

Calpain and Calpastatin Activity Post Mortem and Meat Tenderness: Are the Two Related?

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Abstract: The calpain and calpastatin proteolytic enzyme system is believed to be the main contributor to the tenderness of meat at post mortem. However, little is known about the enzyme calpain and its inhibitor calpastatin and how the two influence meat tenderness. This could be due to the fact that the study of the technology to understand calpain system is still relatively new. When factors that influence conversion of muscle to meat, meat tenderization and the activity of calpain and calpastatin are considered, it is evident that there is overlap of the factors involved in the three aspects. However, not all factors that influence meat tenderization have been shown to affect the calpain and calpastatin enzymatic system. Though there are studies that demonstrate how diet, growth promoters, gender, weather, handling of animals and electrical stimulation influence the calpain and calpastatin enzymatic system, these studies are not conclusive. In view of this, further studies are needed particularly in South Africa to understand the system fully under local conditions and in local beef cattle breeds such as the Nguni, Africander and Bosmara. Furthermore, there is a need for work to establish the relationship between pH and the activity of calpain and calpastatins given the conflicting views held on this aspect.

Key words: Calpain I, calpain II, calpastatin, local beef breeds, animals

INTRODUCTION

One of the main properties the consumer expects of beef is tenderness. It is believed that this property of meat is dependent on actions of the calpain and calpastatin proteolytic enzyme system (Chereta *et al.*, 2007). Studies show that the factors that affect meat tenderness include age, gender, growth promoters, stress, etc. However, not all these factors have been investigated to establish their role in the calpain and calpastatin activity. Furthermore, there seems to be no consensus among the different authors who have written on the factors that influence calpain and calpastatin. For example Kristensen *et al.* (2006) are of the view that calpain and calpastatin activity is stable when meat is stored below -20°C while other researchers (Dransfield, 1994) hold a view that the extractability of the calpains decrease as time progresses during storage post mortem. There are also varying views on the effect of electrical stimulation on calpain I and II (Hope-Jones *et al.*, 2010; Feidt and Brun-Bellut, 1999; Ferguson *et al.*, 2008). In addition, there is paucity of data on the enzyme activity of calpain and its inhibitor calpastatin and how the factors that influence

meat tenderness affect this enzyme system. Presumably this could be due to the fact that the technology to monitor the calpain and calpastatin enzyme activity is relatively new and not well developed. The objective of this review article is to capture the factors that influence muscle conversion to meat and the meat tenderization process and suggest aspects of the calpain and calpastatin enzyme system where research is needed.

CONVERSION OF MUSCLE TO MEAT

The conversion of muscle to meat involves a complex set of interrelated occurrences (Pulford *et al.*, 2008) which have a significant effect on meat quality (Livisay *et al.*, 1996; Simmons *et al.*, 2006).

During post mortem, the pH of the muscle decreases from the normal value of 7.2 to between 5.5 and 5.8 during the 24 h post slaughter (Varnam and Sutherland, 1996; Forrest *et al.*, 1973; Brewer *et al.*, 2001). This decrease is due to the production of lactic acid as described below (Muchenje *et al.*, 2009 a, b). The rate of pH decline differs between animals and even when compared within the same muscle (Simmons *et al.*, 2006). Once the pH becomes

too acidic, the energy metabolism stops because the enzymes involved in the system cannot function at the low pH (Muchenje *et al.*, 2009a, b).

The process of conversion of muscle to meat starts directly after death when the circulation stops. Circulation in the live animal provides nutrients and oxygen to organs, including muscles. Therefore, when the circulation stops neither oxygen nor nutrients can be delivered to the muscles. However, just because the circulation has stopped, it does not mean that metabolism in the muscle stops. The muscles still require energy to maintain their tone as well as to maintain body temperature (Zong *et al.*, 2002; Varnam and Sutherland, 1996; Forrest *et al.*, 1973). Therefore to continue providing muscle with needed energy after the supply of oxygen has stopped, the muscle uses other metabolic pathways to regenerate ATP (adenosine triphosphate). These include firstly the regeneration of ATP through the conversion of creatine phosphate to creatine, resulting in the conversion ADP (Adenosine Di-Phosphate) to ATP, secondly by two ADPs combining to form one ATP and one AMP (adenosine monophosphate) and thirdly through anaerobic respiration that results in lactic acid (Lawrie, 1974; Varnam and Sutherland, 1996).

