Proteolysis, Calpastatin Activity, and µ-Calpain Autolysis in Specific Muscles from the Beef Round

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Summary and Implications

Differences exist in pH, calpastatin activity, and percentage of calpain autolysis between the individual muscles of the round. Some of these individual muscles have biochemical characteristics similar to that of the LD. The LD has been accepted by consumers as a relatively tender cut and has had value added to it by selling this muscle as an individual cut. As a result of this the suitability of the muscles of the round as individual value cuts needs to continue to be investigated**.**

Introduction

Lack of consistent tenderness is a major quality problem that significantly impacts the profitability of the beef industry. Conservative estimates indicate tenderness defects cost the beef industry over $216,000,000 annually (Morgan, 1995). The muscles of the round are particularly prone to being less tender than the higher value cuts of the strip loin and the rib. While muscles of the round traditionally have received similar treatment in regards to aging, differences within tenderness and rate of tenderization will provide insight into adding value to individual cuts from the round. The calpain system, specifically µ-calpain, has been found to be responsible for the majority of postmortem proteolysis of muscle proteins (Geesink et al., 2006). Investigating differences in round muscles for µ-calpain, and calpastatin will allow identification of differences in rate of tenderness and final tenderness of individual muscles of the round. Another factor affecting calpain activity is rate of early postmortem pH decline which influences the rate of µ-calpain activity and autolysis and may play a pivotal role in regulating early postmortem proteolysis and ultimately postmortem tenderization (Melody et al., 2004). Describing the biochemical characteristics of individual muscles of the round will help to identify the suitability of these muscles for various markets.

Materials and Methods

Seven muscles (6 round, 1 reference) were removed from eight cattle at 24 h postmortem. 1) Longissimus dorsi (LD; reference muscle) 2) Adductor (AD), 3)Gracillus (GR), 4) Semimembranosus (SM), 5) Sartorius (SAR), 6) Vastus lateralis (VL), 7)Vastus intermedius (VI) The pH was measured in each muscle. Heated sarcoplasmic protein extracts from each muscle were used to measure calpastatin activity. Whole muscle samples were made from all muscles for Western blot analysis of µ-calpain autolysis and SDS-PAGE analysis of titin degradation.

Results and Discussion

Several muscles in the beef round could be marketed as individual value cuts and/or food service items if they are consistently tender. However, it is not known how these specific muscles tenderize. Therefore, the objective of this study was to understand the biochemical mechanisms that influence beef tenderness, specifically µ-calpain activation, calpastatin activity and subsequent muscle protein proteolysis in specific muscles of the beef round. It was hypothesized that the round muscles studied would demonstrate different rates of protein degradation. At 24 h postmortem, the longissimus dorsi (LD –control muscle), gracillus (GR), adductor (AD), semimembranosus (SM), sartorius (SAR), vastus lateralis (VL), and vastus intermedius (VI) muscles were removed from ten market-weight beef cattle. Across muscles, pH was significantly correlated with 24-h calpastatin activity (0.475, $P<0.0001$) and with the percentage of µ-calpain as the 76 kDa autolysis product (-0.311, $P<0.05$). The AD, GR, and LD had a higher percentage of µ-calpain as the 76 kDa autolysis product, suggesting that calpain was activated earlier in those muscles. The SAR, SM, VL and VI had a lower percentage of the 76 kDa autolysis product at 24 hours postmortem. VI had the highest calpastatin activity, followed by GR, LD, and VL. The lowest calpastatin activity was found in the AD, SAR, and SM muscles. Differences in the degradation of titin, a substrate of µ-calpain, were found. The SAR had little, if any, detectable intact titin at 24 hours postmortem. The results of this study indicate that there are differences in the rate of proteolysis occurring in the muscles studied. These results will help guide progress in determining suitability of these muscles for various markets.

Figure 1. pH at 24h postmortem.

