EXPRESSION OF TISSUE PROTEINASES AND REGULATION OF PROTEIN DEGRADATION AS RELATED TO MEAT QUALITY

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CALPASTATIN-BASED METHODS FOR PREDICTING MEAT TENDERNESS

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Inconsistency in meat tenderness is one of the major problems facing the beef industry. Inconsistency in meat tenderness is probably due to our inability to produce tender meat and our inability to identify carcasses producing tough meat. Therefore, it is essential that methodologies be developed to predict meat tenderness. These methods must be objective and related directly to meat tenderness. Ultimate tenderness of meat is greatly affected by postmortem tenderization, which is caused by proteolysis of key myofibrillar proteins mediated by the calpain proteolytic system. Variation in calpastatin activity rather than variation in calpain activity is related to tenderness. Also, the greatest variation in calpastatin activity exists at 24 h postmortem. Postrigor calpastatin activity is highly heritable and genetically-correlated with tenderness. Therefore, we propose that the efficacy of postrigor calpastatin activity as a predictor of tenderness should be examined. Bovine skeletal muscle calpastatin was cloned and sequenced. It appears that the abundance of calpastatin mRNA may also be related to tenderness and, thus, it could also be used as a predictor. The bovine calpastatin gene is polymorphic, located on chromosome 7 and a member of syntenic group U22. We are now collecting data to determine if either meat tenderness or calpastain activity are associated with polymorphisms found in the bovine calpastatin gene.

Key words: calpain, calpastatin, proteolysis, tenderness, gene

INTRODUCTION

It has recently become clear that inconsistency in meat tenderness is one of the major problems facing the beef industry (Morgan et al., 1991; Morgan, 1992; Savell and Shackelford, 1992). Inconsistency in meat tenderness is probably due to our inability to routinely produce tender meat compounded by our inability to identify carcasses producing tough meat. In the U.S., the beef industry relies on the USDA quality grading system to segment carcasses into groups based on varying levels of expected meat palatability. In young beef (a majority of the production), quality grades are determined primarily by marbling degree (the amount of intramuscular fat visible in a cross section of the longissimus muscle at the 12th rib). The results of numerous investigations of the relationship between marbling and beef palatability indicate that, although there is a positive relationship between marbling degree and tenderness, juiciness, and flavour intensity, this relationship is weak at best (reviewed by Parrish, 1974). Generally, tenderness increases as marbling increases, however, the increments are very small, particularly from one degree marbling to the next. A comparison of the extremes (e.g., 'Practically Devoid' [< 3% intramuscular fat] and 'Moderate' [8% intramuscular fat]) was usually needed to find statistical differences of any practical

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importance. Most importantly, none of the studies have shown meaningful palatability differences between 'Slight' and 'Small' marbling degrees. Thus, the price differentials frequently found in the market place between USDA Select and USDA Choice beef are not justified. Based on the available data, it appears that between 5 and 10% of the variation in tenderness can be accounted for by variation in marbling degree. It is sobering to recognize that the only time the tenderness of meat is known is when the meat is eaten by the consumer. Because consumers consider tenderness to be the major determinant of eating quality of meat, it is essential to develop methodologies to predict objectively meat tenderness prior to its marketing and consumption.

Although marbling is lowly related to tenderness (as determined by shear force or sensory tenderness), there is a negative relationship between marbling and yield of retail product. Furthermore, the price differential between Choice and Select beef encourages producers to feed cattle for longer to improve the percentage which will grade 'Choice', but which also causes the cattle to get fatter. This antagonism between marbling and leanness hinders the industry's efforts to produce a high percentage of lean, highly-marbled carcasses. However, there is virtually no relationship between tenderness and yield of retail product. Therefore, if methodology could be developed to predict tenderness directly, rather than depending on marbling as an indicator, it would not only provide a better estimate of palatability, but it could avoid the antagonism with retail product yield that prevents us from routinely producing lean and palatable beef. The objective of this manuscript is to discuss one of our approaches toward the development of methodologies to predict meat tenderness.

WHY CALPASTATIN?

The first question that needs to be addressed is what factor(s) should be used as the basis for predicting meat tenderness. Factors affecting meat tenderness are numerous and most have a poor relationship to ultimate meat tenderness, and hence, cannot be used as a basis for predicting meat tenderness. Final tenderness of meat is greatly affected by cooler aging or conditioning. It has been about a century since the beneficial effects of postmortem storage on meat tenderness were first described. It is now generally accepted that proteolysis of key myofibrillar proteins is responsible for the improvement in meat tenderness due to postmortem storage. Current data suggest that the differences in the rate of postmortem proteolysis and tenderization is the major reason for variation in the tenderness of aged meat (for detailed review, see Koohmaraie 1992b, 1992c, 1992d). Hence, any trait indicative of the capacity of muscle to undergo proteolysis should be considered when developing methods to predict aged meat tenderness. Results of numerous experiments over the last several years have indicated that of all the proposed proteolytic systems (the calpain, the lysosomal and the multicatalytic systems), only the calpain proteolytic system plays a major role in meat tenderization during postmortem storage (for review, see Koohmaraie, 1988, 1992b, 1992c, 1992d). Since current data indicate that calpains are responsible for postmortem proteolysis and, thus, improvement in meat tenderness, one approach has been to identify a trait that measures the proteolytic capacity of the calpain system.

