CHARACTERIZATION OF *TaqI* POLYMORPHISMS IN THE BOVINE CALPASTATIN GENE

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SUMMARY

Four genotypes in a three allele system are proposed for a *taqI* polymorphism in the bovine calpastatin gene. This polymorphism appears to be related to Warner-Bratzler shear force and therefore may offer a potential genetic screening mechanism for enhancing beef quality.

INTRODUCTION

A variety of factors have been identified which directly or indirectly influence tenderness of beef including age, breed and sex of animal; post-mortem cooler aging and mechanical treatment. However, the majority of these factors are poorly related to ultimate meat tenderness. Recent data have clearly indicated that differences in the rate of postmortem proteolysis give rise to the majority of the variation in tenderness of aged meat (Koohmaraie, 1993, 1992a,b,c). Several endogenous proteolytic systems have been evaluated in research studies in recent years including the calpains, lysosomal and multicatalytic systems. Of these, only the calpains (also known as the calcium-dependent proteases) seem to effect postmortem proteolysis in manners sufficient to give rise to significant variation in final tenderness (reviewed by Koohmaraie, 1988; 1992a,b,c). Furthermore, an endogenous inhibitor of the calpains, called calpastatin, coexists with these enzymes in all cells. In skeletal muscle, calpastatin activity has been shown to triple from 0 to 24 h postmortem, with a concurrent decrease in μM-calpain activity, and has been found to be significantly related (r = .60 or higher) to shear force after 14 days of aging (Whipple et al., 1990; Shackelford et al., 1991). The research literature implicates postrigor calpastatin activity at 24 h postmortem (CA), as a potential marker trait to identify genetic differences in final beef tenderness. However, the primary problem associated with screening animals on the basis of CA is the fact that quantification is difficult. Development and use of a DNA probe for the bovine skeletal muscle calpastatin gene would provide potential opportunities for directly exploiting genetic variation in tenderness. The objectives of these studies were to evaluate whether polymorphisms exist for the bovine calpastatin gene and to provide initial data for defining associations with measures of meat quality.

MATERIALS AND METHODS

PCR Primer Design. Due to the difficulty of obtaining cDNA probes for the calcium-dependent proteases (CDPs) and due to the lack of sequence data specifically for bovine/porcine CDPs, a 19mer cross-species polymerase chain reaction (PCR) primer based on the published DNA sequence available for the CDP inhibitor, calpastatin (Emori et al., 1987; Takano et al., 1988) was designed at Purdue University. The calpastatin probe was designed from available sequence data on pig cardiac calpastatin (Takano et al., 1988). To isolate and amplify porcine calpastatin cDNA in skeletal muscle, bovine/porcine mRNA isolated from *Longissimus dorsi*

muscle was reverse transcribed to single stranded cDNA, and used as the template in the PCR reaction. Two forward and two reverse 19mer PCR primers for the calpastatin genes were designed. The resulting PCR fragment (423 bp) was subcloned and sequenced and hybridization conditions have been validated (Sun et al., 1993).

RFLP Analyses. At least 30 ml of whole blood were collected from each animal into EDTA vacutainer tubes, placed on ice and shipped overnite to Utah State University. The blood was centrifuged, the buffy coat collected and stored at -70°C until the genomic DNA was extracted as described by Muggli-Cockett and Stone (1988). The expected DNA yield from 10 ml of blood was between 100 and 300 ug. Purified DNA (7.5 ug) was digested in a 40 ul volume reaction with a total of 40 units of restriction enzyme (Gibco/BRL, Gaithersburg, MD). Digested samples were electrophoresed overnight (16 to 18 h) at 35 volts in 0.8% agarose (FMC SeaKem LE, Rockland, ME) made in TAE buffer (40 mM Tris-HCl pH 7.8, 5 mM Na acetate, 1 mM EDTA pH 8.0). Southern blot procedures and buffers were essentially as described by Sambrook et al. (1989). Calpastatin cDNA to used as a hybridization probe (described above) was electrophoretically separated from plasmid DNA and radioactively-labelled with [α^{32}P]dCTP(3000 Ci/mM, NEN Research Products, Boston, MA) by nick translation (Rigby et al., 1977). Specific activity of the probe was expected to be 10^9 cpm/ug DNA. Restriction enzymes used were *Taq*I, *Bgl*I and *Bam*HI. Only *Taq*I results are presented in this report.

Tenderness Quantification. Carcasses evaluated in this project were produced by conventional slaughter and dressing procedures. Following a 24 h chill period at 2°C, carcasses were ribbed and factors used to determine USDA quality and yield grades (USDA, 1989) were determined by trained and experienced personnel. A 1.0 in thick sample was obtained from the *l. dorsi* at this time to be used for determination of calpastatin activity. At 48 h postmortem, a loin strip section (*Longissimus dorsi)* approximately 63.5 mm thick and weighing approximately 6.8 kg was obtained from one side of each carcass. Loin strips were boned, vacuum-packaged and stored at 2°C. At 6 and 18 d postmortem, the loinstrips were removed from vacuum packages and fabricated to derive cross-sectional steaks (2.54 cm thick) for sensory panel evaluation and determination of Warner-Bratzler shear force values. Sensory evaluation were performed by a six-member, trained panel. The six panelists were selected and trained in accordance with the AMSA (1978) Guidelines for Cooking and Sensory Evaluation of Meat. Two steaks from each side were thawed (2°C) and broiled on Faberware Open-Hearth broilers to an internal temperature of 70°C. Upon reaching the desired internal temperature, steaks for sensory evaluation were removed from the broilers, trimmed to remove all external fat and connective tissue and sectioned into pieces of uniform dimensions. Panelists assigned scores to each sample for juiciness, initial and overall tenderness, flavor desirability, flavor intensity and overall palatability using eight-point, structured rating scales (Miller et al., 1983). Steaks for shear force determinations were cooked to the same temperature, cooled to 20°C and six 1.27 cm diameter cores were removed for Warner Bratzler shear force measurements (kg).

