 77 amino acids at the COOH-terminal regions of domain II and III, called IS1 and IS2, respectively (Goll et al., 2003). iii) It is thought that CAPN3 is activated at the nanomolar calcium concentration (Garcia Diaz et al., 2006), although Ca^{2+}-dependency of CAPN3 is not clear. iv) CAPN3 is very unstable and undergoes fast autoproteolytic degradation (Sorimachi et al., 1993; Kinbara et al., 1998). CAST has a molecular mass of 60 to 70 kDa and is a specific endogenous inhibitor against CAPN1 and CAPN2 but not CAPN3 (Sorimachi et al., 1993; Moudilou et al., 2010). It contains four inhibitory domains, each of which can inhibit CAPN activity. And three regions (A, B, C) are identified within these domains, which are predicted to interact with CAPNs. Region B between A, and C blocks the active site of CAPNs (Goll et al., 2003; Wendt et al., 2004; Croall and Ersfeld, 2007; Kemp et al., 2010). Its structure is different between species (Ishida et al., 1991; Cong et al., 1998; Goll et al., 2003). Moreover, CAPN3 may regulate the activity of ubiquitous CAPNs via degrading CAST (Ono et al., 2004).

Location and function: What's more, most of CAPNs are intracellular: 70% of CAPN1 is bound to myofibrils, a large portion of CAPN3 is situated in a sarcomere near the N- and M-line, whereas a majority of CAPN2 is located in the cytosol (Ilian et al., 2004c; Xu et al., 2009), and the location of CAST is similar to the CAPNs (Tullio et al., 1999).

Based on the structural features of binding Ca^{2+}, associating membrane/phospholipids and recent studies, CAPNs have been confirmed to be involved in various important cellular processes including cell motility, signal transduction pathways, apoptosis, cell differentiation and regulation of the cytoskeleton through modifying the primary and secondary structural features of target proteins (Croall and Ersfeld, 2007). Unlike other intracellular proteolytic systems (cathepsins and proteasomes), CAPNs act in a limited proteolysis and play a critical role in proteolytic modulating and processing rather than degradation. Therefore, CAPNs are identified as the intracellular "modulator" proteases (Ono et al., 2004; Ono et al., 2010). Even while intensive research on CAPNs is still in development, early results have strongly stimulated increased interest in their role in postmortem meat tenderization. Accumulated evidence indicates that CAPNs contribute to postmortem proteolysis whether in pre- and post-rigor muscle. For this reason, CAPNs are considered to be the major protease responsible for key muscle proteins degradation (Koohmaraie, 1992; Huff-Lonergan et al., 1996; Huff-Lonergan and Lonergan, 1999).

A BRIEF OVERVIEW OF CAPN1, CAPN2, CAPN3 AND CAST IN MEAT TENDERIZATION OF DOMESTIC ANIMAL

It is reported that the CAPN system makes a contribution to postmortem proteolysis and meat tenderization in domestic animals (Table 1) (Koohmaraie, 1992; Huff-Lonergan and Lonergan, 2005). In skeletal muscle, the CAPN system comprises ubiquitous CAPNs (CAPN1 and CAPN2), skeletal-specific CAPN3 (also called p94) and endogenous inhibitor- CAST (Goll et al., 2003).

As far as we know, CAPNs can cleave limited myofibrillar proteins such as titin, desmin and vinculin, and contribute to the improvement of tenderness, whereas, high levels of CAST are related to decreased proteolysis and increased meat toughness (Kent et al., 2004; Kemp et al., 2010). Originally, it has been difficult to determine which isoform is primarily involved in post-mortem proteolysis, because both CAPN1 and CAPN2 can cleave the same myofibrillar proteins (Huff-Lonergan et al., 1996). Afterwards, several experimental investigations showed that the activity of CAPN1 changed with the post-mortem proteolysis of key myofibrillar proteins rather than CAPN2 (Riley et al., 2003). Furthermore, the role of CAPN1 in post-mortem proteolysis was confirmed in knockout mice, and a consistent result was observed (Koohmaraie et al., 2006). Therefore, it is clear that CAPN1 plays the most significant role in postmortem muscle proteolysis and meat tenderization (Koohmaraie et al., 2006; Kemp et al., 2010), and it is also confirmed that SNPs of CAPN1 in bovines are

Table 1. *CAPN1*, *CAPN3* and *CAST* genes associated with meat quality

Meat quality parameters	Model system	Genes	References
Warner-Bratzler Shear force	Ovine, beef cattle, pig	*CAPN1*, *CAPN3*, *CAST*	(Ilian et al., 2001; Veiseth et al., 2001; Page et al., 2002; Ilian et al., 2004a; Ilian et al., 2004b; Page et al., 2004; Veiseth et al., 2004; White et al., 2005; Casas et al., 2006; Schenkel et al., 2006; Corva et al., 2007; Barendse et al., 2008; Lindholm-Perry et al., 2009; Cafe et al., 2010; Pinto et al., 2010; Gandolfi et al., 2011)
Meat tenderness of the descendants	Beef cattle	*CAPN1*, *CAST*	(Casas et al., 2006)
Myofibril Fragmentation index (MFI)	Ovine	*CAPN3*	(Ilian et al., 2004a)
Proteolysis of key myofibril proteins	Ovine, Chicken	*CAPN3*, *CAPN1*	(Ilian et al., 2004a; Ilian et al., 2004b; Lee et al., 2008)
Flavor intensity	Beef cattle	*CAPN1*, *CAST*	(Casas et al., 2006b)
Marbling score	Korean cattle	*CAPN1*	(Cheong et al., 2008)
pH	Yanbian Yellow cattle, pig	*CAPN1*, *CAST*	(Sieczkowska et al., 2010; Jin et al., 2011)
Meat redness	Pig	*CAPN1*	(Gandolfi et al., 2011)
Color scores, fatty acid contents and amino acids	Yanbian Yellow cattle, pig	*CAPN1*, *CAST*	(Skrlep et al., 2010; Jin et al., 2011)
Drip loss	Pig	*CAST*	(Gandolfi et al., 2011)
Muscle mass	Ovine	*CAPN3*	(Bickerstaffe et al., 2008)
Cooking loss and juiciness	Pig	*CAST*	(Ciobanu et al., 2004)

closely associated with meat tenderness (Page et al., 2002; Page et al., 2004; White et al., 2005; Cafe et al., 2010). While CAPN2 plays a minor role in meat tenderization, at least in bovine and ovine muscle (Veiseth et al., 2001; Camou et al., 2007). The detectable activity decrease in the native form of CAPN2 was investigated during post-mortem storage in pork (Pomponio et al., 2008). In addition, a significant association was identified in the CAPN1 3'UTR (c.2151*479C>T) with marbling score (Cheong et al., 2008). Futhermore, a tight correlation has been found between CAPN1 and other meat quality traits such as pH, color scores, fatty acid contents and amino acids in Yanbian Yellow cattle of China (Jin et al., 2011).

