BEEF TENDERNESS: REGULATION AND PREDICTION

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Introduction

Consumers consider tenderness to be the single most important component of meat quality (Figures 1 and 2). This fact is easily confirmed by the positive relationship between the price of a cut of meat and its relative tenderness (Savell and Shackelford, 1992). Inconsistency in meat tenderness has been identified as one of the major problems facing the beef industry (Morgan et al., 1991; Morgan, 1992; Smith, 1992; Savell and Shackelford, 1992). Uniformity, excessive fatness, and inadequate tenderness/palatability were all part of the top 10 quality concerns of the beef industry (Smith, 1992). A recent survey reported that consumers were dissatisfied with the eating quality of beef prepared at home more than 20% of the time (Miller, 1992). One supermarket chain that asks customers to return any meat they are not satisfied with had $364,000 worth of meat returned in a three year period, 78% of which was due to tenderness problems (Morgan, 1992). The real magnitude of the tenderness problem is realized by considering the fact that only 0.1% of unhappy customers actually complain or return the product (Wilkes, 1992). This happens despite the technology that has been developed to improve the consistency of meat tenderness (e.g., postmortem aging, mechanical tenderization, electrical stimulation, and addition of plant enzymes).

The beef industry relies on the USDA quality grading system to segment carcasses into groups based on varying levels of expected meat palatability. However, 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 flavor, this relationship is weak at best (reviewed by Parrish, 1974). There are far too many carcasses with tender meat that are discounted and far too many with tough meat that are not discounted under the current USDA quality grading system (Wheeler et al., 1994a; Figure 3). The data collected at U.S. Meat Animal Research Center (MARC) indicate that both subjective and objective estimates of raw and cooked steak chemical composition are unrelated to meat tenderness (Figures 4 and 5).

Thus, the inconsistency in meat tenderness is due to a combination of our inability to: 1) routinely produce tender meat, and 2) identify carcasses producing tough meat. It is sobering to recognize that the only time the tenderness of meat is known is when the meat is eaten by the consumer, and if the meat is tough, then it is too late. It has been demonstrated that consumers will pay more for steaks that are known to be tender (Boleman et al., 1995), thus, there is an economic incentive for predicting meat tenderness. Because consumers consider tenderness to be the major

determinant of eating quality of meat, it is essential to develop methodologies to objectively predict meat tenderness to supplement or replace the current USDA quality grading system. The objective of this manuscript is to summarize our research results and plans relating to regulation and prediction of beef tenderness. We recognize that palatability has several components which include tenderness, juiciness, and flavor. It is a combination of these eating attributes that determines the degree of eating satisfaction. However, we have concentrated our research efforts on tenderness (for review see Koohmaraie, 1995) because there is twice as much variation in tenderness as in juiciness and flavor (Table 1).

The Sources of Tenderness Variation

To reduce variation in tenderness of aged beef, one must first understand the mechanisms involved. If the causes of variation are identified, then it may be possible to manipulate the process advantageously. Therefore, it is imperative to determine the biological factors regulating meat tenderness. Over the years, a number of parameters, including amount and solubility of connective tissue, and amount of intramuscular fat (marbling), have been associated with meat tenderness. Utilizing the data collected from the Germplasm Evaluation project (GPE), Crouse and coworkers (unpublished data) determined that connective tissue and marbling combined only accounted for 20% of the observed variation in meat tenderness (Figure 6). Therefore, we could not account for 80% of the variation in meat tenderness. In 1984, a project was initiated at the MARC to determine factors regulating tenderness of aged beef. A graphic illustration of the results is reported in Figures 7 and 8 (For review see Koohmaraie 1988, 1992a,b, 1994; Koohmaraie et al., 1994). Based on these results, we hypothesized that differences in the rate and extent of postmortem tenderization were responsible for variation in the tenderness of aged beef. Hence, it was decided that progress toward identifying factors regulating meat tenderness was dependent upon understanding how meat tenderizes during postmortem aging.

The phenomenon of the improvement in meat tenderness with postmortem storage was first described over a century ago. For many decades, meat scientists from throughout the world have conducted research to identify the mechanism(s) of improvement in meat tenderization with postmortem storage. Collectively, these results indicate that there are small, but significant, changes that occur in the muscle that result in tenderization. The following is known about meat tenderization during postmortem aging (for review see Koohmaraie, 1988, 1992a,b, 1994, 1995):

1) Immediately after slaughter meat is tender (low shear force). However, because of the muscle shortening which accompanies rigor mortis development, meat toughens during the first 12 to 24 hours after death. Simultaneously, an opposite phenomenon (i.e., tenderization) also begins either at slaughter or shortly

