Intermuscular Variation In Beef Tenderness: Association With Calpains

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Introduction

The post-mortem rate of tenderisation of different muscles in an individual animal is variable (Ouali and Talmant 1990). For example, the rate tenderisation of *Longissimus dorsi* is significantly different from that of *Psoas major* (Cridge *et al.* 1994). The question is: What drives the intermuscular variations in tenderness? It is generally accepted that improvement in meat tenderness is due to the proteolysis of key myofibr and cytoskeletal proteins (Taylor *et al.* 1995). Numerous experiments over the last two decades have indicated that the calpain system (calpa and calpain II active at μM and mM [Ca^{2+}] respectively and their specific inhibitor calpastatin) could be responsible for improvements in meat tenderness during post-mortem aging (Ouali and Talmant 1990, Koohmaraie 1992, Huff-Lonergan *et al.* 1996). Two factors could explain the intermuscular variation in tenderness; sensitivity of key structural proteins of various muscles to proteolysis and the levels of the calpain syste It is therefore important to determine which of the calpains is responsible for the improvement in tenderness. Unfortunately, the exact catalyt mechanism of breaking down key myofibrillar and associated proteins during meat tenderisation is not yet established. As the activity of calpa in contrast to the activity of calpain I, remains more or less constant throughout post mortem storage and based on the assumption that calpa are proenzymes and once activated by calcium, gradually lose their activity through an autolytic process, Koohmaraie (1992) suggested that calpain I is the primary protease responsible for improvement in tenderness of meat during post mortem storage.
In this paper we investigated the relationship between the activities of the calpain system and rate of tenderisation of various beef muscles du two weeks post-mortem aging.

Objectives

The objectives of this study are to:
1- Determine the effect of muscle type on the degree of meat tenderisation.
2- Determine the role of calpains I, II, and calpastatin in meat tenderness of various muscles.

Materials and Methods:

Eight Angus beef steers were used to conduct this study. These animals were selected based on similarity in age and body size. The animals w killed by captive-bolt stunning, exanguinated and dressed in a commercial slaughter house. The following muscles were exposed for sampling probing about 30 min post-mortem.

Name of Muscle	Fibre type	Commercial Name
Longissimus dorsi (LD)	IIA	Top loin (Back)
Psoas major (PM)	IIB	Tenderloin (Back)
Semitendenosus (ST)	IIB	Eye of round (Thigh)
Supraspinatus (SS)	IA	Chuck tender (Foreleg)

Meat tenderness was determined at 6h, 7d and 14d of post-mortem storage. A steak 2.5cm thick per muscle was cut, frozen at -30°C and shea force was determined according to the procedure of Devine and Graafhuis (1995) using a MIRINZ tenderometer. Calpain analysis was perfo on meat samples at 30min, 12h and 24h post-mortem. Five grams was removed from the muscles of experimental animals (n=6) and homogen immediately in six volumes of homogenisation buffer (100 mM Tris-HCl, 10mM EDTA, 10mM β mercaptoethanol) using an Ultra Turrax. Proteases were separated on DEAE-sepharose FF and assayed using casein as substrate (Koohmaraie, 1990). One unit of calpain activity was defined as the amount of enzyme which caused an increase of one absorbance unit at 278 nm after a 1h incubation at 25°C.The figures were corrected by subtracting the activity in the presence of 1mM EDTA. One unit of calpastatin was defined as the amount of protein which inhib one unit of calpain II under standard assay conditions. Inhibitory units were calculated from the dilution which showed 20 to 50% inhibition. Statistical analysis was carried out using the Minitab computer package (version 10.1).

Results and Discussion

Tenderness, measured as the kg of force (kgF) required to sever a cooked meat sample are given in Figure 1. As expected, considerable differences in the initial tenderness were observed between different muscles, PM was considerably more tender than LD. The speed of tenderisation of different muscles was different in various muscles with LD > SS = ST > PM. The results of activity measurement of calpastati calpain I and calpain II in various muscles are included in Figure 1. Calpain II activity was relatively constant throughout the aging period. In contrast, Calpain I and calpastatin activities were different in various muscles at slaughter time and declined during the first day of aging perio very low levels. It is possible to conclude that the calpain I / calpastatin system could be responsible for improvement in tenderness based on th assumption that calpains are proenzymes and once activated, gradually lose their activity through an autolytic process. In other words, a loss o calpain activity indicates that the enzyme has been active and possibly caused tenderisation. If calpain I / calpastatin system is responsible for t