CAST inhibits CAPN1 and CAPN2 via the interaction between regions A, C and CAPNs when calcium binds to CAPNs: 40 μmol/L and 250 to 500 μmol/L, respectively (Hanna et al., 2008). The possible mechanism is likely to be that CAPNs degrade CAST via cleaving the weaker inhibitory domains, creating specific peptide fragments that retain inhibitory activity (Mellgren, 2008). A significant evidence as to its role in meat tenderness is observed, that is, a high level of CAST is associated with the decrease in postmortem proteolysis and leads to a poor meat quality (Kent et al., 2004). In addition, the relationship between polymorphisms of CAST and meat tenderness in domestic animals has been confirmed (Casas et al., 2006; Gandolfi et al., 2011). Furthermore, as a meat tenderness biomarker, CAST has been quantified by various methods including ELISA, a surface plasmon resonance and the fluorescence resonance energy transfer-based immunosensors (Zór et al., 2009). Meanwhile, it has been determined that several promoters associated with the 5'exons regulate CAST gene expression (Meyers and Beever, 2008), but the exact regulation mechanism at the transcriptional and translational level of these promoters in CAST among species and their contribution to various meat tenderness remains to be researched further (Kemp et al., 2010).

However, CAPN3 is an important muscle-specific Ca^{2+}-dependent cysteine protease (Suzuki et al., 1995) in the CAPN family. It attracts strong interest with its characteristic protein structure (two insertion sequences, IS1 and IS2) and location (binds to connectin at the N2 line, where proteolysis associated with meat tenderization occurs) (Sorimachi et al., 1989; Taylor et al., 1995; Herasse et al., 1999). However, its precise role in improving meat tenderness is still elusive. Some researchers found indications of a positive role in improving meat tenderness (Ilian M et al., 2001; Ilian et al., 2004a; Kemp et al., 2010), whereas some other researchers have not found such a role (Geesink et al., 2005).

In pigs, shear force measurements were carried out to verify the hypothesis that CAPN3 might influence postmortem proteolysis and meat tenderness on porcine longissimus dorsi (LD) muscle (Parr et al., 1999), but no direct evidence was found linking various levels of CAPN3 abundance and changes in porcine tenderness after 8 d of

conditioning. To go further, expression analysis of CAPN3 showed that higher CAPN3 expression was identified in less tender muscles, revealing the indirect involvement of CAPN3 in meat tenderization during postmortem aging (Gandolfi et al., 2011).

There are few available studies on the role of CAPN3 in the tenderness of chicken meat. Previously, Zhang et al. (2009) showed that *CAPN3* might serve as a candidate gene for QTL associated with chicken muscle growth and carcass traits. Muscle growth rate is directly related to three critical factors determining meat tenderness; fiber diameters, the proportion of glycolytic fibers and proteolytic potential. In addition, identification of two SNPs in intron 8 and exon 10 of CAPN3 pave the way for further study of CAPN3 in postmortem tenderization in chicken (Zhang et al., 2009).

So far, the role of CAPN3 in meat tenderization during postmortem aging still remains controversial in domestic animals. The possible causes associated with the divergence of opinion may be as follows:

• the most available evidence results from various animal species, breeds, age, domesticated background and selection history. In addition, the sample size is too small to estimate the relationship between CAPN3 and meat tenderization accurately.

• the difference of methodology may be another important reason leading to the divergence. Perhaps multiple methods and approaches (*in vitro* and *in vivo*) are necessary to evaluate the hypothesis. For example, we can use RNA interference or specific inhibition of CAPN3 in domestic animal myoblasts or satellite cells to detect the target protein degradation rate.

INTERACTION AMONG CAPN3, CAPN1 AND CAST

A hypothesis has been provided that there is likely to be an interaction between CAPN1 and CAST due to their strong biological relationship (two genes produce relevant proteins that physically interact in determining meat tenderness) (Casas et al., 2006). Recent crystallographic observations have identified the nature of the interaction between CAST and CAPN, as to the interaction between their unique aspects (regions A, B and C) as protein inhibitors and proteolytic enzymes (Hanna et al., 2008; Moldoveanu et al., 2008). When CAPNs bind to calcium, they are unable to be active while allowing CAST to interact with the enzyme. In addition, CAPNs can degrade CAST via the cleavage of the weaker inhibitory domains, creating specific peptide fragments that retain inhibitory activity (Doumit and Koohmaraie, 1999; Mellgren, 2008).

Although an interaction between CAPN1 and CAST has been detected affects the Warner-Bratzler shear Force in meat samples from a cattle population, this significant interaction needs further investigation (Casas et al., 2006; Morris et al., 2006).

In addition, CAPNs can play a role in postmortem proteolysis. As we know, CAPN3 binds to connectin (or titin) at the N2 line, where proteolysis associated with meat tenderization occurs (Sorimachi et al., 1989; Taylor et al., 1995), while CAPN1 can degrade the N2 line for meat tenderization (Goll et al., 1991). Degradation of N2 line lead to inability of CAPN3 to anchor to connectin and then too much free CAPN3 is generated, which is toxic to muscle cells (Beckmann and Spencer, 2008).

POSSIBLE MECHANISM OF CAPN3 IN IMPROVING MEAT TENDERNESS

Possible pathways involved which CAPN3 improves meat tenderization

Myofibril type composition: The composition of myofibre type is well-accepted as a vital factor responsible for the variation in meat quality (Karlsson et al., 1999). Three types have been classified via conventional histochemistry in adult skeletal muscle, i.e. types I, IIA and IIB fibres (Brooke and Kaiser, 1970). However, recent studies have demonstrated that a molecular basis of fibre typing based on myosin heavy chains (MyHC) (Larzul et al., 1997; Lefaucheur et al., 2004), would be more reasonable. Four isoforms have been identified, respectively, types I, IIa, IIx and IIb isoforms (Bär and Pette, 1988; Schiaffino et al., 1989). The diameter increases in the rank order I=IIa<IIx<IIb or IIa<I<IIx<IIb (Lefaucheur, 2010), which determined fiber cross-sectional area (CSA). The meat tenderness would be fine, when myofibre was thin and the density was high with higher fat content (Wang and Li, 1994). Therefore, type I and IIa have higher tenderness than IIx and IIb. Moreover, the four types are dynamic structures which exhibit high plasticity and undergo type shift in accordance with an obligatory pathway I→IIa→IIx→IIb (Schiaffino and Reggiani, 1994; Pette and Staron, 2000).

In vitro study has identified that CAPN3 can participate in the muscular regeneration process via decreasing the transcriptional activity of MyOD (Stuelsatz et al., 2010), a protein with a key role in regulating muscle differentiation. Meanwhile, *MyOD* is expressed in myosin heavy chain type IIx-expressing (MyHC-2x-expressing) muscle but less in non-MyHC-2x-expressing muscle (Muroya et al., 2002). Skeletal muscle is characterized by fast and slow muscle based on the expression pattern of MyHC isoforms in muscle fibres (Schiaffino and Reggiani, 1996). A previous study found that MyOD knockout mice showed a shift of MyHC isoform expression toward a slower phenotype (Hughes, 1997). Thus, changes of the expression ratio of

MyHC isofoms may contribute to the transformation of fibre types. Consequently, the tenderness would be improved if the muscle lacks MyOD.