after slaughter, which results in meat tenderization in most but not all animals (Wheeler and Koohmaraie, 1994; Figure 8A).
2) Tenderization occurs because of degradation of a few key structural proteins by endogenous enzymes (this process is called postmortem proteolysis and is the reason for aging meat). These proteins are responsible for maintaining the structural integrity of the muscle. These proteins (more than likely not all have been identified) are involved in inter- (e.g., desmin and vinculin) and intra-myofibril linkages (e.g., titin, nebulin and possibly troponin-T). Although the list of the proteins involved could change over the years, the principle will stand the test of time; that is, proteolysis of key myofibrillar proteins (proteins whose function is to maintain structural integrity of myofibrils) is responsible for postmortem tenderization. As new myofibrillar proteins are discovered, their potential role in postmortem tenderization will systematically be determined.
3) Differences in the rate and extent of postmortem proteolysis of these key myofibrillar proteins is the major source of variation in beef tenderness (Figures 7 and 8).
4) Current data suggest that of all the proteolytic systems endogenous to skeletal muscle, the only enzyme system involved in meat tenderization is the calpain (calcium-dependent) proteolytic system.
5) The calpain system has three components: a low-calcium-requiring enzyme (μ-calpain), a high-calcium-requiring enzyme (m-calpain), and an inhibitor, (calpastatin), which specifically inhibits the activity of the calpains. Calpains have an absolute dependency on calcium for activity.
6) Postmortem tenderization occurs fastest in pork followed by lamb and then beef (Figure 8, Item #2).
7) Although most beef responds to postmortem storage (i.e., tenderization) the rate and extent of tenderization varies such that some beef does not benefit from extended postmortem storage.
8) To improve the consistency of meat quality with respect to tenderness, beef, lamb and pork should be aged at least 14, 10, and 5 days, respectively. This practice alone will eliminate a large portion of the observed variation in meat tenderness (Figure 2B).
9) Tenderization occurs at the same rate for vacuum packaged subprimals as for dry-aged cuts.

Controlling Tenderness Variation

1. Calcium-Activated Tenderization (CAT). Based on our knowledge of the mechanism of postmortem tenderization, we have developed a process that ensures meat tenderness (for review see Koohmaraie et al., 1993). Calpains require calcium for activity. But, conditions in postmortem muscle are not always optimum for calcium to be available to activate calpains. But exogenous calcium can be added to meat, thus, activating calpains and inducing more rapid and extensive tenderization. The process, known as Calcium-Activated Tenderization (CAT), consists of injecting cuts of meat

(either pre-rigor or post-rigor) with 5% (by weight) of a 2.2% solution of food-grade calcium chloride. Following injection, cuts are vacuum-packaged and stored for seven days prior to consumption. For best results, commercial, automatic pickle injectors should be used to ensure uniform distribution of the calcium chloride throughout the cut of meat. If at all possible, one should avoid use of hand held injectors. The process is more effective in prerigor (the first 3 hours after slaughter) meat, but can be used up to 14 days postmortem. It will not affect meat that is already tender, thus, it will not make tender meat "mushy". At the recommended levels of calcium chloride, the process has little effect on other meat quality traits. The process is effective in all cuts of meat regardless of species, breed or sex-class. The process is also effective in cuts of meat expected to be unusually tough. These include meat from sheep and cattle fed ß-agonist, old cows, Brahman cattle, and rounds muscles from bulls. It has been tested under commercial conditions in a large beef processing facility.

Restaurant (Hoover et al., 1995) and supermarket consumer evaluation studies (1,001 participants) have indicated that consumers prefer calcium-injected beef over non-injected control beef due to improved tenderness with no change in flavor desirability or juiciness (Table 2). Supermarket shoppers given the option of selecting steaks labeled "tenderness and juiciness enhanced with the addition of up to 5% of a solution of water and calcium chloride" or control steaks with the same label without the above statement chose the calcium-added steaks 71% of the time (Miller et al., 1995). Fresh pork and chicken products are routinely injected with various ingredients to improve tenderness, juiciness and flavor. Consumer perception of calcium-injected meat should not be a concern. In fact, CAT probably has a positive effect on beef acceptance due to the health benefits of a calcium-added product. The CAT process has enormous potential to help the beef industry in its effort to reduce variation in beef tenderness. In our opinion, there are no barriers to commercial application of the CAT process for ensuring desirable meat tenderness regardless of the product source. We continue to work closely with interested parties to help them implement this process. Meanwhile, we continue to seek a long-term solution to tenderness variation problems by looking for ways to produce tender meat consistently and to identify tough meat.

2. Genetics. Many scientists and producers have suggested that controlling the genetics of the slaughter cattle population would entirely solve the beef industry's tenderness problem. We agree that genetics makes a large contribution to the total variation in tenderness. However, genetic analyses indicate that environmental factors make a much larger contribution to variation in tenderness. Thus, it may be more efficient to improve tenderness through management and processing procedures than genetic selection.

On average, some breeds of cattle produce more tender meat and some produce less tender meat relative to other breeds (Koch et

al., 1976, 1979, 1982b; Wheeler et al., 1995a). It is well documented that the mean shear force and variation in shear force increases as the percentage of *Bos indicus* inheritance increases (Figure 9; Crouse et al., 1989). Furthermore, meat from 1/2 or greater *Bos indicus* (Brahman, Nellore, Sahiwal) cattle is usually significantly less tender than meat from cattle with less than 1/2 *Bos indicus*. On the other hand, several breeds (Jersey, Pinzgauer, South Devon, and Piedmontese) tend to produce meat that is more tender than meat from other breeds. But, on average, most breeds are fairly similar in meat tenderness. However, there is more variation within each breed, than among the most different breeds (Figure 10). Figure 10A indicates the amount of change that could be expected in shear force by selecting Pinzgauer instead of Nellore purebred cattle (4.76 genetic standard deviations) relative to the within-breed variation (6 genetic standard deviations). For F1 progeny this same comparison results in 2.38 genetic standard deviations between Pinzgauer- and Nellore-sired progeny (Figure 10B), although only 1.43 phenotypic standard deviations are realized among Pinzgauer- and Nellore-sired progeny (Figure 10C). Thus, the realized improvement in tenderness from selecting one breed over another will be small (at most 1.44 kg; to change from half-blood Nellore to half-blood Pinzgauer). To make additional improvement within a breed requires identifying those sires (and dams) whose progeny produce more tender meat, either through progeny testing or some direct measure on the sire and dam to predict the tenderness of their progeny.