The calpain proteolytic system is a major cytosolic proteolytic system and has been isolated from a wide variety of cells, tissues and species. Its properties have been detailed elsewhere in this volume and will not be further discussed. The calpain proteolytic system has been implicated in initiation of myofi-

bril disassembly during growth (for review, see Goll et al., 1989, 1992). The calpains also cause hydrolysis of the myofibrillar proteins resulting in destabilization of myofibrils during postmortem storage (for review, see Koohmaraie, 1988, 1992b, 1992c, 1992d).

Although regulation of the calpain proteolytic system in living (for review, see Croall and DeMartino, 1991) or postmortem muscle is not yet understood, we have suggested that calpastatin (for detailed review, see Koohmaraie, 1992b, 1992c, 1992d) and autolysis (Koohmaraie, 1992a) are probably the main regulators in postmortem muscle. Therefore, calpastatin seems to provide the greatest opportunity for direct prediction of meat tenderness.

CALPAIN/CALPASTATIN ACTIVITIES DURING POSTMORTEM STORAGE

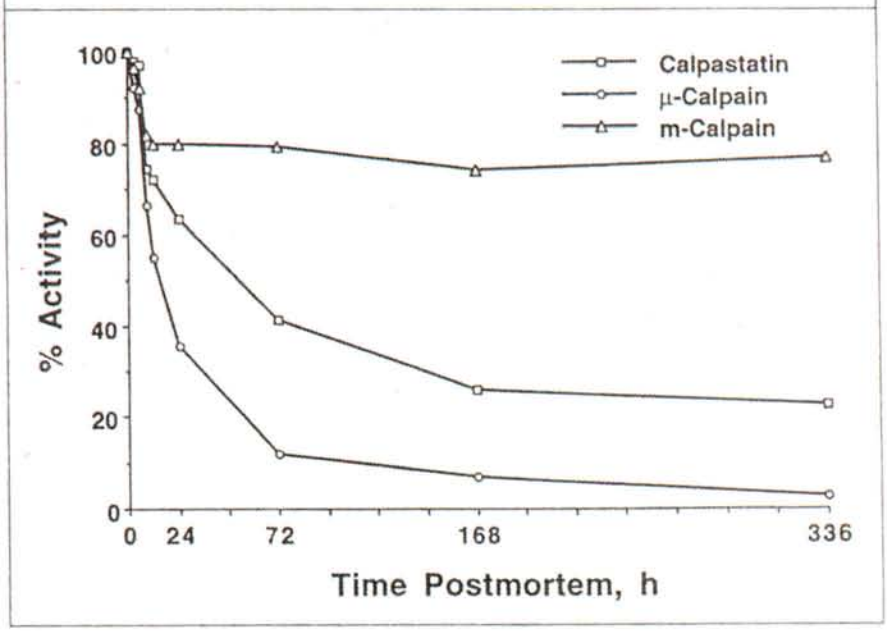
Calpastatin coexists with the calpains in all cells. Calpastatin concentration varies between cells and tissues within an animal (for review, see Croall and DeMartino, 1991) and also within the same tissue (e.g. skeletal muscle) from different species (Ouali and Talmant, 1990; Koohmaraie et al., 1991b).

Studies conducted to determine the effect of postmortem storage on activities of components of the calpain proteolytic system have indicated that while m-calpain is rather stable, there is a gradual decline in the activity of μ-calpain and calpastatin.

It is important to note that the pattern of decline in the activity of μ-calpain and particularly calpastatin is highly variable. For the data shown in Figure 1, the standard deviation of calpastatin activity

FIGURE 1

THE EFFECT OF POSTMORTEM STORAGE ON μ-CALPAIN, m-CALPAIN AND CALPASTATIN ACTIVITIES IN BOVINE LONGISSIMUS MUSCLE (FROM KOOHMARAIE, ARBONA AND WHIPPLE, UNPUBLISHED). (FIG. 1 VIDALENC ET AL., 1983; DUCASTAING ET AL., 1985; KOOHMARAIE ET AL., 1987, 1989, 1991B).

