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 1 (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; Koohmaraiia and Geesink, 2006).

CAPNs are a large family of intracellular Ca²⁺-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; Koohmaraiia, 1996). Numerous studies have shown that the proteolytic CAPNs activity is responsible for meat tenderization (Sentandreu et al., 2002; Koohmaraiia and Geesink, 2006). Therefore, the CAPN system is regarded as the main endogenous protease system contributing to postmortem proteolysis and meat tenderization (Koohmaraiia 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 (Koohmaraiia 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
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 protease 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 archaeabacteria (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\textsuperscript{2+}-binding areas (Ohno et al., 1986; Blanchard et al., 1997; Lin et al., 1997). Meanwhile, CAPN1 and CAPN2 are activated \textit{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\textsuperscript{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\textsuperscript{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).

\textit{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\textsuperscript{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).

\textbf{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, because both CAPN1 and CAPN2 can cleave the same myofibrillar proteins (Huff-Lonergan et al., 1996). 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 intensity                       | Korean cattle    | CAPN1          | (Cheong et al., 2008)                                                      |
| pH                                       | Yanbian Yellow cattle, pig | CAPN1, CAST | (Siewczowska et al., 2010; Jin et al., 2011)                                 |
| Meat redness                             | Pig              | CAPN1, CAST    | (Gandolfi et al., 2011)                                                    |
| Color scores, fatty acid contents and    | Yanbian Yellow cattle, pig | CAPN1, CAST | (Skrlep et al., 2010; Jin et al., 2011)                                     |
| amino acids                              | Pig              | CAST           |                                                                           |
| 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 postmortem 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). Furthermore, 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 Ca²⁺-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 (Lian 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 Koolmaraie, 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; LeFauve 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 I<IIa<IIx<IIB (LeFauve, 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 *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
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 (CaCl₂) (Morgan et al., 1991; Whipple and Koohmaraie, 1991; Koohmaraie, 1992; Boelman 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 Ca²⁺-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 Ca²⁺ 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 filament-, actin- 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 muscle contraction in living cells (Goll et al., 1984). After slaughter, ATP can not be supplied and the shorten process of myofibrils is influenced. 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). However, 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 juiciness 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...
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.

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