Traditional animal breeding theory indicates that the most effective genetic selection is made through progeny testing. Due to the time required, progeny testing may not be a practical method to improve tenderness. If we make the following assumptions: use 13 sires, hold inbreeding to less than 1%, 100 head cow herd size, heritability estimates of 0.30 for shear force and 0.42 for marbling, the genetic correlation 0.25 between shear force and marbling (Koch et al., 1982a and the references therein), standard deviation of 1.0 kg for shear force, then it would take 12.0 years and 40.7 years to improve shear force by 1.0 kg by selection for shear force or marbling, respectively. If we increase the size of cow herd to 500, the above estimates will be 6.8 and 23.1 years, respectively. Obviously, a significant change in the above parameters will affect these estimates. There is evidence to suggest that significant improvement in shear force measurement can be made (Wheeler et al., 1994, 1995b; Koohmaraie et al., 1995d) to improve its accuracy, which may increase the heritability estimates for shear force and thus, change the above estimates. Furthermore, data collected at the MARC indicate that extreme culling would have to be imposed to eliminate all tenderness problems through genetics. The rate of genetic improvement in a given trait is a function of the heritability of the trait, the generation interval, and the selection differential. MARC data indicate that the maximum selection differential which could be imposed for tenderness is relatively small. In fact, the distributions of shear force values overlap for the progeny of the toughest and most tender ten percent of

sires (Figure 11). Moreover, if we culled the toughest 10% of sires we would only decrease the frequency of shear values above 4 kg from 20% to 16% (Figure 12). Thus, extreme culling would have to be imposed to eliminate all tenderness problems through genetics. Undoubtedly, it would be impossible to select heavily for tenderness without compromising other economically important traits. It appears to us that the beef industry should 1) exploit breed complimentary and heterosis through crossbreeding to balance production, carcass, and meat traits and 2) use appropriate production, processing, and evaluation procedures to guarantee tenderness. This should not be interpreted to mean, that the genetic contribution to tenderness is not important. The major impact that genetics can have on meat tenderness is well documented. The effect of *Bos indicus* inheritance on meat tenderness was cited above. Another good example is the case of the callipyge gene in sheep. Callipyge is a gene recently identified in lamb which has a major effect on carcass composition by increasing total muscle weight by approximately 30%. However, carrier lambs produce meat that has extremely high longissimus shear force value (248% of control), even after 21 days of postmortem storage (Figure 13; Koohmaraie et al., 1995b,c). Thus, the application of molecular genetic approaches (as described in pages to follow) could hasten our ability to control the genetic aspects of meat tenderness.

Direct Methods of Predicting Beef Tenderness

1. Shear Force-Based Classification of Beef. We have determined that the tenderness of beef longissimus measured directly by shear force at 1 day postmortem is strongly related ($r = .75$) to tenderness of longissimus muscle at 14 days postmortem (i.e., if a carcass is tough initially, it will be tough after aging). Over the course of the last several years, we have collected day-1 shear values on 400 steer carcasses. Analysis of these data indicated that we can accurately segregate cattle into expected aged longissimus muscle tenderness groups (day-14 shear force $<$ 6.00 kg vs day-14 shear force $\geq$ 6.00 kg). The success rate of this procedure was 85% which was much higher than the present quality grading system (60%). This procedure allows for the creation of a tenderness grade which contains 100% tender beef. In contrast, 20% of upper Choice carcasses (Modest and Moderate marbling scores) are relatively tough.

Shear force could be used to segregate carcasses into any number of expected tenderness groups. But, if the industry were to use a tenderness-based classification system, we suggest a system that includes three tenderness grades (Figure 14). The highest grade would consist of carcasses that are already acceptably tender before aging. These carcasses, which had a mean day-14 shear value of 4.1 kg, could be identified as "Guaranteed Tender". The middle grade would consist of carcasses that are not tender before aging but that will probably be tender after aging. These carcasses, which had a mean day-14 shear value of 5.1 kg, could be identified as "Probably Tender." The lowest grade would consist

of carcasses that are extremely tough before aging and that will probably still be tough even after extensive aging. These carcasses, which had a mean day-14 shear value of 7.3 kg, could be identified as "Probably Tough" and would require tenderization before marketing.

Because day-1 shear is a much better predictor of aged longissimus shear force than any visual, physical, or chemical measurement heretofore examined, we believe that day-1 shear force could be used as a tenderness grading criterion. Thus, we have outlined an automated system for measuring shear force at 1 day postmortem at commercial beef processing speeds. This automation will require some changes to the current shear force measurement protocol and, thus, a series of experiments are being conducted to ensure accuracy is maintained with automation. This procedure would decrease the value of a portion of the product and would be much more expensive than the present quality grading system. Based on a rough cost estimate this procedure would require a $9/cwt increase in the price of ribeye and striploin to recoup reduced value on a portion of the product. As indicated earlier, consumers are willing to pay more for guaranteed tender meat (Boleman et al., 1995).