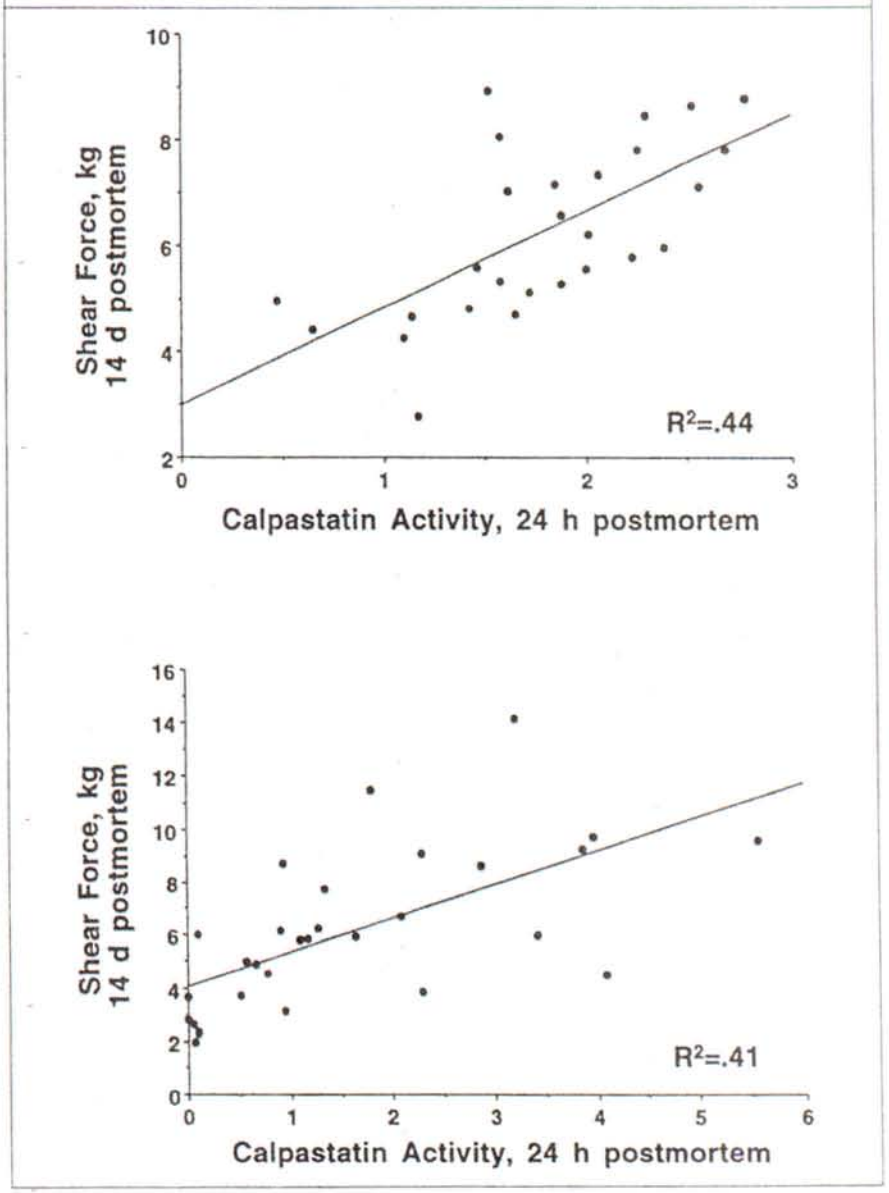


increased from .47 units at 0 h postmortem to a maximum of 1.39 at 24 h postmortem. Thus, the activity of calpastatin at 24 h postmortem was found to be highly related to tenderness of aged beef (Figure 2; Whipple et al., 1990; Shackelford et al., 1991).

For the data reported in Figure 1, the correlation coefficient between calpastatin activity at 24 h postmortem (hereafter referred to as postrigor calpastatin activity) and shear force after 14 d of aging was 0.61. Additionally, correlation coefficients of 0.66 and 0.39 were reported by Whipple et al. (1990) and Shackelford et al. (1991).

FIGURE 2

THE RELATIONSHIP BETWEEN CALPASTATIN ACTIVITY AT 24 H POSTMORTEM AND WARNER-BRATZLER SHEAR FORCES OF MEAT FROM LONGISSIMUS MUSCLE OF BEEF (TOP; WHIPPLE ET AL., 1990) AND LAMB (BOTTOM; KOOHMARAIE AND SHACKELFORD, 1991).



POSTRIGOR CALPASTATIN IS A HIGHLY HERITABLE TRAIT

At present, we do not fully understand the biological reasons for the association between postrigor calpastatin activity and tenderness of aged meat. However, we have suggested that higher levels of calpastatin result in reduced proteolytic capacity of μ-calpain and, thus, reduced tenderization. We hypothesized that selection against postrigor calpastatin activity could result in improved meat tenderness and, therefore, conducted an experiment to determine the heritability of calpastatin and its genetic relationship to tenderness. Heritability estimates for postrigor calpastatin activity, Warner-Bratzler Shear (WBS) force, and intramuscular fat content were 0.65, 0.53, and 0.93, respectively (Table 1; Shackelford et al., 1993).

TABLE 1 HERITABILITIES AND GENETIC AND PHENOTYPIC CORRELATIONS[a]

Trait	Calpastatin activity	Intramuscular fat content	Shear force	Retail product	Average daily gain
Calpastatin activity	(.65)	-.19	.27	.21	-.15
Intramuscular fat content	-.34	(.93)	-.27	-.46	.12
Shear force	.50	-.57	(.53)	.15	-.06
Retail product yield	.44	-.63	.70	(.45)	-.18
Average daily gain	-.52	-.04	-.40	-.62	(.32)

a Heritability estimates are shown along the diagonal. Genetic correlations are in the lower triangle and phenotypic correlations are in the upper triangle. Parenthetical values are standard errors of the estimates.

Furthermore, the genetic correlation between postrigor calpastatin activity and WBS was 0.50 (Shackelford et al., 1993). Collectively, these results indicate that it should be possible to select for improvements in postrigor calpastatin activity, intramuscular fat content, and meat tenderness. However, selection against calpastatin activity should be a more suitable mechanism for improving meat tenderness than selection for increased intramuscular fat content because: 1) the level of genetic antagonism between postrigor calpastatin activity and retail product yield is not as great as that between intramuscular fat content and retail product yield (Shackelford et al., 1993) and 2) calpastatin activity is more highly related to tenderness than is intramuscular fat (Shackelford et al., 1991).

It should be obvious that the above data imply that the factor(s) responsible for inactivation of calpastatin is(are) a heritable trait(s). Presently, we do not understand the nature of calpastatin inactivation during postmortem storage, particularly during the first 24 h after slaughter. However, several lines of evidence suggest that μ-calpain is responsible for inactivation of calpastatin (for review, see Koohmaraie 1992b, 1992c, 1992d). These include: 1) infusion of carcasses or injection of cuts of meat with a solution of calcium chloride, which activates calpains, inactivated calpastatin (Koohmaraie et al., 1988, 1989,

1990; Koohmaraie and Shackelford, 1991; Wheeler and Koohmaraie, 1992) and 2) infusion of carcasses with zinc chloride, which inhibits calpains, also prevented inactivation of calpastatin during a 14-d storage (Koohmaraie, 1990). The accuracy of the above hypothesis needs to be determined. According to Mellgren and Lane (1990), in contrast to degradation catalyzed by other proteases, calpain degradation of calpastatin results in little or no loss of calpastatin activity towards calpains. Therefore, calpains may not be responsible for calpastatin inactivation. In light of the relationship between the inactivation of calpastatin and tenderness of aged meat, it is important to determine the mechanism of calpastatin inactivation in postmortem muscle. Factors responsible for inactivation of calpastatin could then be used as a basis for predicting meat tenderness as well as genetic selection for tenderness.