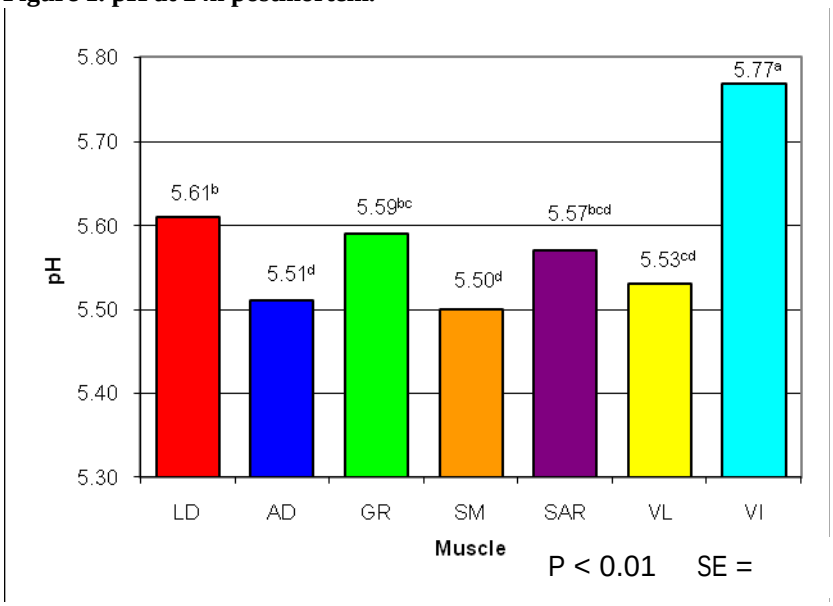

Figure 2. Calpastatin activity at 24h.

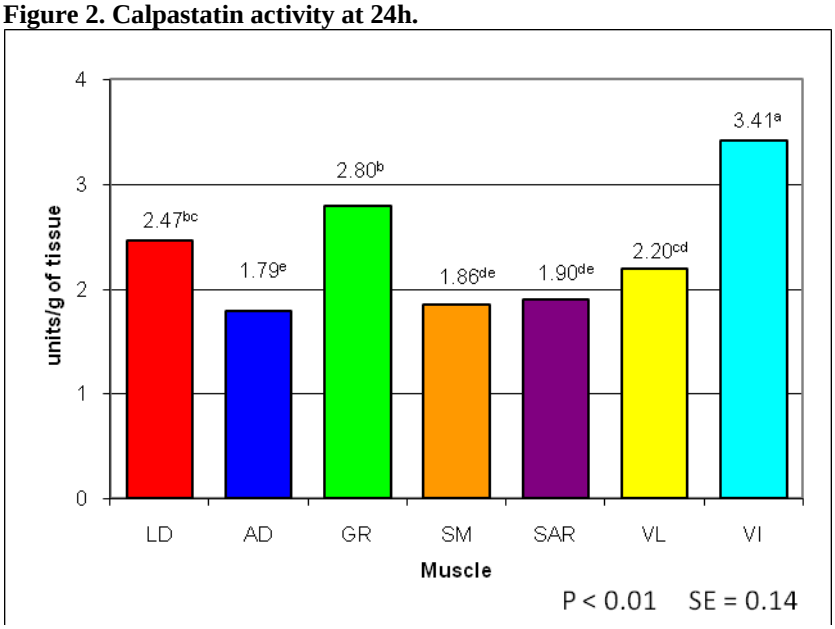

Figure 3. Percentage of autolyzed 80 kDa calpain subunit at 24h postmortem.