Calcium ion: Calcium ions can improve tenderness when they are introduced into meat by means of injection, infusion and pickling for a short time with the concentration of 0.3 mol/L solutions ($CaCl_2$) (Morgan et al., 1991; Whipple and Koohmaraie, 1991; Koohmaraie, 1992; Boleman et al., 1995; Rees et al., 2002). Infusing calcium can increase tenderness and increased proteolysis or increased post-mortem glycolysis can all be the potential causes. Nevertheless, CAPN3, especially those associated with the sarcoplasmic reticulum (SR) membrane (Kramerova et al., 2006), has a confirmed role in regulating calcium release in skeletal muscle in the maintenance of a protein complex (AldoA and RyR) (Kramerova et al., 2008). Once calcium release is significantly reduced, the concentration of calcium ions in the sarcoplasm will be decreased. Consequently, the activation of Ca^{2+}-dependent proteolytic systems such as the CAPN system will be influenced and the key proteins involved in tenderization will not be degraded or modified. It was reported that CAPN1 was inactive when the Ca^{2+} concentration in sarcoplasm was below 10^{-7} mol after slaughter (Dransfield, 1994). Finally, structural changes of myofibril or collagen will not occur, when the degree of degradation is insufficient (Morgan et al., 1991). Thereby, meat tenderness can not be improved.

Tenderization-related protein: Early researches show that final tenderness is determined by the rate of proteolysis and weakening of myofibril structure (Taylor et al., 1995; Faulkner et al., 2000). The degradation of key proteins, for instance, titin, nebulin, filamin, desmin and troponin-T, are responsible for tenderization during aging (Koohmaraie, 1992; Huff-Lonergan et al., 1996; Wheeler et al., 2000; Lametsch et al., 2002). According to the theory of enzymatic meat tenderization, the CAPN system can play a role in postmortem proteolysis and meat tenderness. Recent studies have indicated that CAPN3 could cleave titin (related to the remodeling of sarcomere), filamin C (the Z-disc of the myofibrillar apparatus by binding directly to the Z-disc proteins FATZ (a filamin-, actinin-, and telethonin-binding protein of the Z-disc of skeletal muscle) and myotilin) and nebulin (the autolysis of myofibrillar CAPN3 and CAPN1), respectively (Faulkner et al., 2000; Van Der Ven et al., 2000; Guyon et al., 2003; Salmikangas et al., 2003; Ilian et al., 2004a; Kramerova et al., 2004). From the above studies, a role of CAPN3 in improving meat tenderness is inferred from modulating the proteolysis of key myofibrillar proteins.

Other factors: ATP is the key element that affects the meat aging. Kramerova et al. (2009) identified that mitochondria were dysfunctional in the CAPN3 knockout (C3KO) mice and this then lead to oxidative stress and ATP deficiency. ATP provides the energy for shortening the myofibril which results in mucle contraction in living cells (Goll et al., 1984). After slaughter, ATP can not be supplied and the shorten process of myofibrils is influnenced. Meat tenderness is affected by the amount of shortening of the myofibrils. It will be tougher, if the myofibril is shorter (Locker and Hagyard, 1963). Howerver, if the muscle stores much more ATP before slaughter, it will take a longer time to deplete the ATP. In that way, the muscle tends to produce less shortened myofibrils. The converse occurs if there is little stored ATP.

In addition, it is reported that CAPN3 can cleave titin *in vitro*. Titin serves as a template and molecular ruler for thick filament assembling and sarcomere formation and plays a central role in myofibrillogenesis (Kramerova et al., 2004). Specific degradation of titin causes an increase in the myofilament lattice spacing (Cazorla et al., 2001). With the intact proteinaceous connections associated with cell membrane, the increased spacing makes it possible for the myofibril to hold much water, thereby reduce the drip loss (Wang and Ramirez-Mitchell, 1983; Huff-Lonergan and Lonergan, 2005). Ultimately, meat juiceness will be improved.

So far, the regulation role of CAPN3 in meat tenderization is still controversial. Causes of the divergence are diverse. In the current review, possible pathways of CAPN3 involved in improving meat tenderness are speculated (Figure 1).

CONCLUSION AND PROSPECTIVES

Meat tenderness is a multifactorial economic trait, involving heredity, nutrition and environment factors. In spite of this, several researches have been conducted in domestic animals based on the theory of enzymatic meat tenderization especially the CAPN protease system. So far, it is clear that *CAPN1* and *CAST* genes are responsible for postmortem proteolysis, but the role of CAPN3, a member of CAPN family, in postmortem meat tenderization is unclear. In the present review, the role of CAPN1, CAPN3, CAST in postmortem meat aging is discussed, and four possible pathways of CAPN3 are suggested based on the recent findings, however, the precise regulation role of CAPN3 remains to be investigated and validated further involving multi-methods with sufficiently large sample sizes.

If CAPN3 does contribute to postmortem meat tenderization, and the identified SNP markers linked to meat tenderness developed for the *CAPN1*, *CAPN3* and *CAST* genes, they can ultimately be applied in identifying

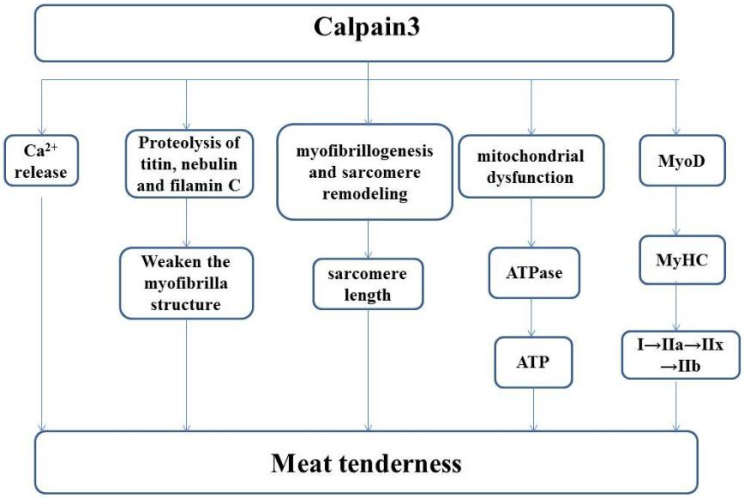

Figure 1. Possible mechanism of CAPN3 in meat tenderization.

animals with the genetic potential to produce better meat, i.e. marker-assisted breeding programmes. In addition, the various protein isoforms from CAST are likely to be responsible for variations in meat tenderness.

REFERENCES

Bär, A. and D. Pette. 1988. Three fast myosin heavy chains in adult rat skeletal muscle. FEBS Lett. 235:153-155.

Barbut, S., A. A. Sosnicki, S. M. Lonergan, T. Knapp, D. C. Ciobanu, L. J. Gatcliffe, E. Huff-Lonergan and E. W. Wilson. 2008. Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. Meat Sci. 79:46-63.

Barendse, W., B. E. Harrison, R. J. Bunch and M. B. Thomas. 2008. Variation at the calpain 3 gene is associated with meat tenderness in zebu and composite breeds of cattle. BMC Genet. 9:41.

Beckmann, J. S. and M. Spencer. 2008. Calpain 3, the "gatekeeper" of proper sarcomere assembly, turnover and maintenance. Neuromuscul. Disord. 18:913-921.

Bernard, C., I. Cassar-Malek, M. L. Cunff, H. Dubroeucq, G. Renand and J. F. Hocquette. 2007. New indicators of beef sensory quality revealed by expression of specific genes. J. Agric. Food Chem. 55:5229-5237.