Because of the apparent relationship between calpastatin activity at 24 h postmortem and tenderness of aged meat, it would seem logical that the possibility of using postrigor calpastatin activity as a basis for segregating carcasses into expected palatability groups should be pursued actively. To test the accuracy of this hypothesis, we are in the process of developing a sandwich Enzyme-Linked Immunosorbent-Assay (ELISA) for rapid calpastatin quantification. Nakamura et al. (1989) demonstrated that calpastatin fragments (fragmentation was produced by incubation with m-calpain) as small as 15,000 daltons retained their inhibitory activity. Therefore, the ELISA antibody will have to be polyclonal and cross-react with intact calpastatin and all calpastatin fragments capable of inhibiting calpains. Once the ELISA is developed, the efficacy of this method for predicting tenderness of aged meat will be determined utilizing a large number of phenotypically-diverse cattle.

CLONING AND SEQUENCE ANALYSIS OF BOVINE SKELETAL MUSCLE CALPASTATIN

To better understand the role of the calpain/calpastatin proteolytic system in muscle protein degradation during growth and postmortem storage (i.e., meat tenderization), calpastatin from bovine skeletal muscle was cloned and sequenced. Although calpastatin had been cloned and sequenced in other species, (Asada et al., 1989; Takano et al., 1988; Emori et al., 1987), we could not obtain cDNA from other species and were only able to determine the cross-reactivity of bovine mRNA with calpastatin cDNA from rabbit heart muscle (kindly provided by Dr. Emori, The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan). Northern blot analysis revealed that rabbit cDNA cannot be used to study expression of calpastatin from bovine skeletal muscle. Therefore, we cloned and sequenced calpastatin cDNA (Figure 3) from a bovine skeletal muscle cDNA library that recognizes the mRNA for calpastatin from bovine and ovine but not porcine skeletal muscle (Killefer and Koohmaraie, 1994).

Some of the characteristics of bovine calpastatin cDNA include: 1) two clones (pCR41 and pBSA1) that encompass the entire coding sequence (3.4 kb corresponding to 706 amino acids), 2) 70 to 80% sequence identity to published nucleotide sequences for human, rabbit and pig calpastatin, 3) exon 3, which corresponds to a highly conserved 22 amino acid region, is deleted from domain L, and 4) Northern blot analysis indicates the presence of three (3.8, 3.0 and 1.5 kb) and four (3.8, 3.0, 2.5 and 1.5 kb) calpastatin mRNA transcripts in bovine and ovine skeletal muscle, respectively (Figure 4).

FIGURE 3

SCHEMATIC OF THE DOMAIN STRUCTURE OF BOVINE SKELETAL MUSCLE CALPASTATIN CDNA (KILLEFER AND KOOHMARAIE, 1993A). DOMAINS ENCOMPASSED BY CDNA CLONES PCR41 AND PBSA1 ARE REPRESENTED FOR REFERENCE. THE ENTIRE CODING REGION ENCODES A 706 AMINO ACID PROTEIN HAVING A PREDICTED MR OF 76,000.

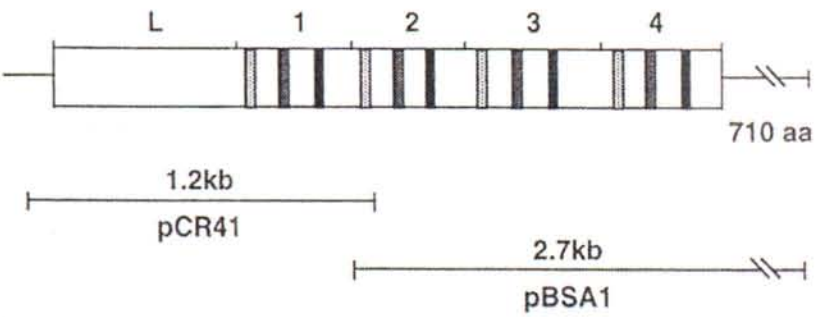
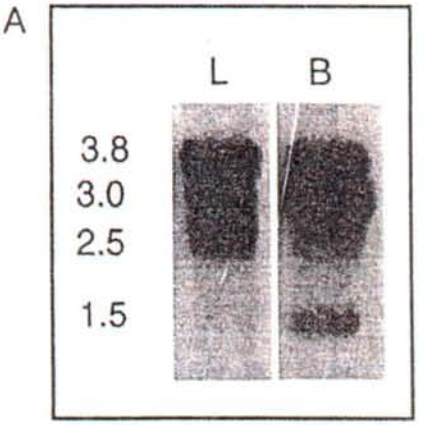
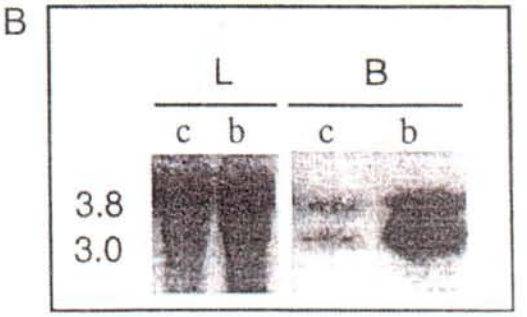


FIGURE 4

NORTHERN BLOT ANALYSIS OF 3 μG POLY(A+) RNA/LANE (PANEL A) OR 25 μG TOTAL CELLULAR RNA (PANEL B) FROM LAMB (L) OR BEEF (B) LONGISSIMUS MUSCLE PROBED WITH CALPASTATIN CDNA. IN PANEL B REPRESENTS C AND B REPRESENTS CONTROL AND β-ADRENERGIC AGONIST-FED, RESPECTIVELY.