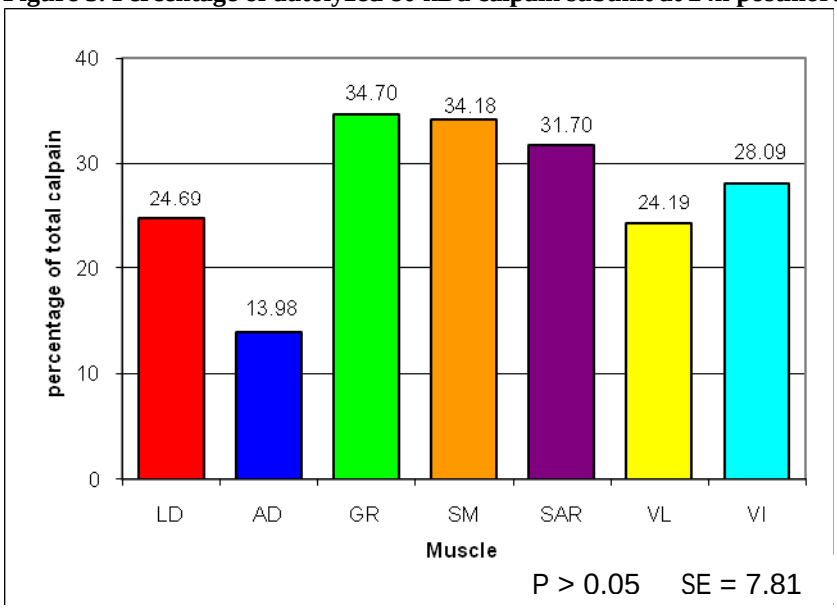

Figure 4. Percentage of autolyzed 78 kDa calpain subunit at 24h postmortem.

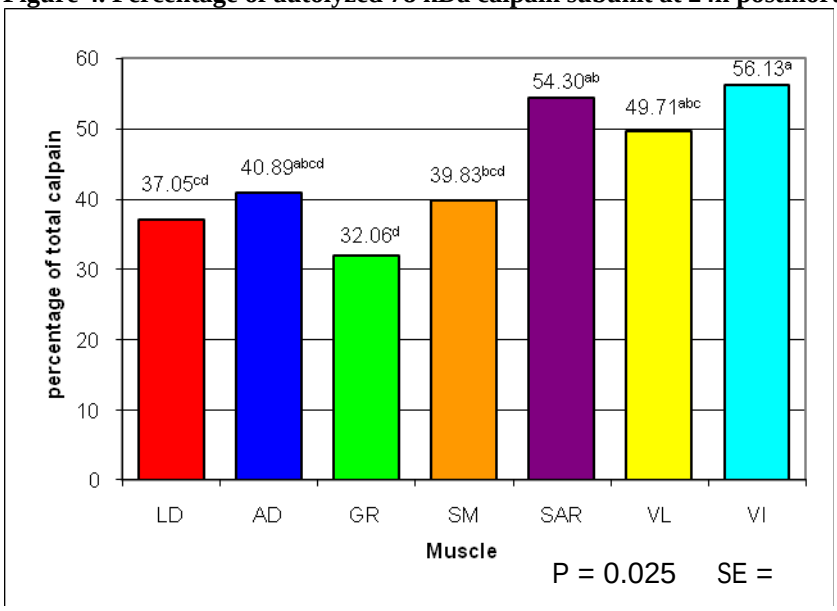

Figure 5. Percentage of autolyzed 76 kDa calpain subunit at 24h postmortem.