Indirect Methods of Predicting Beef Tenderness

1. Predicting Beef Tenderness with Carcass Traits. After studying sources of variation in tenderness of youthful, grain-fed beef (the majority of block beef in the United States), we, and others, have found that marbling will account for at most 15% of the variation in aged beef tenderness. Other carcass traits, proposed to be related to beef tenderness, such as skeletal and lean maturity, fat thickness, carcass weight, and lean color, texture, and firmness, are even more weakly related to aged beef tenderness. Concomitantly, our data indicate live animal performance traits such as slaughter weight, weight per day of age, average daily gain, and time-on-feed will not account for a significant portion of the variation in aged beef tenderness. The one historical trait that will consistently explain a large percentage of the variation in aged beef tenderness is the percentage of *Bos indicus* inheritance in the cattle. Numerous experiments have demonstrated that the frequency of unacceptably tough meat is greater for cattle possessing high levels of *Bos indicus* inheritance (Koch et al., 1982b; Crouse et al., 1989; Cundiff et al., 1993). However, most research indicates that cattle containing 25% or less *Bos indicus* inheritance are similar to their *Bos taurus* counterparts in palatability (Crouse et al., 1989; Johnson et al., 1990). Thus, if one adheres to sound crossbreeding principles, the production advantages of *Bos indicus* crossbred cattle may be reaped without compromising product quality.

2. Calpastatin-Based Methods of Predicting Beef Tenderness. As noted above, our studies have indicated that differences in the rate and extent of postmortem tenderization are responsible for

variation in tenderness of aged beef. Furthermore, our results have demonstrated that the calpain enzyme system is responsible for the changes that result in meat tenderization. Thus, our approach to tenderness prediction has been to identify a trait that measures the capacity of this enzyme system. The principal regulator of the calpain enzyme system, in postmortem muscle, is its endogenous and specific inhibitor called calpastatin. In several studies (Whipple et al., 1990a,b; Shackelford et al, 1991a,b) designed to determine the biological reason for differences in meat tenderness between *Bos indicus* and *Bos taurus* cattle, it was determined that calpastatin activity at 24 hours postmortem (referred to as postrigor calpastatin) would explain a greater proportion of the variation (up to 44%; Figure 15) in aged beef tenderness than any other trait measured in those experiments. In a subsequent experiment (Shackelford et al., 1994), postrigor calpastatin was shown to be highly heritable (heritability = 0.65). Furthermore, the genetic correlation between postrigor calpastatin and Warner-Bratzler shear force was 0.50. Collectively, these results demonstrate that selection against postrigor calpastatin activity could result in improved meat tenderness. Furthermore, it suggests that postrigor calpastatin activity could be used as a predictor of beef tenderness. Unfortunately, current methods of calpastatin quantification are laborious and time consuming. We have just developed a rapid method for quantification of calpastatin using an Enzyme-Linked Immunosorbant Assay (ELISA). We are now in the process of determining the efficacy of postrigor calpastatin as a predictor of beef tenderness using the ELISA.

Because of the apparent importance of calpastatin in regulating the tenderness of aged beef, we initiated a project in which we, for the first time, successfully cloned and sequenced bovine skeletal muscle calpastatin (Killefer and Koohmaraie, 1994). Additionally, we have localized the calpastatin gene to chromosome 7 of the beef genome, and more importantly, we have demonstrated that the calpastatin gene is polymorphic (i.e., there are several forms of the calpastatin gene; Bishop et al., 1993). It may be possible to exploit the polymorphisms in the calpastatin gene to develop methodology for predicting tenderness of aged beef and to genetically select for tenderness. These goals can be accomplished only if the polymorphisms in the calpastatin gene are associated with variation in tenderness of aged beef. If the polymorphisms in the calpastatin gene are not associated with variation in tenderness, then the polymorphisms would not provide us with any useful information. Using 83 crossbred steers from sires representing 8 different breeds, we found no association between polymorphisms at the calpastatin loci and tenderness of aged beef or calpastatin activity (Lonergan et al., 1995). Thus, these polymorphisms do not have the potential to be used as tenderness predictors because they are not related to Warner-Bratzler shear force at 14 d postmortem. The lack of relationship between polymorphisms at the calpastatin loci and meat tenderness must be distinguished from the well documented relationship between calpastatin activity and meat tenderness. It simply means

that there are different forms of the calpastatin gene, but that they are not related to expression of the protein (calpastatin). The level of this protein, however, is highly related to tenderness of aged meat. It important to recognize that our study was designed to evaluate the potential application of calpastatin RFLP's in the prediction of meat tenderness. Therefore, a sample of unrelated animals was chosen to represent the diversity of beef breeding in the U.S. industry. Evaluations of defined populations (i.e., family studies) may help further characterize the calpastatin gene. Such an analysis may increase the understanding of the inheritance patterns of calpastatin and may explain differences in calpastatin activity within that population. However, such models will not improve the value of calpastatin RFLPs as a predictor of meat tenderness at the industry level.

We are in the process of determining the elements that regulate calpastatin gene expression. These studies should provide information about the regulation of calpastatin gene expression and possibly on how to manipulate its expression.