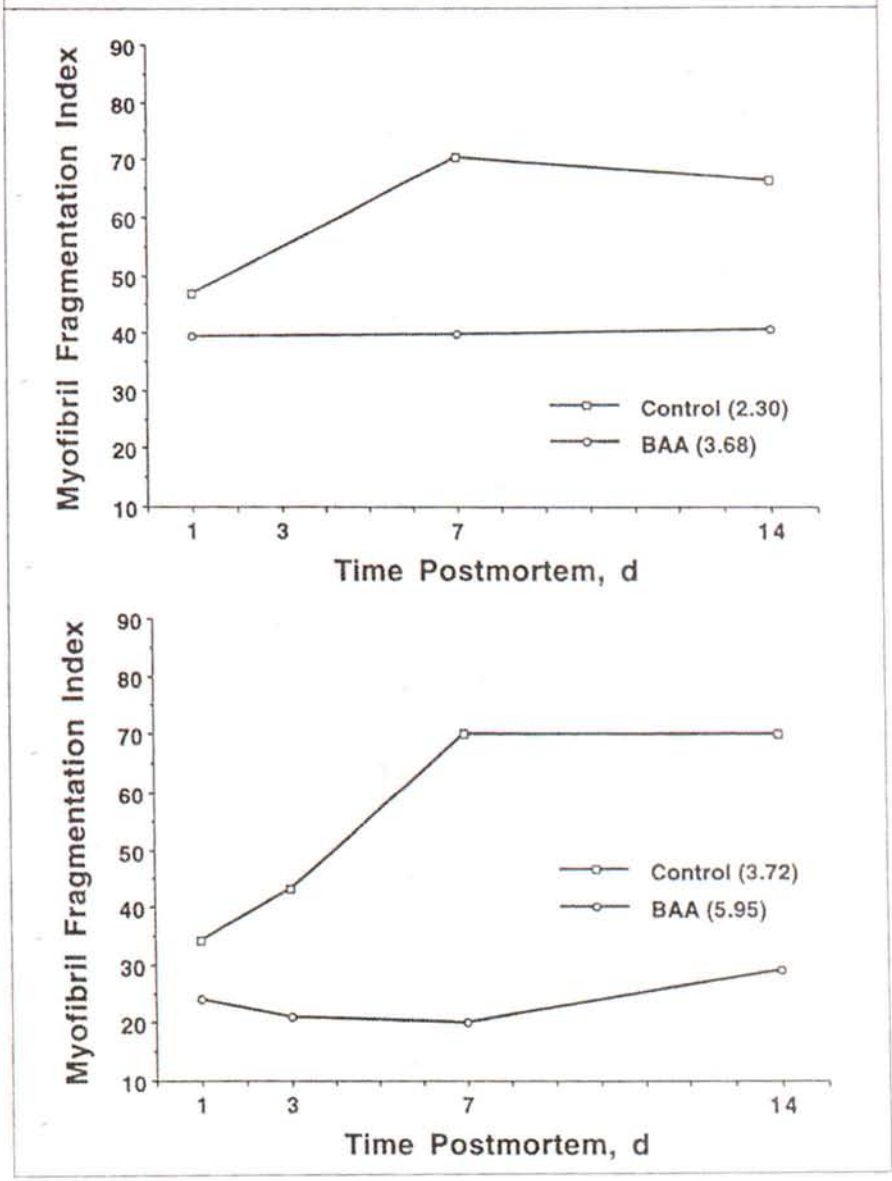


CORRELATION BETWEEN CALPASTATIN mRNA AND MEAT TENDERNESS

The most consistent effects of dietary administration of some β-adrenergic agonists (BAA: $L_{644,969}$, cimaterol and clenbuterol) are increased calpastatin activity from skeletal muscle and decreased rate and extent of postmortem proteolysis (Figure 5; Higgins et al., 1988; Kretchmar et al., 1989, 1990; Koohmaraie et al., 1991a; Koohmaraie and Shackelford, 1991; Wheeler and Koohmaraie, 1992; Pringle et al., 1993).

FIGURE 5

EFFECT OF POSTMORTEM STORAGE ON MYOFIBRIL FRAGMENTATION INDEX OF THE LONGISSIMUS MUSCLE FROM CONTROL AND β-ADRENERGIC AGONIST FED ($L_{644,969}$, MERCK, SHARP AND DOHME LABORATORIES, RAHWAY, NJ) LAMB (TOP; KOOHMARAIE ET AL., 1991A) OR STEERS (BOTTOM; WHEELER AND KOOHMARAIE, 1992). NUMERICAL VALUES IN PARENTHESIS ARE CALPASTATIN ACTIVITY (UNITS/G FRESH MUSCLE).