Calpastatin Activity. Determination of calpastatin activity was performed on *Longissimus dorsi* muscle samples obtained 24 h postmortem after refrigerated storage. Calpastatin activity was quantified using procedures similar to Shackelford et al. (1993) and Koohmaraie (1990).

Experimental Animals-- PHASE I. Fifty-four beef steers were identified from a known source at Excel's slaughter facility, Plainview, TX. Animals were chosen to represent several different biological types as an initial sample to screen for polymorphisms in the calpastatin gene. Blood samples (30 ml) were collected into EDTA vacutainer tubes at time of slaughter and shipped for RFLP analyses as described above. At 48 h postmortem, loin strips were transported to the Texas Tech University Meat Laboratory for tenderness quantification as described (only d 7 postmortem). RFLP analyses revealed five genotypes on the basis of scoring for presence or absence of four restriction fragments. Data analysis included estimation of genotypic frequencies and

estimation of genotype effects on meat quality traits. Sire effects were ignored in the analysis due to lack of pedigree information.

Experimental Animals -- PHASE II: A second study was conducted in collaboration with Colorado State University (Tatum et al., 1992). One-hundred, thirteen steers representing 25 or 50% Brahman inheritance when crossed with Hereford were fed in this study. Standard feedlot data and carcass data, including calpastatin activity at 24 h postmortem, were collected by the Colorado State research team. Blood samples for the RFLP analyses with the calpastatin probe were collected prior to slaughter. All growth, feed efficiency, carcass and sensory data were analyzed for association of polymorphisms in the calpastatin gene with these traits. Again, sire information was not available. The analytical model evaluated the effect of percentage Brahman and genotype in one comparison with a gene substitution model employed in a second comparison.

RESULTS AND DISCUSSION

Phase I. Four restriction fragments and five genotypes were detected from this group of animals. Frequencies of the genotypes and means for shear force and sensory panel traits are presented in table 1. Overall, differences of 21, 21 and 28% were observed between the most divergent genotypes for sensory tenderness, sensory overall palatability and shear force, respectively. Since pedigree information was not available, it was impossible to determine codominance of alleles, however, it preliminarily appeared that fragment 1 (allele 1), fragments 2 and 4 combined (allele 2) and fragments 3 and 4 combined (allele 3) constitute a three allele system for the calpastatin gene.

TABLE 1. FREQUENCIES OF CALPASTATIN GENOTYPES AND MEANS OF MEAT QUALITY TRAITS BY GENOTYPE (Phase I)

	Genotype[1]				
Trait	1000	0111	1011	0101	1101
Frequency (%)	6.0	18.0	2.0	54.0	20.0
Shear Force (kg)	5.70^{a}	4.71^{ab}	4.45^{ab}	4.56^{b}	4.58^{b}
Sensory Panel (1 to 8)					
Overall Tenderness	5.40^{a}	6.35^{ab}	5.67^{ab}	6.51^{b}	6.40^{ab}
Juiciness	6.00^{a}	6.13^{a}	6.50^{a}	6.43^{a}	6.28^{a}
Flavor Desirability	5.83^{a}	6.09^{ab}	6.17^{abc}	6.54^{c}	6.47^{bc}
Overall Palatability	5.28^{a}	5.87^{ab}	5.50^{ab}	6.38^{b}	6.32^{ab}

[1]Genotypes are scored for absence (0) or presence (1) of four restriction bands, i.e. genotype 1011 has bands 1, 3 and 4.

^{abcd}Means within a row having differing superscripts are different (P <.10).

Phase II. Results from phase II animals are presented in table 2. The same five genotypes were detected from these animals as observed in phase I. Shear force differences between genotypes were also similar to phase I results for both d 6 and 18 postmortem. However, taste panel results did not agree with the shear force results within this phase or the sensory panel results from the previous phase. Calpastatin activity was not different between genotypes with no trend evident. Feedlot average daily gain (kg/d) was associated with

genotype although difficult to interpret since the lowest calpastatin activity genotype had the highest average daily gain, contrary to expected effects of calpastatin activity on protein turnover.

TABLE 2. FREQUENCIES OF CALPASTATIN GENOTYPES AND MEANS OF MEAT QUALITY TRAITS BY GENOTYPE (Phase II)

	Genotype				
Trait	1000	0111	1011	0101	1101
Frequency (%)	4.4	7.9	3.2	53.9	30.4
Shear Force (d 6, kg)	5.42^{a}	4.22^{b}	4.82^{ab}	4.33^{b}	4.15^{b}
Shear Force (d 18, kg)	3.80^{ab}	3.62^{ab}	4.00^{a}	3.49^{ab}	3.39^{b}
Calpastatin Activity	3.19^{a}	2.98^{a}	2.74^{a}	3.00^{a}	3.04^{a}
Avg. Daily Gain (kg/d)	1.33^{a}	1.51^{ad}	1.74^{bc}	1.55^{bd}	1.59^{bd}

^{abcd}Means within a row having differing superscripts are different (P <.10).

Assuming a three-allele model for this gene locus, a gene substitution analysis was performed. Results indicated the importance of allele 2 whose presence assured more favorable shear forces (-1.11 kg on d 6 and -.44 kg on d 18)) and sensory tenderness (.46 score unit on d 18).The most important conclusion for this phase of the study was that there were significant differences in shear force between the genotypes, verifying the preliminary results obtained in phase I.

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