Bickerstaffe, R., K. Gately and J. D. Morton. 2008. The association between polymorphic variations in calpain 3 with the yield and tenderness of retail lamb meat cuts. Proceedings of 54th International Congress of Meat Science and Technology, Helsinki, Finland, 1-4.

Blanchard, H., P. Grochulski, Y. Li, J. S. C. Arthur, P. L. Davies, J. S. Elce and M. Cygler. 1997. Structure of a calpain Ca^{2+}-binding domain reveals a novel EF-hand and Ca^{2+}-induced conformational changes. Nat. Struct. Biol. 4:532-538.

Blanchard, H., Y. Li, M. Cygler, C. M. Kay, J. S. C. Arthur, P. L. Davies and J. S. Elce. 1996. Ca^{2+}-binding domain VI of rat calpain is a homodimer in solution: Hydrodynamic, crystallization and preliminary X-ray diffraction studies. Protein Sci. 5:535-537.

Boleman, S. J., S. L. Boleman, T. D. Bidner, K. W. McMillin and C. J. Monlezun. 1995. Effects of postmortem time of calcium chloride injection on beef tenderness and drip, cooking, and total loss. Meat Sci. 39:35-41.

Brooke, M. H. and K. K. Kaiser. 1970. Muscle fiber types: How many and what kind? Arch. Neurol. 23:369-379.

Brulé, C., E. Dargelos, R. Diallo, A. Listrat, D. Béchet, P. Cottin and S. Poussard. 2010. Proteomic study of calpain interacting proteins during skeletal muscle aging. Biochimie 92:1923-1933.

Cafe, L. M., B. L. McIntyre, D. L. Robinson, G. H. Geesink, W. Barendse, D. W. Pethick, J. M. Thompson and P. L. Greenwood. 2010. Production and processing studies on calpain-system gene markers for tenderness in brahman cattle:1. Growth, efficiency, temperament, and carcass characteristics. J. Anim. Sci. 88:3047-3058.

Camou, J. P., J. A. Marchello, V. F. Thompson, S. W. Mares and D. E. Goll. 2007. Effect of postmortem storage on activity of μ-and m-calpain in five bovine muscles. J. Anim. Sci. 85: 2670-2681.

Casas, E., S. N. White, T. L. Wheeler, S. D. Shackelford, M. Koohmaraie, D. G. Riley, C. C. Chase Jr, D. D. Johnson and T. P. L. Smith. 2006. Effects of calpastatin and μ-calpain markers in beef cattle on tenderness traits. J. Anim. Sci. 84:520-525.

Cazorla, O., Y. Wu, T. C. Irving and H. Granzier. 2001. Titin-based modulation of calciumsensitivity of active tension in mouse skinned cardiac myocytes. Circ. Res. 88:1028-1035.

Cheong, H. S., D. H. Yoon, B. L. Park, L. H. Kim, J. S. Bae, S. Namgoong, H. W. Lee, C. S. Han, J. O. Kim and I. C. Cheong. 2008. A single nucleotide polymorphism in CAPN 1 associated with marbling score in Korean cattle. BMC Genet. 9:33.

Ciobanu, D. C., J. W. M. Bastiaansen, S. M. Lonergan, H. Thomsen, J. C. M. Dekkers, G. S. Plastow and M. F. Rothschild. 2004. New alleles in calpastatin gene are associated with meat quality traits in pigs. J. Anim. Sci. 82: 2829-2839.

Cong, M., V. F. Thompson, D. E. Goll and P. B. Antin. 1998. The bovine calpastatin gene promoter and a new N-terminal region of the protein are targets for cAMP-dependent protein kinase