However, the mechanism of BAA-induced increase in calpastatin activity has not been determined. Possible mechanisms by which BAA increases calpastatin activity include increases in the rate of calpastatin gene transcription, translation and/or activity. Using bovine calpastatin cDNA, we were able to demonstrate that both mRNA abundance and activity of calpastatin increased in a coupled fashion in wether lambs and steers with dietary administration of $L_{644,969}$. Northern blot analysis indicated that BAA treatment affected differentially the expression of calpastatin isoforms (Figure 4). Specifically, BAA treatment increased the expression of the 3.0 and the 3.8 kb isoforms. Hence, these results demonstrated that regulation of calpastatin activity with BAA ($L_{644,969}$) is at the transcriptional level. The reasons for differences in calpastatin isoforms are not yet apparent. Because of the differential effects of BAA on these isoforms, it may be important to know sequence differences among isoforms to better understand the role of the proteins translated from these transcripts. Questions such as stability (half-life) of the three different transcripts will need to be investigated. The importance of these findings is realized when one considers the fact that BAA treatment results in decreased rate of muscle protein degradation during both growth (thus, improving the efficiency of production) and postmortem storage (thus, resulting in minimum or no detectable postmortem proteolysis and tough meat). The fact that calpastatin isoforms are affected differently with BAA treatment suggests that BAA-sensitive mechanisms are controlling exon splicing and/or selection of polyadenylation sites, which in turn may control stability and cellular function of calpastatin.

In the same data set, calpastatin mRNA abundance was highly correlated with shear force of aged meat (Table 2).

TABLE 2

CORRELATION COEFFICIENTS BETWEEN WARNER-BRATZLER SHEAR FORCE AT 14 D POSTMORTEM AND CALPASTATIN MRNA ABUNDANCE[a]

		n	Correlation coefficients
Beef			
	Overall	7	.95 **
	Control	3	.99 **
	BAA	3	.92
Lamb			
	Overall	13	.78 **
	Control	7	.70
	BAA	6	.83 *
Beef and Lamb			
	Overall	21	.71 **
	Control	12	.42
	BAA	9	.68 *

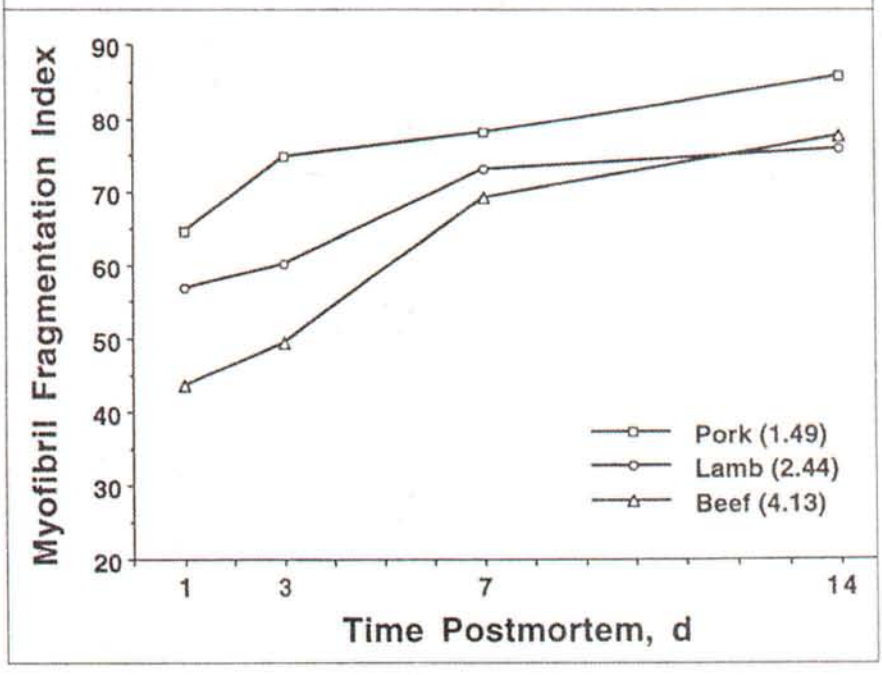
a From Killefer and Koohmaraie (not yet published).
*P < .05; **P < .01

Because these correlation values are unexpectedly high and because limited numbers of animals were used to derive them, these data should be interpreted with caution. Further studies are under way to test the repeatability and accuracy of the correlation between calpastatin mRNA abundance and tenderness of aged meat. To date, we have analyzed 24 additional animals and the correlation, between calpastatin mRNA and shear force of aged meat, still remains high (r = .64).

The association between calpastatin activity determined immediately after slaughter (hereafter referred to as prerigor calpastatin activity to distinguish from postrigor calpastatin activity) and tenderness of aged meat is not unprecedented. A similar relationship exists when postmortem events are studied in the same muscle from different species. Immediately after slaughter, bovine, ovine and porcine skeletal muscle contains about 4.0, 2.4 and 1.5 units of calpastatin/gram muscle, respectively (Koohmaraie et al., 1991b). Ouali and Talmant (1990) have reported similar observations. Prerigor calpastatin activity in the above species is highly related to the rate of postmortem tenderization (Figure 6).

FIGURE 6

EFFECT OF POSTMORTEM STORAGE AND SPECIES ON MYOFIBRIL FRAGMENTATION INDEX OF THE LONGISSIMUS MUSCLE FROM BEEF, LAMB AND PORK (KOOHMARAIE ET AL., 1991B). VALUES IN PARENTHESES REPRESENT PRERIGOR CALPASTATIN ACTIVITY (UNITS/G FRESH MUSCLE).



It is known that the rate of postmortem proteolysis differs among species (Dransfield et al., 1981; Etherington et al., 1987). For example, 80% of the tenderization process is completed in about 5 days for pork, 14 days for beef and lamb requires an intermediate amount of time to achieve the same degree of tenderization.