A myofibril (also known as a muscle fibril) is a basic rod-like unit of a muscle. Muscles are composed of tubular cells called myocytes also known as muscle fibers and these cells in turn contain many chains of myofibrils (<http://en.wikipedia.org/wiki/Myofibril>). Since, ATP is required for muscle contraction and relaxation (Varnam and Sutherland, 1996) as the levels of ATP decrease, more actomyosin (the bond between actin and myosin in the muscle fiber) forms that cannot be released again (Lawrie, 1974). This is when rigor mortis sets in.

Rigor mortis also occurs when the energy supply is depleted (Hwang *et al.*, 2003). There is a relationship between glycolytic rate and the muscle contraction process (Rosenvold *et al.*, 2003). Structural changes of meat occurring during the rigor mortis development are both longitudinal and lateral contraction of the myofibrillar portion (Tornberg, 1996).

The onset of rigor mortis is not the end of the conversion from muscle to meat. After the onset or even before the onset of rigor mortis enzymes start breaking down the proteins in the muscle (Lawrie, 1974). This proteolytic action is responsible for loosening up the myofibrillar held together laterally, thus weakening the myofibrillar length and as a result, myofibril fragmentation occurs (Tornberg, 1996). The enzymes involved are mainly calpains and cathepsins. The latter breaks down muscle fibers at very low pH level which means that cathepsin may play a role in Pale, Soft, Exudative (PSE) meat (Varnam and Sutherland, 1996). Table 1 illustrates the activity of these two enzymes.

Table 1: The activity of the enzyme calpains and cathepsins during post mortem (Varnam and Sutherland, 1996)

Location	Protease	Activity
Sarcoplasmic	Calpain I (μ -calpain)	Releases α -actinin, Z-nin Degrades desmin, filamin, connectin, nebulin
	Calpain II (m-calpain)	Degrades troponins, tropomyosin Degrades C- and M-proteins
Lysosomal	Cathepsin B	Degrades myosin, actin, troponin T Degrades collagen
	Cathepsin L	Degrades myosin, actin, troponins Degrades tropomyosin, α -actinin Degrades collagen
	Cathepsin D	Degrades myosin, actin, α -actinin Degrades troponins, tropomyosin Degrades collagen

Proteins in the meat are degraded at different rates (Pulford *et al.*, 2008). The enzymes involved in the process, breakdown different parts of the muscle as shown in the Table 1. However, the actomyosin stays intact, as no known enzyme is able to break this bond (Dransfield, 1994). Based on this, the end result of the conversion of muscle to meat and mainly the enzyme activity will determine the resulting tenderness.

THE ROLE OF CALPAIN IN THE TENDERIZATION EFFECT

The post mortem tenderization process is influenced by the pH and calcium of the meat which may involve activation or inactivation of the proteolytic enzymes. As mentioned, cathepsins are thought to be more reactive in low pH conditions thus play a role in PSE while calpains are more reactive in higher pH conditions (Dransfield, 1994). Therefore in meat tenderization, calpain plays a more significant role as compared to cathepsins.

Calpain I and II are calcium dependent proteases and are responsible for the myofibrillar degradation observed during post mortem ageing (Lvisay *et al.*, 1996). Calpain I is activated first (at pH 6.3) when the calcium levels are still low. As the calcium levels increase calpain II is activated (Dransfield, 1994). However, both of these calpains are unstable at temperatures above -4°C and therefore their activities are bound to decrease over time in meat stored at such temperatures or higher (Dransfield, 1994). This is because calpains eventually become inactive at these temperatures, resulting in lower calpain activity and consequently lower meat tenderness (Dransfield, 1994).

Ouali and Talmant (1990) implicate the ratio between calpastatin and calpain as having a positive correlation with shear force. But according to Du Toit (2011) as the activity of calpain I and II decrease, the shear force increase.

Based on the discussion, muscle pH and the level of calcium in the muscle at post mortem enhance the calpain activity and increase muscle tenderness.