activity. J. Biol. Chem. 273:660-666.
Corva, P., L. Soria, A. Schor, E. Villarreal, M. P. Cenci, M. Motter, C. Mezzadra, L. Melucci, C. Miquel, E. Paván, G. Depetris, F. Santini and J. G. Naón. 2007. Association of *CAPN1* and *CAST* gene polymorphisms with meat tenderness in *Bos Taurus* beef cattle from Argentina. Genet. Mol. Biol. 30:1064-1069.
Costello, S., E. O'Doherty, D. J. Troy, C. W. Ernst, K. S. Kim, P. Stapleton, T. Sweeney and A. M. Mullen. 2007. Association of polymorphisms in the calpain I, calpain II and growth hormone genes with tenderness in bovine *M. Longissimus Dorsi*. Meat Sci. 75:551-557.
Croall, D. E. and G. N. DeMartino. 1991. Calcium-activated neutral protease (calpain) system: Structure, function, and regulation. Physiol. Rev. 71:813-847.
Croall, D. E. and K. Ersfeld. 2007. The calpains: Modular designs and functional diversity. Genome Biol. 8: 218.
Destefanis, G., A. Brugiapaglia, M. T. Barge and E. Dal Molin. 2008. Relationship between beef consumer tenderness perception and warner-bratzler shear force. Meat Sci. 78:153-156.
Doumit, M. E. and M. Koohmaraie. 1999. Immunoblot analysis of calpastatin degradation: Evidence for cleavage by calpain in postmortem muscle. J. Anim. Sci. 77:1467-1473.
Dransfield, E. 1994. Modelling post-mortem tenderization-V: Inactivation of calpains. Meat Sci. 37:391-409.
Dutaud, D., L. Aubry, M. A. Sentandreu and A. Ouali. 2006. Bovine muscle 20S proteasome: I. Simple purification Procedure and Enzymatic Characterization in Relation with Postmortem Conditions. Meat Sci. 74:327-336.
Eisenberg, D., R. M. Weiss, T. C. Terwilliger and W. Wilcox. 1982. Hydrophobic moments and protein structure. Faraday Symp. Chem. Soc. 17:109-120.
Emori, Y., H. Kawasaki, S. Imajoh, S. Kawashima and K. Suzuki. 1986. Isolation and sequence analysis of cDNA clones for the small subunit of rabbit calcium-dependent protease. J. Biol. Chem. 261:9472-9476.
Fürst, D. O., M. Osborn, R. Nave and K. Weber. 1988. The organization of titin filaments in the half-sarcomere revealed by monoclonal antibodies in immunoelectron microscopy: A map of ten nonrepetitive epitopes starting at the Z line extends close to the M line. J. Cell Biol. 106:1563-1572.
Faulkner, G., A. Pallavicini, A. Comelli, M. Salamon, G. Bortoletto, C. Ievolella, S. Trevisan, S. Koji, F. Dalla Vecchia and P. Laveder. 2000. FATZ, a filamin-, actinin-, and telethonin-binding protein of the Z-disc of skeletal muscle. J. Biol. Chem. 275:41234-41242.
Fougerousse, F., M. Durand, L. Suel, O. Pourquié, A. L. Delezoide, N. B. Romero, M. Abitbol and J. S. Beckmann. 1998. Expression of genes (CAPN3, SGCA, SGCB, and TTN) involved in progressive muscular dystrophies during early human development. Genomics 48:145-156.
Gandolfi, G., L. Pomponio, P. Ertbjerg, A. H. Karlsson, L. Nanni Costa, R. Lametsch, V. Russo and R. Davoli. 2011. Investigation on CAST, CAPN1 and CAPN3 porcine gene polymorphisms and expression in relation to post-mortem calpain activity in muscle and meat quality. Meat Sci. 88:694-700.
Garcia Diaz, B. E., S. Gauthier and P. L. Davies. 2006. $Ca2^{+}$ dependency of calpain 3 (p94) activation. Biochemistry 45: 3714-3722.
Geesink, G. H., R. G. Taylor and M. Koohmaraie. 2005. Calpain 3/p94 is not involved in postmortem proteolysis. J. Anim. Sci. 83:1646-1652.
Goll, D. E., R. M. Robson and M. H. Stromer. 1984. Skeletal muscle, nervous system, temperature regulation, and special senses. In: Duke's physiol. domestic anim (Ed. M. J. Swensen). Ithaca, N.Y: Cornell University Press. pp. 548-580.
Goll, D. E., W. R. Dayton, I. Singh and R. M. Robson. 1991. Studies of the alpha-actinin/actin interaction in the Z-Disk by using calpain. J. Biol. Chem. 266:8501-8510.
Goll, D. E., Y. Otsuka, P. A. Nagainis, J. D. Shannon, S. K. Sathe and M. Muguruma. 1983. Role of muscle proteinases in maintenance of muscle integrity and mass. J. Food Biochem. 7:137-177.
Goll, D. E., V. F. Thompson, H. Li, W. Wei and J. Cong. 2003. The calpain system. Physiol. Rev. 83:731-801.
Gollasch, M. and M. T. Nelson. 1997. Voltage-dependent Ca^{2+} channels in arterial smooth muscle cells. Kidney Blood Press. Res. 20:355-371.
Grobbel, J. P., M. E. Dikeman, M. C. Hunt and G. A. Milliken. 2008. Effects of packaging atmospheres on beef instrumental tenderness, fresh color stability, and internal cooked color. J. Anim. Sci. 86:1191-1199.
Guroff, G. 1964. A neutral, calcium-activated proteinase from the soluble fraction of rat brain. J. Biol. Chem. 239:149-155.
Guyon, J. R., E. Kudryashova, A. Potts, I. Dalkilic, M. A. Brosius, T. G. Thompson, J. S. Beckmann, L. M. Kunkel and M. J. Spencer. 2003. Calpain 3 cleaves filamin C and regulates its ability to interact with γ-and δ-sarcoglycans. Muscle Nerve 28: 472-483.
Hanna, R. A., R. L. Campbell and P. L. Davies. 2008. Calcium-bound structure of calpain and its mechanism of inhibition by calpastatin. Nature 456:409-412.
Herasse, M., Y. Ono, F. Fougerousse, E. Kimura, D. Stockholm, C. Beley, D. Montarras, C. H. Sorimachi, K. Suzuki, J. S. Beckmann and I. Richard. 1999. Expression and functional characteristics of calpain 3 isoforms generated through tissue-specific transcriptional and posttranscriptional events. Mol. Cell Biol. 19:4047-4055.
Hosfield, C. M., J. S. Elce, P. L. Davies and Z. C. Jia. 1999. Crystal structure of calpain reveals the structural basis for Ca^{2+}-dependent protease activity and a novel mode of enzyme activation. EMBO J. 18:6880-6889.
Huff-Lonergan, E. and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. Meat Sci. 71:194-204.
Huff-Lonergan, E., T. Mitsuhashi, D. D. Beekman, F. Parrish Jr, D. G. Olson and R. M. Robson. 1996. Proteolysis of specific muscle structural proteins by mu-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. J. Anim. Sci. 74:993-1008.
Huff-Lonergan, E. and S. M. Lonergan. 1999. Postmortem mechanisms of meat tenderization: The roles of the structural proteins and the calpain system. In: Quality attributes muscle foods (Ed. Y. L. Xiong, C. -T. Ho and F. Shahidi). New York: Kluwer Academic/Plenum Publishers. pp. 229-251.
Huff Lonergan, E., W. Zhang and S. M. Lonergan. 2010. Biochemistry of postmortem muscle-Lessons on mechanisms