Although we had observed the relationship between postrigor calpastatin and tenderness of aged beef and lamb, the association between prerigor calpastatin activity within control animals was not observed until recently. In light of this new information, the relationship between mRNA abundance and tender-

ness of aged beef is being currently investigated in our laboratory to determine its efficacy for predicting meat tenderness.

It is appropriate at this time to mention a recent noteworthy observation. All calpastatin activity data reported from our laboratory has been determined using skeletal muscle m-calpain for quantification of calpastatin. We have always used DEAE-Sephacel-purified m-calpain as our source of calpain in the calpastatin assay because this fraction is relatively pure. Though known for sometime, within the last two years it has become apparent to us that skeletal muscle calpastatin has more activity toward m- than μ-calpain (Koohmaraie, unpublished observation). The significance of this finding is currently being investigated in our laboratory.

GENOMIC POLYMORPHISMS AT THE BOVINE CALPASTATIN LOCUS

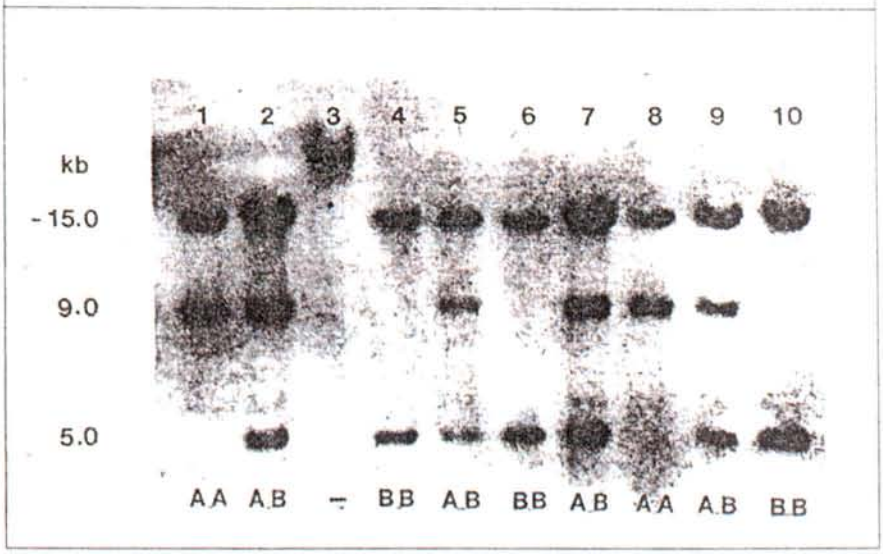
Mutations in the coding sequence for structural genes have been reported for a number of loci in the bovine genome (Fries et al., 1993). The types of mutations which occur include insertion or deletion of exons or simple base changes (point mutations) within exons which lead to conformational changes in form and function of the encoded gene product. In pigs, a classic example of a point mutation (C to T) in the coding sequence of the ryanodine receptor leads to the onset of malignant hyperthermia or pale, soft and exudative (PSE) pork (Fujii et al., 1991). Of course, point mutations in the intronic sequences leading to alteration of enzyme restriction sites can also occur, yielding different restriction fragment sizes among individuals. Their presumed impact on the transcription of a gene is seemingly of less consequence; however, little is known about point mutation effects on transcription on a locus-by-locus basis. For bovine calpastatin, no polymorphisms in the L Domain or for Domains 2 through 4 have previously been reported in farm animals.

Polymorphisms at the bovine calpastatin locus were identified utilizing the method of Southern (1975). Two cDNA probes sequenced by Killefer and Koohmaraie (1994) were used to independently screen restriction enzyme digested DNA from forty unrelated animals (Hereford, Angus, Piedmontese, Simmental, Gelbvieh, Red Poll, Braunvieh, Charolais, Limousin, Brahman, Pinzgauer) for polymorphisms that were easily identified and had potential for following Mendelian inheritance. DNA from bovine full-sib families (created by multiple ovulation embryo transfer; MOET) was analyzed to provide meiotic recombination data for linkage studies as part of a larger study to develop a genetic linkage map of the bovine genome at the U.S. Meat Animal Research Center.

Analysis for restriction sites within the cDNA sequenced by Killefer and Koohmaraie (1994) indicated that restriction sites were present for EcoRI and TAQI in the 3', 2.2 kb cDNA (pBSA1; Figure 3) spanning Domains 2 through 4 plus a 3', untranslated region of the bovine calpastatin gene (Bishop et al., 1993). A total of 9 enzymes (BamHI, EcoRI, TaqI, PstI, PvuII, MspI, HindIII, DraI and EcoRV) were used to screen panels of genomic DNA from forty unrelated animals. Three enzymes (BamHI, EcoRI and TaqI) yielded restriction fragment length polymorphisms (RFLP) that were allelic and easy to identify. One enzyme (BamHI; Figure. 7) provided a RFLP consisting of three bands, one band being common (monomorphic) in all individual animal DNA (> 15 kb) and two others at 9.0 and/or at 5.0 kb that were easily identified for presence or absence and, subsequently, used for genetic linkage analysis and chromosomal assignment of the bovine calpastatin gene.