mortem aging and the terminal activation theory is valid, it is logical to expect that a higher decline rate in enzyme should result in a higher aging response and vice versa. Our results do not show such relationship as both LD and PM showed about 70% decline in calpain I activity however improvement in tenderness was 56% for LD and 22% for PM. Furthermore, the LD of β-agonist treated animals does not tenderise significantly with aging although their calpain I and calpastatin levels decline in a similar magnitude as for the LD of non-treated animals which tenderises with aging (Koohmaraie *et al.* 1991). To conclude, intermuscular variation in tenderness is a complex process and may be caused, in part, by differences in levels of calpain I. Further research is needed to illustrate the mechanism of calpain catalysis of myofibrillar protein degradation.

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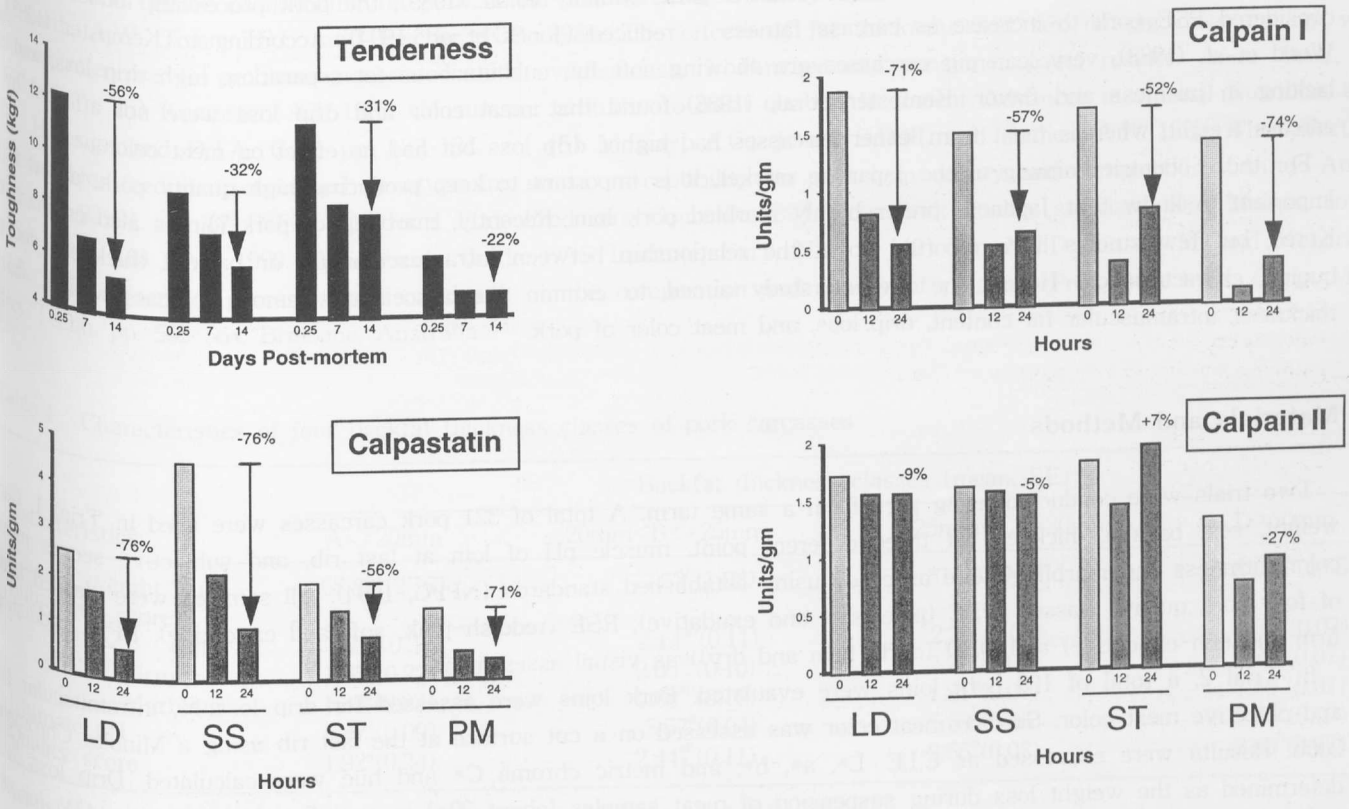

Figure 1. Effect of post-mortem storage on the shear force and activities of the calpain system of four beef muscles
Longissimus dorsi = (LD), Psoas major = (PM), Semitendenosus = (ST), Supraspinatus = (SS)