of meat tenderization. Meat Sci. 86:184-195.
Hughes, S. M., K. Koishi, M. Rudnicki and A. M. Maggs. 1997. MyoD protein differentially accumulated in fast and slow skeletal muscle fibers and required for normal fiber type balance in rodents. Mech. Dev. 61:151-163.
Hwang, I. H., C. E. Devine and D. L. Hopkins. 2003. The biochemical and physical effects of electrical stimulation on beef and sheep meat tenderness. Meat Sci. 65:677-691.
Ilian, M. A., A. E. D. Bekhit and R. Bickerstaffe. 2004a. The relationship between meat tenderization, myofibril fragmentation and autolysis of calpain 3 during post-mortem aging. Meat Sci. 66:387-397.
Ilian, M. A., A. E. D. A. Bekhit, B. Stevenson, J. D. Morton, P. Isherwood and R. Bickerstaffe. 2004b. Up-and down-regulation of *longissimus* tenderness parallels changes in the myofibril-bound calpain 3 protein. Meat Sci. 67:433-445.
Ilian, M. A., R. Bickerstaffe and M. L. Greaser. 2004c. Postmortem changes in myofibrillar-bound calpain 3 revealed by immunofluorescence microscopy. Meat Sci. 66:231-240.
Ilian, M. A., J. D. Morton, A. E. D. Bekhit, N. Roberts, B. Palmer, S. Sorimachi and R. Bickerstaffe. 2001. Effect of preslaughter feed withdrawal period on longissimus tenderness and the expression of calpains in the ovine. J. Agric. Food Chem. 49: 1990-1998.
Ilian, M. A., J. D. Morton, M. P. Kent, C. E. Le Couteur, J. Hickford, R. Cowley and R. Bickerstaffe. 2001. Intermuscular variation in tenderness: Association with the ubiquitous and muscle-specific calpains. J. Anim. Sci. 79:122-132.
Imajoh, S., K. Aoki, S. Ohno, Y. Emori, H. Kawasaki, H. Sugihara and K. Suzuki. 1988. Molecular cloning of the cDNA for the large subunit of the high-calcium-requiring form of human calcium-activated neutral protease. Biochemistry 27:8122-8128.
Ishida, S., Y. Emori and K. Suzuki. 1991. Rat calpastatin has diverged primary sequence from other mammalian calpastatins but retains functionality important sequences. Biochim. Biophys. Acta. 1088:436-438.
Jin, X., L. C. Zhang, Z. H. Li, X. H. Liu, H. G. Jin and C. G. Yan. 2011. Association of polymorphisms in the calpain I gene with meat quality traits in Yanbian yellow cattle of China. Asian-Aust. J. Anim. Sci. 24:9-16.
Karlsson, A. H., R. E. Klont and X. Fernandez. 1999. Skeletal muscle fibres as factors for pork quality. Livest. Prod. Sci. 60: 255-269.
Kemp, C. M., P. L. Sensky, R. G. Bardsley, P. J. Buttery and T. Parr. 2010. Tenderness-An enzymatic view. Meat Sci. 84:248-256.
Kent, M. P., M. J. Spencer and M. Koohmaraie. 2004. Postmortem proteolysis is reduced in transgenic mice overexpressing calpastatin. J. Anim. Sci. 82:794-801.
Kinbara, K., S. Ishiura, S. Tomioka, H. Sorimachi, S. Y. Jeong, S. Amano, H. Kawasaki, B. Kolmerer, S. Kimura, S. Labeit and K. Suzuki. 1998. Purification of native p94, a muscle-specific calpain, and characterization of its autolysis. Biochem. J. 335: 589-596.
König, N., F. Raynaud, H. Feane, M. Durand, N. NMestre-Francès, M. Rossel, A. Ouali and Y. Benyamin. 2003. Calpain 3 is expressed in astrocytes of rat and *microcebus* brain. J. Chem. Neuroanat. 25:129-136.
Koohmaraie, M. 1992. The role of Ca^{2+}-dependent proteases (calpains) in post mortem proteolysis and meat tenderness. Biochimie 74:239-245.
Koohmaraie, M. 1996. Biochemical factors regulating the toughening and tenderization processes of meat. Meat Sci. 43 (Supp. 1):193-201.
Koohmaraie, M., A. H. Chishti, G. H. Geesink and S. Kucha. 2006. μ-Calpain is essential for postmortem proteolysis of muscle proteins. J. Anim. Sci. 84:2834-2840.
Koohmaraie, M., M. E. Doumit and T. L. Wheeler. 1996. Meat toughening does not occur when rigor shortening is prevented. J. Anim. Sci. 74:2935-2942.
Koohmaraie, M. and G. H. Geesink. 2006. Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Sci. 74:34-43.
Koohmaraie, M., S. C. Seideman, J. E. Schollmeyer, T. R. Dutson and A. S. Babiker. 1988. Factors associated with the tenderness of three bovine muscles. J. Food Sci. 53:407-410.
Koohmaraie, M., G. Whipple, D. H. Kretchmar, J. D. Crouse and H. J. Mersmann. 1991. Postmortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. J. Anim. Sci. 69: 617-624.
Kramerova, I., E. Kudryashova, J. G. Tidball and M. J. Spencer. 2004. Null mutation of calpain 3 (p94) in mice causes abnormal sarcomere formation *in vivo* and *in vitro*. Hum. Mol. Genet. 13:1373-1388.
Kramerova, I., E. Kudryashova, B. Wu and M. J. Spencer. 2006. Regulation of the M-cadherin-beta-catenin complex by calpain 3 during terminal stages of myogenic differentiation. Mol. Cell Biol. 26:8437- 8447.
Kramerova, I., E. Kudryashova, B. Wu, C. Ottenheijm, H. Granzier and M. J. Spencer. 2008. Novel role of calpain-3 in the triad-associated protein complex regulating calcium release in skeletal muscle. Hum. Mol. Genet. 17:3271-3280.
Kramerova, I., E. Kudryashova, B. Wu, S. Germain, K. Vandenborne, N. Romain, R. G. Haller, M. A. Verity and M. J. Spencer. 2009. Mitochondrial abnormalities, energy deficit and oxidative stress are features of calpain 3 deficiency in skeletal muscle. Hum. Mol. Genet. 18:3194-3205.
Lametsch, R., P. Roepstorff and E. Bendixen. 2002. Identification of protein degradation during post-mortem storage of pig meat. J. Agric. Food Chem. 50:5508- 5512.
Larzul, C., L. Lefaucheur, P. Ecolan, J. Gogue, A. Talmant, P. Sellier, P. Le Roy and G. Monin. 1997. Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in large white pigs. J. Anim. Sci. 75:3126-3137.
Lee, H. L., V. Santé-Lhoutellier, S. Vigouroux, Y. Briand and M. Briand. 2008. Role of calpains in postmortem proteolysis in chicken muscle. Poult. Sci. 87:2126-2132.
Lefaucheur, L. 2010. A second look into fibre typing-relation to meat quality. Meat Sci. 84:257-270.
Lefaucheur, L., D. Milan, P. Ecolan and C. Le Callennec. 2004. Myosin heavy chain composition of different skeletal muscles in large white and meishan pigs. J. Anim. Sci. 82:1931-1941.
Lin, G. D., D. Chattopadhyay, M. Maki, K. K. Wang, M. Carson, L. Jin, P. W. Yuen, E. Takano, M. Hatanaka, L. J. DeLucas and S. V. Narayana. 1997. Crystal structure of calcium bound domain VI of calpain at 1.9 A resolution and its role in enzyme

assembly, regulation, and inhibitor binding. Nat. Struct. Biol. 4:539-547.

Lindholm-Perry, A. K., G. A. Rohrer, J. W. Holl, S. D. Shackelford, T. L. Wheeler, M. Koohmaraie and D. Nonneman. 2009. Relationships among calpastatin single nucleotide polymorphisms, calpastatin expression and tenderness in pork longissimus. Anim. Genet. 40:713-721.

Locker, R. H. and C. J. Hagyard. 1963. A cold shortening effect in beef muscles. J. Sci. Food Agric. 14:787-793.

Ma, H., C. Fukiage, M. Azuma and T. R. Shearer. 1998. Cloning and expression of mRNA for calpain Lp82 from rat lens: Splice variant of p94. Invest. Ophthalmol. Vis. Sci. 39:454-461.

Mayes, P. A. 1993. Metabolism of glycogen. In: Harpers biochemistry 28th. edn. New Jersy: Appleton and Lange Publishing Division of Prentice Hall, Englewood Cliffs, pp. 50-400.

McCormick, R. J. 2009. Collagen, applied muscle biology and meat science. In: CRC Press, Boca Raton, FL, pp. 129-148.

Mellgren, R. L. 2008. Structural biology: Enzyme knocked for a loop. Nature 456:337-338.

Melody, J. L., S. M. Lonergan, L. J. Rowe, T. W. Huiatt, M. S. Mayes and E. Huff-Lonergan. 2004. Early postmortem biochemical factors influence tenderness and water holding capacity of three porcine muscles. J. Anim. Sci. 82:1195-1205.

Meyers, S. N. and J. E. Beever. 2008. Investigating the genetic basis of pork tenderness: Genomic analysis of porcine CAST. Anim. Genet. 39:531-543.

Moeller, S. J., R. K. Miller, K. K. Edwards, H. N. Zerby, K. E. Logan, T. L. Aldredge, C. A. Stahl, M. Boggess and J. M. Box-Steffensmeier. 2010. Consumer perceptions of pork eating quality as affected by pork quality attributes and end-point cooked temperature. Meat Sci. 84:14-22.

Moldoveanu, T., K. Gehring and D. R. Green. 2008. Concerted multi-pronged attack by calpastatin to occlude the catalytic cleft of heterodimeric calpains. Nature 456:404- 408.

Morgan, J. B., R. K. Miller, F. M. Mendez, D. S. Hale and J. W. Savell. 1991. Using calcium chloride injection to improve tenderness of beef from mature cows. J. Anim. Sci. 69:4469-4476.

Morris, C. A., N. G. Cullen, S. M. Hickey, M. Dobbie, B. A. Veenvliet, T. R. Manley, W. S. Pitchford, Z. A. Kruk, C. D. K. Bottema and T. Wilson. 2006. Genotypic effects of calpain 1 and calpastatin on the tenderness of cooked *M. Longissimus Dorsi* steaks from Jersey×Limousin, Angus and Hereford-cross cattle. Anim. Genet. 37:411-414.