FACTORS EFFECTING CALPAIN AND CALPASTATIN ACTIVITY

According to Kouakou *et al.* (2005), calpain activity is influenced (inhibited) by pH_u (ultimate pH) and calpastatin activity. According to Kendall *et al.* (1993), both calpastatin and calpain II activity decreases as the pH decreases. In view of this, since the pH decreases as time progresses after death, it follows that the calpastatin and calpain activity is also expected to decrease. However, on the contrary, Du Toit (2011) in her study found that as the pH decrease the activity of both calpain and calpastatin increases. This difference between the authors may demonstrate that a threshold pH value exist, meaning that above this threshold pH value the decrease in pH stimulates an increase in activity but below this threshold pH value, an increase in pH stimulates a decrease in the activity. Or it can be explained by the fact that calpain and calpastatin become inactive as time progress post mortem.

There is a direct link between the activities of calpain and its inhibitor calpastatin on one hand and the conversion of muscle to meat on the other. This is because the activities of the enzyme calpain and its inhibitor calpastatin like the conversion of muscle to meat are calcium dependent, meaning that the amount of free calcium on the muscle does influence the activity of calpain enzymes as well as its inhibitor calpastatin (Koochmaraie and Geesink, 2006).

Diet does not seem to influence the activities of calpain and calpastatin. This has been confirmed by Volpelli *et al.* (2005) who state that diet has no effect on calpastatin and calpain activity. However the researchers are of the view that since diet influences the energy status at slaughter that in turn should affect the pH value. Because calpain and calpastatin activity are influenced by the pH, therefore diet should affect the activity of calpain and calpastatin.

Glycolytic potential is defined as the amount of energy in the muscle at a specific time that has the potential to be used by the body (it can be calculated as follow: Glycolytic potential = 2×(glucose-6-phosphate + glucose + glycogen) + lactic acid (Wulf *et al.*, 2002). According to Du Toit (2011), muscle energy status (glycolytic potential) at slaughter may play a role in the activity of calpastatin and calpain. The higher the glycolytic potential, glycogen and glucose-6-phosphate concentration of the muscles the higher the activity of the inhibitor calpastatin. Whereas the lower the muscle

creatine phosphate concentration the higher the activity of calpastatin. In view of this, muscle glucose concentration has a negative effect on the enzyme calpain I. However, Du Toit (2011) was able to demonstrate that glycolytic potential has a positive correlation with calpain I. Which means that the higher the glycolytic potential the higher the calpain I activity. The same researcher also found that while muscle lactic acid concentration has a positive effect on calpain activity post mortem, glycogen concentration has a negative effect on calpain activity.

Genetics definitely play an important role in the expression of calpains and their inhibitor calpastatin (Mazzucco *et al.*, 2010). Other factors that have been reported to play a role in the expression of calpain and its inhibitor are age. According to Dransfield (1994) as animals grow and get older the levels of calpains increase. Growth promoters as well as muscle type may also influence the calpain system. For example, β -agonists have been shown to have a tendency to increase calpain II and calpastatin activities but decrease calpain I activity (Dransfield, 1994). This view, is however opposed by Hope-Jones *et al.* (2010) who state that growth promoters increase calpastatin activity but have no effect on calpain activity.

One study reported that electrical stimulation does not affect the activity of calpain I and II but it decreases the activity of calpastatin (Ferguson *et al.*, 2000). However, Hope-Jones *et al.* (2010) are of the view that electrical stimulation decreases calpastatin activity and increases calpain I and II activity.

Calpain I activity is effected by thawing rate and with time post mortem. With rapid thawing there is a reduction of 14% of calpain I activity (Dransfield, 1996). Furthermore, the extractability of the calpains decrease as time progress post mortem (Dransfield, 1996). Adrenalin concentration in the blood ante-mortem and exertion ante-mortem increases the calpain activity (Ertbjerg *et al.*, 1999).

Strydom *et al.* (2009) and Zamora *et al.* (1996) in their studies on carcass characteristics and meat quality concluded that calpastatin activity and not calpain has an effect on tenderness. On the other hand, Neath *et al.* (2007) and Ilian *et al.* (2004), suggest that shear force (tenderness) is influenced by calpain activity.

Based on the discussion of the factors that influence calpain, it is clear that there is no consensus in some instances among the different researchers. This could be attributed to the fact that these factors are probably not well understood. In view of this, more research needs to be done on factors like diet, electrical stimulation and growth promoters, to gain a better understanding of their influence on calpain and calpastatin.