Similar to shear-force based classification of beef, a calpastatin-based classification system would likely include only three tenderness classes, unacceptably tough, average, and desirably tender. It appears that any individual consumer has a threshold for acceptable meat tenderness. Meat below the threshold would be unacceptable, and meat above the threhold would be acceptable. However, this threshold may vary with the eating circumstances (i.e., restaurant or at home). In addition, the threshold for acceptable tenderness will vary for different consumers. For these reasons a simple acceptable/unacceptable grading system is not sufficient. More than three grades may attempt greater classification than is needed or feasible. Three grades would allow the identification of meat that is clearly unacceptable in tenderness that would be discounted in price or targeted for the CAT treatment. The top grade would represent meat that would be acceptably tender to almost everyone. The middle grade would be for meat that encompasses the range between individual consumers for acceptably tender meat (i.e., the lower boundary would be equal to the least tender meat that a consumer considers the threshold for acceptable, and the upper boundary would be the most tender meat that a consumer considers the threshold for acceptable).

4. Predicting Beef Tenderness with Multiple Traits. As mentioned previously, based on current knowledge, with the exception of measuring shear force at 1 day postmortem, no single trait consistently explains greater than 50% of the observed variation in tenderness of aged beef. To improve our chance of developing a method for predicting beef tenderness, we are using several approaches in addition to those based on calpastatin. We are currently collecting data on a large number of carcasses in order to develop an accurate tenderness prediction model. Because the value of the loin and rib drive the value of beef carcasses, we chose to predict the tenderness of top loin (longissimus) steaks.

Moreover, because most rib and loin cuts are aged for at least 10 days postmortem with the national average being about 17 days, we chose tenderness at 14 days postmortem as our endpoint for prediction.

The dependent variables that we are using to predict meat tenderness include historical data about the cattle (age, time-on-feed, dietary energy density, percentage *Bos indicus* inheritance, etc.), live animal performance data (average daily gain and weight per day of age), pH and temperature at 3, 6, 9, 12, and 24 hours postmortem, and the following traits determined at 24 hours postmortem: calpastatin activity, myofibril fragmentation index, fragmentation index, osmotic pressure, water-holding capacity, sarcomere length, and standard carcass grade traits (quality and yield grade factors). These traits were selected because they are the traits which are most commonly thought to be responsible for animal-to-animal variation in the tenderness of youthful, grain-fed beef. Other traits, such as collagen (connective tissue) amount and solubility and fiber type and size, were not included in this experiment because we have a substantial amount of data that indicates that variation in these traits is not related to variation in the tenderness of youthful, grain-fed beef. Some combination of these traits may allow us to explain additional variation not accounted for by calpastatin measures.

Genetic Approaches to Predicting Meat Tenderness

The genetic contribution to tenderness or any other trait can be evaluated by using the candidate gene approach and/or a whole genome approach. With the current capabilities, these two approaches are not mutually exclusive and, thus, can be pursued simultaneously.

Candidate gene approach. The candidate gene approach takes advantage of the existing knowledge of the biochemical basis of meat tenderness. As stated, current data indicate that calpain-mediated proteolysis of key myofibrillar proteins is responsible for postmortem tenderization; thus, differences in the potential proteolytic activity of the calpain system result in differences in the rate and extent of postmortem tenderization. We have collected evidence indicating that, within a species, postmortem calpastatin activity is related to meat tenderness. In beef, for example, calpastatin activity at 24 hours postmortem is highly related to beef tenderness after 14 days of postmortem storage (for review see Koohmaraie et al., 1995a). Across species, however, at-death calpastatin activity is highly related to meat tenderness (Koohmaraie et al., 1991 and Ouali et al., 1990). In some special circumstances, at-death calpastatin is also related to tenderness of meat within a species such as: dietary administration of some beta-adrenergic agonists (such as L644,969 and cimaterol; for review see Koohmaraie et al., 1991) and expression of callipyge gene in lamb (Koohmaraie et al., 1995b,c).

The estimates for the relationship between calpastatin activity and meat tenderness vary, but up to 40% of the variation in beef tenderness is explained by calpastatin activity at 1-day postmortem (Koohmaraie et al., 1995a). Such a high degree of association could be the justification for using calpastatin in a candidate gene approach for predicting meat tenderness. The drawbacks to the candidate gene approach are twofold. Undoubtedly, more than one gene is involved in regulation of tenderness and this approach only allows for examination of one gene at a time. Secondly, the factors affecting the expression of the gene of interest (e.g., calpastatin) could be located on an entirely different chromosome; thus, such regulatory factors would not be identified in a candidate gene approach.

Gene mapping. Genetic maps are rapidly being constructed as a basis for identification of markers associated with Quantitative-Trait-Loci (QTLs) for use in Marker-Assisted-Selection (MAS) in cattle breeding programs. Several hundred markers spaced randomly throughout the cattle genome have been identified, sequenced and used to trace the inheritance of DNA segments from parent to offspring in cattle families designed for development of a linkage map. A linkage map characterizing heterozygous, well-spaced markers enables efficient selection of markers for identification of QTLs segregating in cattle resource populations. Resource populations are well defined large families of animals having traceable heritage through pedigree analysis and segregating alleles of genes affecting phenotypic characteristics of interest (i.e., meat tenderness, carcass retail yield, etc.). These resource populations may be derived from within breed, breed crosses or interspecies crosses. However, the type of resource population used or constructed will influence the level of heterozygosity within parental genomes. Several hundred more markers must be available for parental screening for a within breed (such as Angus or Hereford) search of QTLs than for an interspecies cross (such as Brahman x Angus) search due to the lower level of heterozygosity in the purebred genome. Depending on the objectives for use of the marker information, resource populations must either be created in a research setting or identified in the field from cattle populations currently in production.

