THE RELATIONSHIP BETWEEN VARIATIONS IN CALPAIN SYSTEM ACTIVITY AND POSTMORTEM RATE OF MYOFIBRE FRAGMENTATION IS THE SAME IN BOTH SHEEP AND CATTLE

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Abstract

The effect of variations in the ratio of calpain I to calpastatin activity (CI:CS) at 1 hour post-slaughter on the rate of meat tenderisation determined in two separate trials. CI:CS explained over 50% (P<0.001) of the variation in the rate of myofibril fragmentation, assessed as slope of the progressive increase in Myofibril Fragmentation Index measured at successive times in both electrically stimulated bo longissimus dorsi and cold shortened ovine longissimus dorsi muscle. Over 30% of the variation in the postmortem rate of decrease Warner-Bratzler shear force (P<0.001) from both bovine and ovine groups was also explained by CI:CS. The relationship between CI:CS myofibrillar fragmentation rate did not differ between trials (P=0.816). When adjusted for CI:CS and initial post-rigor shear force, relationship between CI:CS and change in shear force also did not differ (P=0.202) between trials. These results suggest that regardless treatment and species differences, the biochemistry of calpain specific postmortem proteolysis as measured by myofibre fragmentation and the subsequent effects on change in shear force, was similar between trials. However, additional factors which affect the physi integrity of postmortem muscle are also important in the tenderisation process.

Introduction

Of the endogenous muscle proteinase systems, only the calpain system can reproduce many of the effects on myofibres and muscle prote that are seen in meat during aging (Koohmaraie, 1994). In particular, evidence suggests that the degradation of costamere proteins aroun bands by calpain is responsible for proteolytically induced tenderisation in aging meat (Taylor *et al*, 1995). It has been suggested that degradation of these structures by calpain, along with changes in the actin/myosin interaction, result in changes in toughness during postmortem storage of meat (Goll *et al*, 1995). This paper reports similarities between the effects of variations in the activity of the calp system on rate of tenderisation in cold shortened lamb and electrically stimulated, non cold shortened beef muscle.

Methods

Experiment 1: Heifers from the Trangie yearling weight selection lines have been selected over a 22 year period for their individual yearl growth performance (Parnell *et al,* 1994). Fifteen low, 12 control and 9 high yearling weight selection line heifers were either implanted w 200mg trenbolone acetete and 20mg oestradiol-17β (Revelor®h, Rousel Uclaf, Paris) (treatment group, HGP+) or not implanted (cont group, HGP-). Heifers were slaughtered 90 days after implantation and carcasses were electrically stimulated (200 milliamps, peak volta 45v, 40 seconds). Core samples for calpain analysis were removed from the longissimus dorsi p12 site at 1 hour post slaughter and stored a 70°C for 2 hours. Myofibrillar fragmentation index (MFI) (Olson *et al*, 1976) and Warner-Bratzler shear force values (Bouton *et al*, 19 were determined at 1, 3 and 10 days postmortem. Myofibril fragmentation rate was defined as the slope of the line of best fit through MF values plotted at 1, 3 and 10 days postmortem for each animal. Similarly, rate of change in shear force was defined as the slope of the line best fit through shear force values at 1, 3 and 10 days postmortem for each animal.

Samples were prepared for separation of the calpain system following the procedure described by Koohmaraie (1990) for ion-exchang chromatography. Using a stepwise NaCl gradient, optimal separation of the calpain system components was achieved using 100mM NaCl (p 8.0) for calpastatin, 150 mM NaCl (pH 7.0) for calpain I and 350 mM NaCl (pH 7.0) for calpain II. Proteolytic activity of the calpains w determined using casein as substrate.

Experiment 2: Twenty four Dorset x Border Leister x Merino wether lambs with an average starting weight of 27.0 ± 3.3 kg were divide into control, β-agonist or IGF analog treatment groups. Within groups, lambs were continuously fed at either 0.6 or 1.8 X maintenance for week. β-agonist treated lambs were orally administered with clenbuterol (0.2mg/kg liveweight) once daily during the experimental feedi period. For IGF-1 treatment, jugular vein catheters were inserted on day 5 of the experiment. Catheters were filled with 100 IU heparin an 0.01% tetracycline in sterile saline (0.9% w/v NaCl). Long R^3 IGF-1 (media grade, GroPep) was continuously infused at 150 ug/hr for hours directly prior to slaughter on day 7 of the experiment.

The longissimus dorsi was removed from the left side of each carcass at 1 hour postmortem, vacuum packaged and stored at 1°C. MFI an Warner Bratzler shear force values were determined as with cattle samples at 1, 3, 5 and 9 days postmortem. Rate of change MFI an Warner-Bratzler shear force was calculated as described for cattle.