Moudilou, E. N., N. Mouterfi, J. M. Exbrayat and C. Brun. 2010. Calpains expression during Xenopus Laevis development. Tissue Cell 42:275-281.

Muroya, S., I. Nakajima and K. Chikuni. 2002. Related expression of MyoD and Myf5 with myosin heavy chain isoform types in bovine adult skeletal muscles. Zool. Sci. 19:755-761.

Neath, K. E., A. N. Del Barrio, R. M. Lapitan, J. R. V. Herrera, L. C. Cruz, T. Fujihara, S. Muroya, K. Chikuni, M. Hirabayashi and Y. Kanai. 2007. Protease activity higher in postmortem water buffalo meat than Brahman beef. Meat Sci. 77:389-396.

Ohno, S., Y. Emori and E. Suzuki. 1986. Nucleotide sequence of a cDNA coding for the small subunit of human calcium-dependent protease. Nucleic Acids Res. 14:5559.

Ohno, S., S. Minoshima, J. Kudoh, R. Fukuyama, Y. Shimizu, S. Ohmi-Imajohs, N. Shimizu and K. Suzuki. 1990. Four genes for the calpain family locate on four distinct human chromosomes. Cytogenet. Genome Res. 53:225-229.

Ono, Y., K. Kakinuma, F. Torii, A. Irie, K. Nakagawa, S. Labeit, K. Abe, K. Suzuki and H. Sorimachi. 2004. Possible regulation of the conventional calpain system by skeletal muscle-specific calpain, p94/calpain 3. J. Biol. Chem. 279:2761-2771.

Ono, Y., K. Ojima, F. Torii, E. Takaya, N. Doi, K. Nakagawa, S. Hata, K. Abe and H. Sorimachi. 2010. Skeletal muscle-specific calpain is an intracellular Na^+-dependent protease. J. Biol. Chem. 285:22986-22998.

Page, B. T., E. Casas, M. P. Heaton, N. G. Cullen, D. L. Hyndman, C. A. Morris, A. M. Crawford, T. L. Wheeler, M. Koohmaraie, J. W. Keele and T. P. L. Smith. 2002. Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle. J. Anim. Sci. 80:3077-3085.

Page, B. T., E. Casas, R. L. Quaas, R. M. Thallman, T. L. Wheeler, S. D. Shackelford, M. Koohmaraie, S. N. White, G. L. Bennett, J. W. Keele, M. E. Dikeman and T. P. Smith. 2004. Association of markers in the bovine CAPN1 gene with meat tenderness in large crossbred populations that sample influential industry sires. J. Anim. Sci. 82:3474-3481.

Parr, T., P. L. Sensky, G. P. Scothern, R. G. Bardsley, P. J. Buttery, J. D. Wood and C. Warkup. 1999. Relationship between skeletal muscle-specific calpain and tenderness of conditioned porcine longissimus muscle. J. Anim. Sci. 77:661-668.

Peaston, A. E. and E. Whitelaw. 2006. Epigenetics and phenotypic variation in mammals. Mamm. Genome 17:365-374.

Pette, D. and R. S. Staron. 2000. Myosin isoforms, muscle fiber types, and transitions. Microsc. Res. Techniq. 50: 500-509.

Pinto, L. F. B, J. B. S. Ferraz, F. V. Meirelles, J. P. Eler, F. M. Rezende, M. E. Carvalho, H. B. Almeida and R. C. G. Silva. 2010. Association of SNPs on *CAPN1* and *CAST* genes with tenderness in Nellore cattle. Genet. Mol. Res. 9:1431-1442.

Pomponio, L., R. Lametsch, A. H. Karlsson, L. N. Costa, A. Grossi and P. Ertbjerg. 2008. Evidence for post-mortem m-calpain autolysis in porcine muscle. Meat Sci. 80:761-764.

Poussard, S., M. Duvert, D. Balcerzak, S. Ramassamy, J. J. Brustis, P. Cottin and A. Ducastaing. 1996. Evidence for implication of muscle-specific calpain (p94) in myofibrillar integrity. Cell Growth Differ. 7:1461-1469.

Ravulapalli, R., R. Campbell, S. Y. Gauthier, S. Dhe-Paganon and P. L. Davies. 2009. Distinguishing between calpain heterodimerization and homodimerization. FEBS J. 276:973-982.

Ravulapalli, R., B. Garcia Diaz, R. L. Campbell and P. L. Davies. 2005. Homodimerization of calpain 3 penta-EF-hand domain. Biochem. J. 388:585-591.

Rees, M. P., G. R. Trout and R. D. Warner. 2002. Effect of calcium infusion on tenderness and ageing rate of pork *M. Longissimus Thoracis* et *Lumborum* after accelerated boning. Meat Sci. 61: 169-179.

Riley, D. G., C. C. Chase, T. D. Pringle, R. L. West, D. D. Johnson, T. A. Olson, A. C. Hammond and S. W. Coleman. 2003. Effect of sire on μ-and m-calpain activity and rate of tenderization as indicated by myofibril fragmentation indices of steaks from Brahman cattle. J. Anim. Sci. 81:2440-2447.

Roberts, N., B. Palmer, J. G. H. Hickford and R. Bickerstaffe. 1996. PCR-SSCP in the ovine calpastatin gene. Anim. Genet.

27:211.

Salmikangas, P., P. F. M. Van der Ven, M. Lalowski, A. Taivainen, F. Zhao, H. Suila, R. Schr der, P. Lappalainen and D. O. Fürst. 2003. Myotilin, the limb-girdle muscular dystrophy 1A (LGMD1A) protein, cross-links actin filaments and controls sarcomere assembly. Hum. Mol. Genet. 12:189-203.

Schenkel, F. S., S. P. Miller, Z. Jiang, I. B. Mandell, X. Ye, H. Li and J. W. Wilton. 2006. Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. J. Anim. Sci. 84:291-299.

Schiaffino, S., L. Gorza, S. Sartore, L. Saggin, S. Ausoni, M. Vianello, K. Gundersen and T. LØmo. 1989. Three myosin heavy chain isoforms in type 2 skeletal muscle fibres. J. Muscle Res. Cell Motil. 10:197-205.

Schiaffino, S. and C. Reggiani. 1994. Myosin isoforms in mammalian skeletal muscle. J. Appl. Physiol. 77:493-501.

Schiaffino, S. and C. Reggiani. 1996. Molecular diversity of myofibrillar proteins: Gene regulation and functional significance. Physiol. Rev. 76:371-423.

Seideman, S. C., H. R. Cross and J. D. Crouse. 1989. Variations in the sensory properties of beef as affected by sex condition, muscle and postmortem aging. J. Food Qual. 12:39-58.

Sentandreu, M. A., G. Coulis and A. Ouali. 2002. Role of muscle endopeptidases and their inhibitors in meat tenderness. Trends Food Sci. Technol. 13:400-421.

Shackelford, S. D., T. L. Wheeler, M. K. Meade, J. O. Reagan, B. L. Byrnes and M. Koohmaraie. 2001. Consumer impressions of tender select beef. J. Anim. Sci. 79:2605-2614.