Evidence is growing that we will be successful in identifying markers with proximity to loci having substantial effect on economically important traits. For instance, in plants (tomatoes, corn, soybeans), several QTLs have been identified and markers implemented through MAS to improve disease resistance and drought tolerance in breeding programs (Tanksley et al., 1982; Paterson et al., 1990). Markers for several debilitating human diseases have been discovered and are used for genetic screening and parental identification purposes. Recently, a region on pig chromosome 4 was shown to contribute to breed difference in growth rate, fatness and length of the small intestine. A region on cattle chromosome 1 may contain genes responsible for "polledness." Information will soon be released detailing the identification of

markers flanking QTLs responsible for milk component and yield variation within elite dairy families. Based on these discoveries, and those that are sure to follow, it is reasonable to assume that MAS for economically important traits will be implemented in both beef and dairy cattle selection programs in the very near future.

Strategies for identifying loci affecting economically important traits, in the examples cited above, have relied on the concept of "whole-genome-linkage-scanning" (Andersson et al., 1994; Figure 16). This concept is contrary to the "candidate gene" approach in that it allows, at the DNA level, an assessment of genetic variation at multiple intervals simultaneously with phenotypic records across all regions of the genome flanked with markers. Because of their ease of use, high utility and high throughput, microsatellites are the current marker of choice in whole-genome-linkage-scanning. They allow rapid efficient dissection of a plant or animal genome into interval parts for determining their direct contribution to variation in quantitative and disease related traits. The strategy begins with identification of a set of heterozygous microsatellite markers (from fully developed linkage maps) which span the parental genomes with reasonable interval distance between them. Once a set of markers have been selected, linkage scanning for chromosomal regions in the progeny genomes contributing to the variation of a phenotype (i.e., meat tenderness) can begin. Depending on the structure and size of the population used for dissection of a particular quantitative trait, statistical analysis techniques have been derived which yield conclusive results. Those techniques involve the use of linkage analyses along with maximum-likelihood and simple regression methodologies to identify regions of the genome contributing to the variation of a given trait. A method of searching for markers involve the use of a large number of half-sibs from interspecies backcrosses involving only a few sire families. To discover what region(s) of the genome are contributing to meat tenderness, phenotypic observations on tenderness (i.e., shear force) will be collected and associated with variation at the DNA level. Once found, markers for meat tenderness can be implemented in various MAS schemes and the gene(s) responsible determined.

Conclusions

Undoubtedly variation in tenderness of aged-beef at the consumer level must be controlled to improve customer satisfaction with beef. It has been shown that consumers are willing to pay more for higher or guaranteed tenderness. Several processes can be implemented immediately to reduce this variation, while others require further research.

Over the years, numerous factors have been reported to affect tenderness of aged beef. We must sort through those factors and determine which factors are most relevant. Those factors determined to be of most importance for controlling variation in meat tenderness should then be established as Critical Control

Points. Critical Control Points would likely include some or all of the following: genetics, male sex-condition, age, time-on-feed, type of ration, implant protocol, preslaughter handling procedures, slaughter/dressing, electrical stimulation, chilling, postmortem tenderization technologies ($CaCl_2$-injection, blade tenderization, etc.), and aging.

In addition, our data suggest that even if all critical points are controlled, we will still have tough beef. Within all breeds there are animals that will not produce tender meat even when the best processing procedures are followed. This means that we must develop methodology to identify such animals. Thus, we must be able to predict tenderness of aged beef prior to or within 24 hours of slaughter. We believe the best prediction of aged beef tenderness will be obtained by combining shear force at one day after slaughter with the ELISA for calpastatin activity. These techniques can be used to segregate carcasses into aged beef tenderness groups with greater than 85% accuracy. Because this method is invasive and results in devaluation of one top loin steak per carcass, some have argued against this method of tenderness-based classification. However, a prediction method that is highly-accurate should not be discarded simply because it is invasive. Rather this system should be compared to noninvasive systems on a cost/benefit basis. Beef that is classified into tenderness groups would meet consumer expectations better because they would more consistently get what they paid for.

Genome mapping and other projects to identity markers associated with tenderness of aged beef are progressing rapidly. Once these markers are identified they could be used to: 1) select for tenderness, 2) sort feeder cattle to optimize quality and yield, and 3) predict tenderness. However, markers may only be useful within the family in which they were generated. But, by sequencing the location of these markers in the cattle genome the identity of the gene(s) affecting beef tenderness will be determined. It is only at this level of knowledge that we truly can maximize the genetic effects on beef tenderness. One never knows what the future holds, maybe the identity of these genes will allow us to sort cattle into expected tenderness groups prior to slaughter. When knowledge of genetics is combined with critical control of environmental sources of variation in tenderness we should be able to consistently produce tender beef.