FIGURE 7

RESTRICTION FRAGMENT PATTERNS OF TEN FULL-SIB CATTLE AFTER GENOMIC DNA WAS DIGESTED WITH BAMHI AND HYBRIDIZED TO THE BOVINE CALPASTATIN PROBE. ANIMAL 1 REPRESENTS THE AA GENOTYPE, ANIMAL 2 REPRESENTS THE AB GENOTYPE AND ANIMAL 4 REPRESENTS THE BB GENOTYPE. LANE 3 CONTAINS THE STANDARD MARKER LAMBDA HINDIII DIGESTED DNA



The sizes of the EcoRI and TaqI alleles were 6.0 and 4.0 kb, and 1.9, 3.5, 4.0 and 5.0 kb, respectively (data not shown). In Figure 7, the type of banding pattern expected from a digestion of bovine genomic DNA with the *E. Coli* restriction enzyme BamHI is shown. The three alleles found with BamHI are coded AA (2 alleles of size 9.0 kb), AB (1 allele of size 9.0 kb and 1 allele of size 5.0 kb) and BB (2 alleles of size 5.0 kb). Frequency of the BamHI alleles A and B in the 40 unrelated animals were 38% and 62%, respectively. We are now collecting data from offspring produced and slaughtered from the Genome Mapping Project and other projects at the U.S. Meat Animal Research Center to determine if either (or both) meat tenderness or calpastain activity is associated with polymorphisms found in the bovine calpastatin gene.

For the 5', 1.2 kb cDNA (Figure 3) spanning the L domain of calpastatin, no RFLP have been discovered utilizing the enzymes listed earlier. Therefore, screening of cosmid libraries has begun to identify clones containing portions of the bovine calpastatin locus for subsequent identification of polymorphic microsatellites (di-, tri-, or tetra-nucleotide repeats). If found, a polymorphic microsatellite would provide a convenient tool for following inheritance of this portion of the bovine genome to determine its contribution as an economic trait loci (ETL) to the phenotypic variance of meat tenderness.

LINKAGE GROUP AND CHROMOSOMAL ASSIGNMENT

To determine the relative genomic location of bovine calpastatin the BamHI RFLP was utilized to screen bovine full-sib families created by MOET. A total of ten paternal half-sib families were screened. Sixty-three informative meioses were detected on progeny DNA blots. Analysis of progeny inheritance of the 9.0 and 5.0 kb bands from their parents indicated that bovine calpastatin is part of a linkage group

currently spanning 50.8 cM (centimorgans) located on Chromosome 7 and is a member of bovine syntenic group U22 (Fries et al., 1993). Anchoring this linkage group is a gene coding for RAS p21 protein activator (GTPase activating protein; Fries et al., 1993). Knowledge of linkage among genes allows for study of effects of alleles at linked loci on the quantitative trait of interest. It further allows the study of a larger portion of the bovine genome for other gene loci which may be contributing to the total additive genetic variance and, thus, the heritability of an economic trait such as meat tenderness.

CONCLUSIONS

What has been achieved?

It has finally been recognized that variation in meat tenderness at the consumer level is unacceptable and ways to overcome this problem need to be developed. Significant progress has been made in identification and manipulation of factors regulating tenderness of aged meat. It is now accepted that the rate and extent of postmortem proteolysis of key myofibrillar proteins will determine the tenderness of aged meat. Based on knowledge of the postmortem tenderization process, methodology has been developed to produce tender meat consistently by postmortem injection of cuts of meat with a calcium chloride solution (200 mM at 5% of the cut weight). However, this methodology (Calcium-Activated Tenderization) should perhaps be considered a short-term solution to the tenderness problem. This is a significant achievement because efforts can now be directed toward development of methods to predict the capacity of the muscle to undergo postmortem proteolysis and, therefore, its ultimate tenderness.

What has been neglected?

The importance of the variation in meat quality was not recognized until recently. Therefore, the inadequacy of current methods for predicting meat palatability, although known for sometime, has not been accepted as a major concern. Now that it has finally been recognized, we must work toward the development of an objective method for predicting meat tenderness. The significance of this need is emphasized by the fact that the only time that the actual eating quality of meat is known is when it is consumed. Successful development of an accurate method of predicting meat tenderness requires a thorough understanding of regulation of the proteolytic system responsible for postmortem proteolysis. These problems are basic in nature. Therefore, we must patiently support basic research. The extent to which we progress toward our present goals is greatly dependent upon identification and quantification of factors regulating the activity of the proteases involved (i.e., the calpains and calpastatin).

What needs to be done?

It is urgent that inconsistency in meat tenderness be controlled. We must develop procedures to accurately predict the tenderness of aged beef with measurements made during the first 24 h postmortem and, eventually, before the animal is slaughtered. As we progress toward development of methodology for predicting meat tenderness, we must bear in mind that perhaps the most effective approach for elimination of tenderness inconsistency is through genetic control. Mapping of the bovine genome has been initiated at several institutions. Meat scientists and muscle biologists must participate in these projects, because the collaboration could prove to be beneficial to the industry.

Congresses, such as this one, should be promoted as a forum for exchange of information at an international level. As a result of these congresses, opportunities arise to form research collaborations that would not otherwise have been conceived.

Note: Mention of a trade name, proprietary product or specific equipment is necessary to report factually on the available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name USDA implies no approval of the product to the exclusion of others that also may be suitable.

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DISCUSSION WITH THE AUTHORS

Greaser

You attempt to predict tenderness at 14 days. From an industry point of view, would it not be better to try to predict tenderness at an earlier time?

Authors

Surveys in the USA show that the earliest the consumer receives meat is 5 days, the mean being about 10 days and the majority 18 days after slaughter. We wait to get a final value at 14 days to remove ageing variability. From an industry point of view, we need to get a predictive measure of tenderness within 24 hours.

Ouali

Are the mRNA correlated to levels of expression and activity in the tissue and do they vary from day to day?