Sieczkowska, H., A. Zybert, E. Krzecio, K. Antosik, M. Kocwin-Podsiadla, M. Pierzchala and P. Urbanski. 2010. The expression of genes *PKM2* and *CAST* in the muscle tissue of pigs differentiated by glycolytic potential and drip loss, with reference to the genetic group. Meat Sci. 84:137-142.

Simmons, N. J., C. C. Daly, T. L. Cummings, S. K. Morgan, N. V. Johnson and A. Lombard. 2008. Reassessing the principles of electrical stimulation. Meat Sci. 80:110-122.

Škrlep, M., M. Čandek-Potokar, T. Kavar, B. Žlender, M. HortÓs, P. Gou, J. Arnau, G. Evans, O. Southwood, A. Diestre and N. Robert. 2010. Association of *PRKAG3* and *CAST* genetic polymorphisms with traits of interest in dry-cured ham production: Comparative study in France, Slovenia and Spain. Livest Sci. 128:60-66.

Sorimachi, H., S. Imajoh-Ohmi, Y. Emori, H. Kawasaki, S. Ohno, Y. Minami and K. Suzuki. 1989. Molecular cloning of a novel mammalian calcium-dependent protease distinct from both m- and mu-types. Specific expression of the mRNA in skeletal muscle. J. Biol. Chem. 264:20106-20111.

Sorimachi, H., K. Kinbara, S. Kimura, M. Takahashi, S. Ishiura, N. Sasagawa, N. Sorimachi, H. Shimada, K. Tagawa, K. Maruyama and K. Suzuki. 1995. Muscle-specific calpain, p94, responsible for limb girdle muscular dystrophy type 2A, associates with connectin through IS2, a p94-specific sequence. J. Biol. Chem. 270:31158-31162.

Sorimachi, H., N. Toyama-Sorimachi, T. C. Saido, H. Kawasaki, H. Sugita, M. Miyasaka, K. Arahata, S. Ishiura and K. Suzuki. 1993. Muscle-specific calpain, p94, is degraded by autolysis immediately after translation, resulting in disappearance from muscle. J. Biol. Chem. 268:10593-10605.

Strobl, S., C. Fernandez-Catalan, M. Braun, R. Huber, H. Masumoto, K. Nakagawa, A. Irie, H. Sorimachi, G. Bourenkow, H. Bartunik, K. Suzuki and W. Bode. 2000. The crystal structure of calcium-free human m-calpain suggests an electrostatic switch mechanism for activation by calcium. Proc. Natl. Acad. Sci. 97:588-592.

Stuelsatz, P., F. Pouzoulet, Y. Lamarre, E. Dargelos, S. Poussard, S. Leibovitch, P. Cottin and P. Veschambre. 2010. Down-regulation of MyoD by calpain 3 promotes generation of reserve cells in C2C12 myoblasts. J. Biol. Chem. 285:12670-12683.

Suzuki, K., H. Sorimachi, T. Yoshizawa, K. Kinbara and S. Ishiura. 1995. Calpain: Novel family members, activation, and physiological function. Biol. Chem. Hoppe-Seyler 376:523.

Taylor, R. G., G. H. Geesink, V. F. Thompson, M. Koohmaraie and D. E. Goll. 1995. Is Z-disk degradation responsible for postmortem tenderization? J. Anim. Sci. 73:1351-1367.

Tompa, P., Y. Emori, H. Sorimachi, K. Suzuki and P. Friedrich. 2001. Domain III of calpain is a Ca^{2+}-regulated phospholipid-binding domain. Biochem. Biophys. Res. Commun. 280:1333-1339.

Tullio, R. D., M. Passalacqua, M. Averna, F. Salamino, E. Melloni and S. Pontremoli. 1999. Changes in intracellular localization of calpastatin during calpain activation. Biochem. J. 343:467-472.

Van der Ven, P. F. M., S. Wiesner, P. Salmikangas, D. Auerbach, M. Himmel, S. Kempa, K. Haye, D. Pacholsky, A. Taivainen, R. Schröder, O. Carpén and D. O. Fürst. 2000. Indications for a novel muscular dystrophy pathway. J. Cell Biol. 151:235-248.

Veiseth, E., S. D. Shackelford, T. L. Wheeler and M. Koohmaraie. 2001. Effect of postmortem storage on mu-calpain and m-calpain in ovine skeletal muscle. J. Anim. Sci. 79:1502-1508.

Veiseth, E., S. D. Shackelford, T. L. Wheeler and M. Koohmaraie. 2004. Factors regulating *lamb longissimus* tenderness are affected by age at slaughter. Meat Sci. 68:635-640.

Wang, K. and R. Ramirez-Mitchell. 1983. A network of transverse and longitudina intermediate filaments is associated with sarcomeres of adult vertebrate skeletal-muscle. J. Cell Biol. 96:562-570.

Wang, K. and J. Wright. 1988. Architecture of the sarcomere matrix of skeletal muscle: immunoelectron microscopic evidence that suggests a set of parallel inextensible nebulin filaments anchored at the Z line. J. Cell Biol. 107:2199-2212.

Wang, Y. M. and L. F. Li. 1994. The relationship between muscle histology characteristics and meat quality on Yushan pigs of JiangXi. Acta J. X. Agric. Univ. 16:284-287.

Wendt, A., V. F. Thompson and D. E. Goll. 2004. Interaction of calpastatin with calpain: A review. Biol. Chem. 385:465-472.

Wheeler, T. L. and M. Koohmaraie. 1999. The extent of proteolysis is independent of sarcomere length in lamb longissimus and psoas major. J. Anim. Sci. 77:2444-2451.

Wheeler, T. L., S. D. Shackelford and M. Koohmaraie. 2000. Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. J. Anim. Sci. 78: 958-965.

Whipple, G. and M. Koohmaraie. 1991. Degradation of myofibrillar proteins by extractable lysosomal enzymes and m-calpain, and the effects of zinc chloride. J. Anim. Sci. 69: 4449-4460.

White, S. N., E. Casas, T. L. Wheeler, S. D. Shackelford, M.

Koohmaraie, D. G. Riley, C. C. Chase, D. D. Johnson, J. W. Keele and T. P. L. Smith. 2005. A new single nucleotide polymorphism in *CAPN1* extends the current tenderness marker test to include cattle of *Bos Indicus*, *Bos Taurus*, and crossbred descent. J. Anim. Sci. 83:2001-2008.

Xu, X. X., X. Shui, Z. H. Chen, C. Q. Shan, Y. N. Hou and Y. G. Cheng. 2009. Development and application of a real-time PCR method for pharmacokinetic and biodistribution studies of recombinant adenovirus. Mol. Biotechnol. 43:130-137.

Zhang, Z. R., Y. P. Liu, Y. G. Yao, X. S. Jiang, H. R. Du and Q. Zhu. 2009. Identification and association of the single nucleotide polymorphisms in calpain3 (*CAPN3*) gene with carcass traits in chickens. BMC Genet. 10:10.

Zór, K., R. Ortiz, E. Saatci, R. Bardsley, T. Parr, E. Csöregi and M. Nistor. 2009. Label free capacitive immunosensor for detecting calpastatin-A meat tenderness biomarker. Bioelectrochemistry 76:93-99.