FACTORS EFFECTING TENDERNESS

Tenderness is the most important factor determining the satisfaction of consumer-eating beef (Jelenikova *et al.*, 2008). Tenderness of beef is determined by a variety of factors which factors are related to each other. Factors influencing tenderness starts at the farm level. If the animals are treated correctly there is a greater possibility that the meat will be tender. The next place where tenderness is influenced is when the animals are transported to the abattoir, followed by when they are at the abattoir and then during and after slaughter (Hollung *et al.*, 2007). The animal's age, their feeding system, gender, breed and genetics play an important role in tenderness as well as the weather conditions before slaughter (Muchenje *et al.*, 2009a, b; Du Toit, 2011). According to Du Toit (2011), the energy status in the muscle at slaughter have no effect on meat tenderness. This can be due to the fact that threshold values were not exceeded in this study and the animals were slaughtered under optimum slaughter conditions (animals were not too stressed).

On the farm the animals must be handled frequently so that they are used to handling. If they are not handled frequently they tend to be wild and use more energy (because they are stressed) than animals that are amiable to handling (Kadim *et al.*, 2004). The other on farm factor that influences the tenderness of the beef is the nutrition on the farm (Muchenje *et al.*, 2009a, b). All of the above components (handling and nutrition) differ among the different production systems (Du Toit, 2011). This therefore suggests that production systems do play a role in the tenderness of meat.

During transportation of animals, the animals must not be mixed, they must have enough space to move around in truck. However, this movement must not be limited and not excessive. If done correctly, this will decrease on the likelihood of ending up with poor quality carcass, due to bruising and stress. The distance that the animals travel may influence the tenderness of the meat as well as the attitude of the driver or any other treatment of the animal that can cause stress (Lawrie, 1974; Muchenje *et al.*, 2009a, b; Jelenikova *et al.*, 2008; Hollung *et al.*, 2007).

At the abattoir, the animals should not be mixed. Mixing causes stress in the animals (Jelenikova *et al.*, 2008). The amount of time that animals are without food also influences the tenderness of the meat. The animals should always have some water to drink or they will dehydrate severely (Hollung *et al.*, 2007). Dehydration causes stress which impacts on meat tenderness. The animals must be handled gently to prevent as much stress as possible (Silva *et al.*, 1999). Another important factor

at the abattoir is the weather conditions. If the weather is hot, more meat defects occur (resulting in meat with poor tenderness values or scores).

The animals must be slaughtered in such a way to minimize stress. Most common practice in abattoir is to use a captive bolt to stun the animals before the jugular vein is cut (Janz *et al.*, 2001). Depending on the type of stress the animals is exposed to, meat can be affected in two ways. With long term stress, energy is depleted in the muscle before slaughter and Dark Firm Dry (DFD) meat results with a high pH_u (ultimate pH) and variability affects tenderness. With short term stress lactic acid forms in the muscle ante mortem resulting in Pale Soft Exudative (PSE) meat with a very low pH_u and tough meat (Du Toit, 2011).

There are other factors that influence the meat tenderness after slaughter. These include the use of electrical stimulation (Hwang *et al.*, 2003; Janz *et al.*, 2001; Pearce *et al.*, 2009), the temperature of storage and conditioning, humidity during storage and conditioning, if hot boning is used if wrapping is used and the time the meat is matured/conditioned (Simmons *et al.*, 2006; Rosenvold *et al.*, 2003, 2008). The cooking process also plays an important role in the ultimate tenderness of the piece of meat.

According to the process in which muscles are converted to, calpain and calpastatin should influence tenderness. From the proceeding paragraphs it can be concluded that factors that influences meat tenderness should also affect calpain and calpastatin activity post mortem. However, no studies have been done to confirm this for many of these factors.

CONCLUSION

Though there seems to be no agreement in literature on how both calpain and calpastatin influences tenderness, this review shows with certainty that both calpain and calpastatin through their enzymatic activity do impact on the resulting tenderness. It can thus be concluded that the calpastatin and calpastatin enzyme system is probably the main mechanism by which meat tenderness is achieved. However, not all the factors influencing tenderness have been investigated so as to assess their impact on the proteolytic system.

The calpain/calpastatin proteolytic system is influenced by muscle pH, muscle calcium concentration, genetics (type of animal), age of the animal and thawing rate, there is no evidence to prove that factors like diet, stress, growth promoters, ante mortem exercise, muscle temperature, electrical stimulation and breed that are known to influence meat tenderness, play a role in calpain and calpastatin activity.

Finally, some of the inconsistencies found in the literature may be due to old calpain stock solution used given that old stock is unstable. The study of the calpain system is still relatively new and further studies are required to understand the system.

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