Figure 1

Figure 2

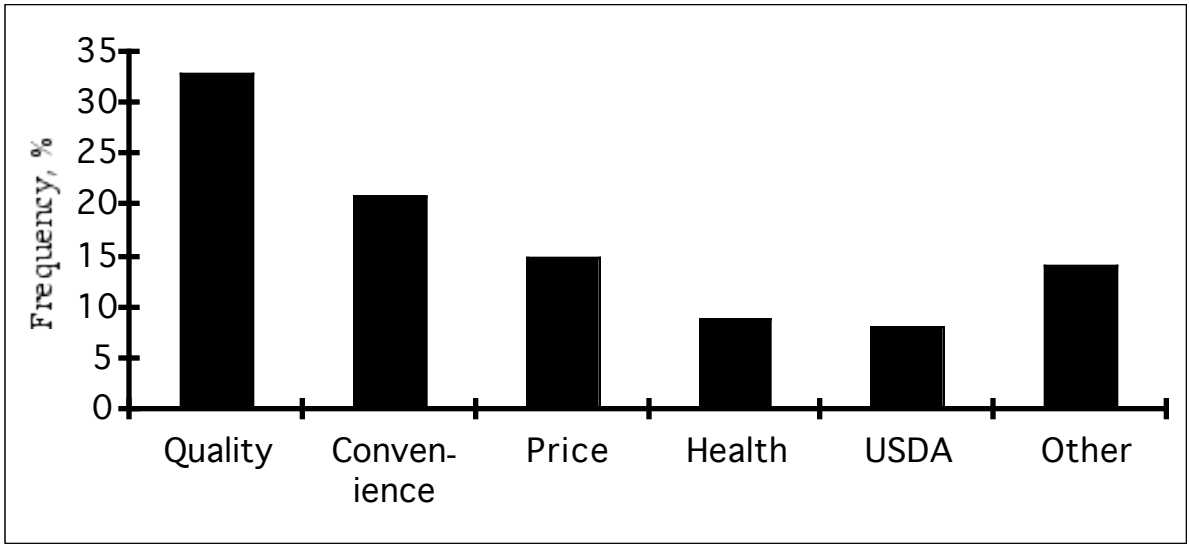


Figure 3

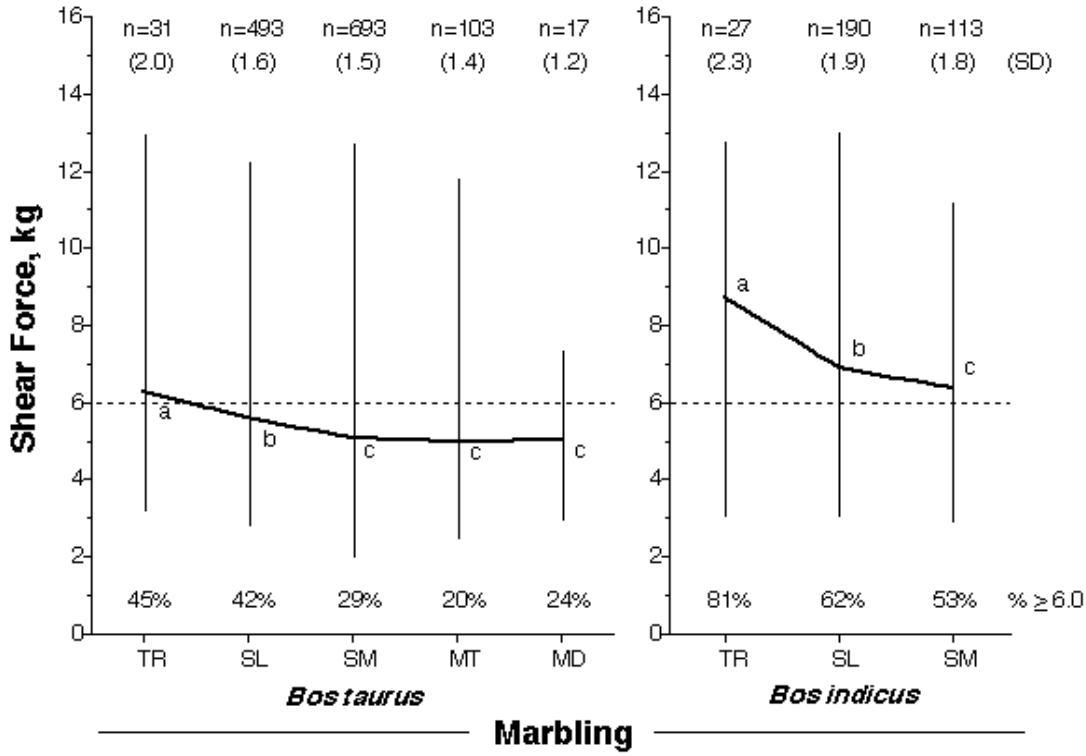


Figure 4