The procedure for analysis of the calpain system in lamb was similar to that previously described for cattle with the following exceptio Using a stepwise NaCl gradient, optimal separation was achieved using110mM NaCl (pH 7.0) for calpastatin, 200mM NaCl (pH 7.0) fo calpain I and 400 mM NaCl (pH 7.0) for calpain II.

Results and Discussion

Treatment with HGP significantly increased (P<0.001) calpastatin activity across all three lines of heifers (results not shown). No effect o line or HGP treatment was seen on calpain I or calpain II activity. In lambs, calpastatin activity was significantly reduced (P<0.005) by sup maintenance feeding, and increased (P<0.05) by β-agonist treatment at supra-maintenance feeding levels (results not shown). IGF-1 and β agonist treatments lowered (P<0.005) calpain I levels across both levels of nutrition. Neither plane of nutrition or pharmacological treatme had an effect on calpain II activity.

In both heifer and lamb trials, variations in calpain I and calpastatin activity between treatment groups were not associated with significant differences in any postmortem MFI or shear force measurements (data not shown). However, between animals, as the activity of calpain I relative to calpastatin activity (CI:CS) increases, the rate of postmortem myofibrillar fragmentation also increases ($P<0.001$) (Figure 1.). CI:CS explained over 50% of the variation in myofibril fragmentation rate in both trials. Also, the vast differences in the postmortem handling of the longissimus dorsi muscle from both species failed to produce significant differences in the slope of regression lines plotted through each data set ($P=0.816$). Indeed the slope of the regression lines are markedly similar, suggesting that regardless of treatment and species differences, the biochemistry of calpain specific postmortem proteolysis as measured by myofibre fragmentation rate was similar. The intercept of each regression line does not significantly different from 0, suggesting that the calpain system is unique and essential in the degradation of myofibres in postmortem meat.

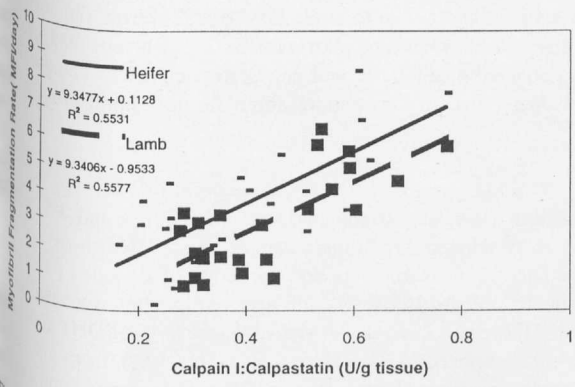

Figure 1: The ratio of calpain I:calpastatin is positively correlated with the rate of myofibril fragmentation in both electrically stimulated bovine and cold shortened ovine longissimus dorsi. Each point represents individual lambs (J) or heifers (–). Regression lines and equations for both lamb (– – –) and heifer (———) groups are shown.

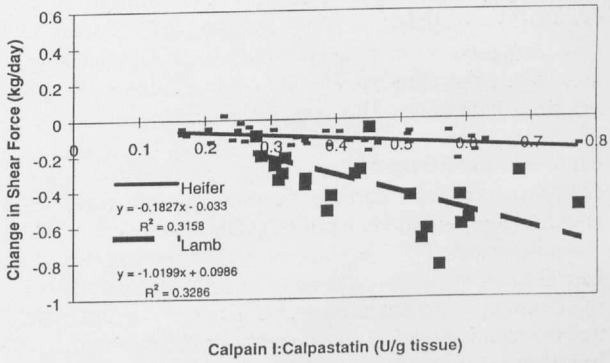

Figure 2: Correlation between the ratio of calpain I:calpastatin and rate of decrease in shear force in both electrically stimulated bovine and cold shortened ovine longissimus dorsi. Each point represents individual lambs (J) or heifers (–). Regression lines and equations for both lamb (– – –) and heifer (———) groups are shown

On the other hand, CI:CS explained only 30% of the variation in postmortem rate of decrease in shear force in both trials (Figure 2). Additional factors affecting the physical integrity of postmortem muscle, such as changes in the actin/myosin interaction during postmortem storage as suggested by Goll *et al* (1995) must also be important in meat tenderisation. The rate of decrease in shear force was also not significantly different between both trials ($P=0.202$) when initial post-rigor shear force (24hr shear force) and CI:CS were included as covariates. Therefore, at the same CI:CS and 24hr shear force, species and treatment differences did not change the interaction between CI:CS and rate of decrease in shear force.

Conclusions

The calpain system appears to be the essential proteolytic system involved in the post-mortem fragmentation of myofibres and explains over 50% of myofibril fragmentation rate. Despite species and treatment differences, CI:CS has a similar effect on the rate of postmortem degradation of myofibres and muscle. However, CI:CS explained only 30% of the decrease in shear force in postmortem muscle. Clearly other factors are also involved in physically detectable meat tenderisation.

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