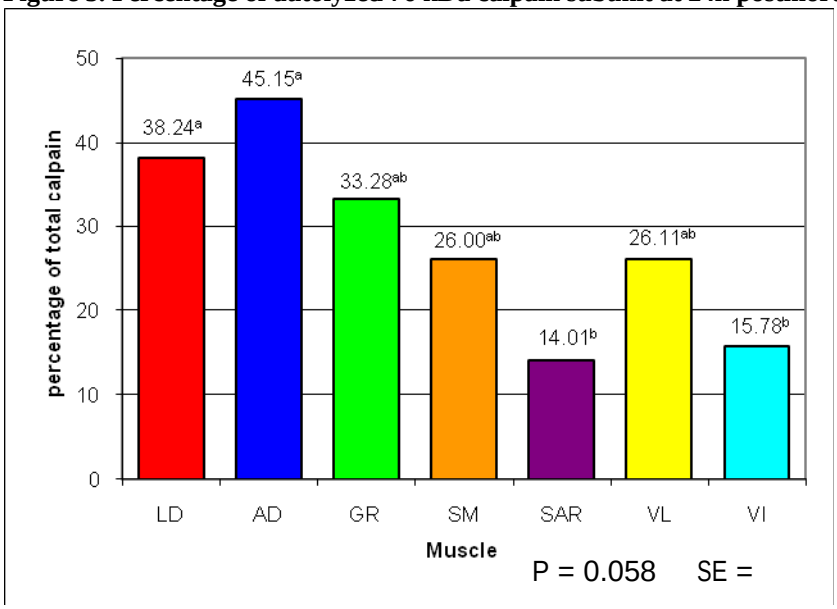

Figure 6. Titin degradation at 24h across muscles.

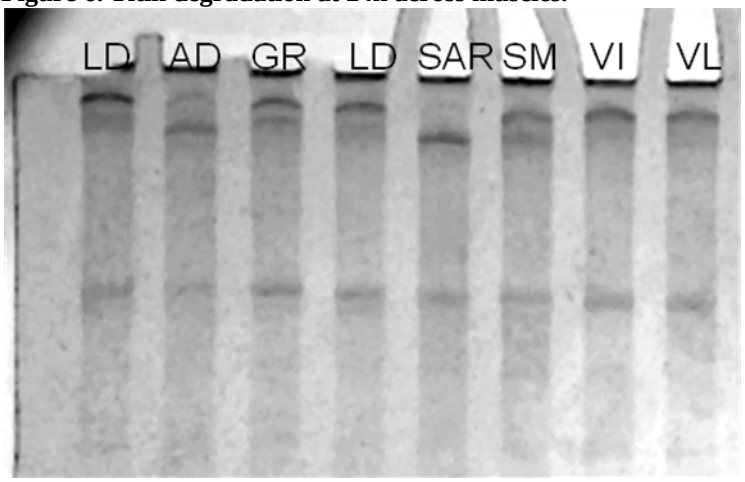

Figure 7. Western blot of µ-calpain autolysis across muscles.

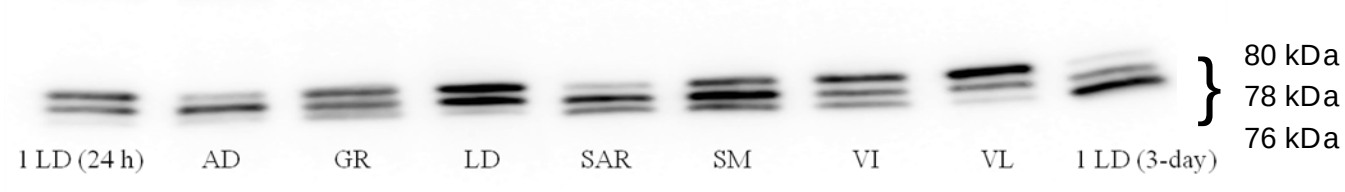

Table 1. Correlations between Calpastatin, pH, and Calpain (80, 78, and 76 kDa)

	Calpastatin	pH	80 kDa Calpain	78 kDa Calpain	76 kDa Calpain
Calpastatin		0.5213**	0.2342†	0.0743	-0.2782*
pH	0.5213**		0.2102	0.1489	-0.3113*
80 kDa Calpain	0.2342†	0.2102		-0.3258*	-0.7079**
78 kDa Calpain	0.0743	0.1489	-0.3258*		-0.4371**
76 kDa Calpain	-0.2782*	-0.3113*	-0.7079**	-0.4371**	

†P < 0.10; *P < 0.05; **P < 0.01

Acknowledgements

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