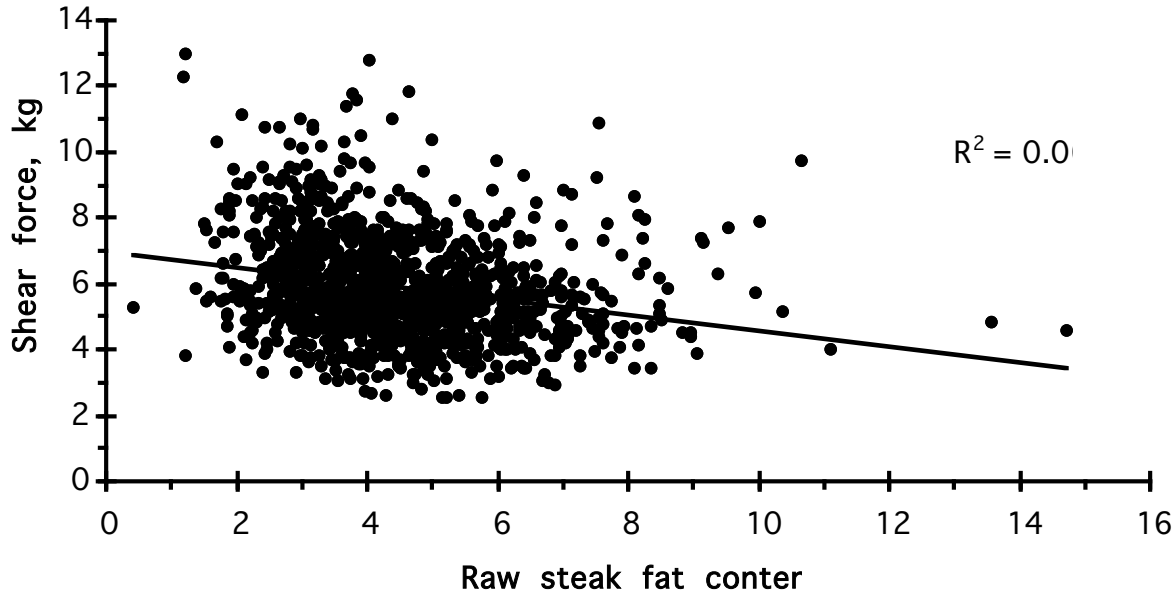


Figure 5

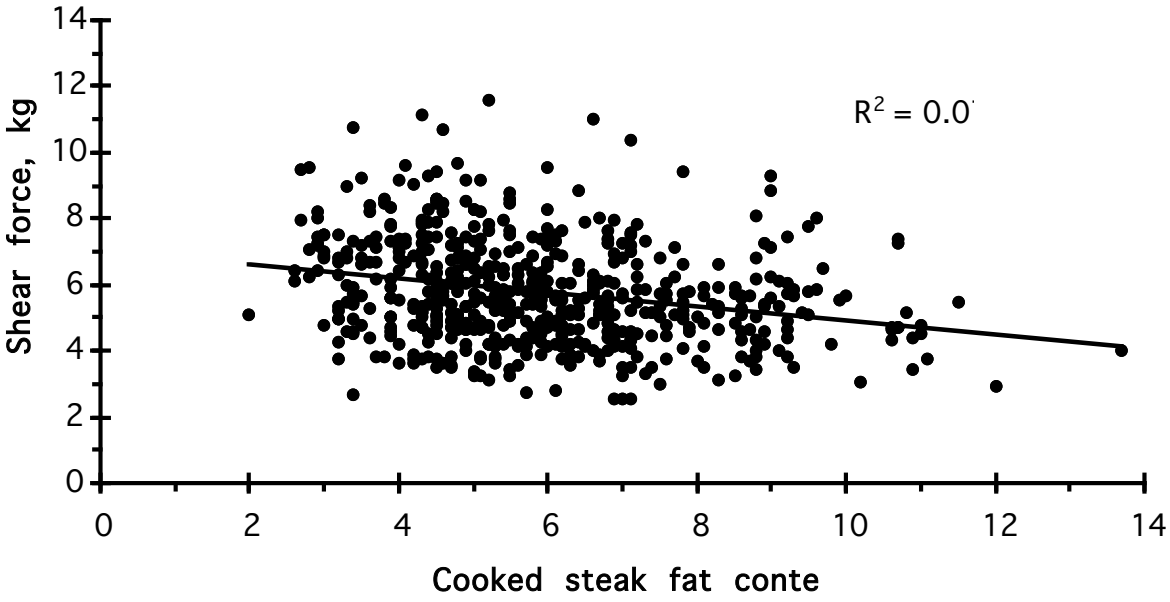


Figure 6

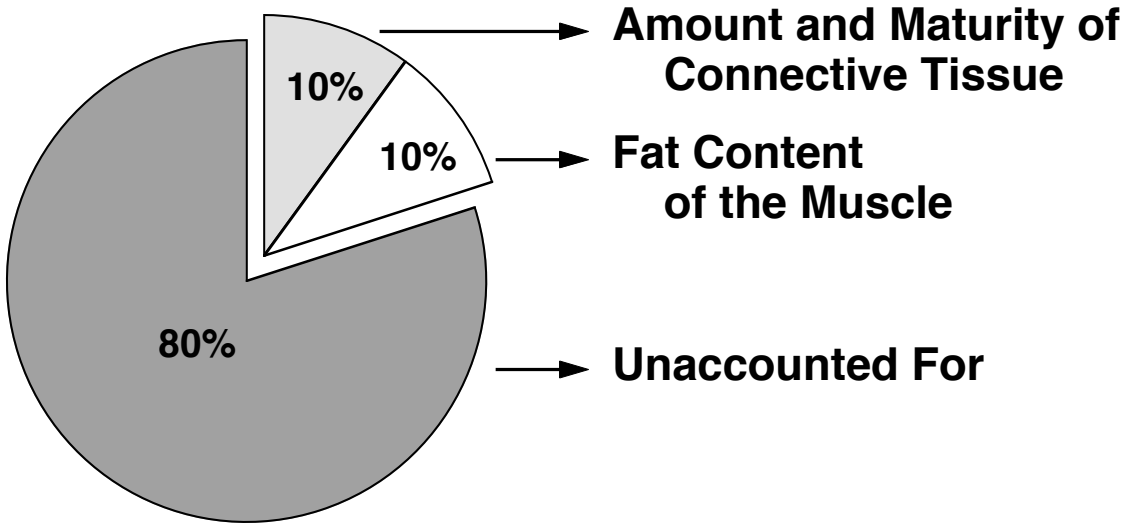
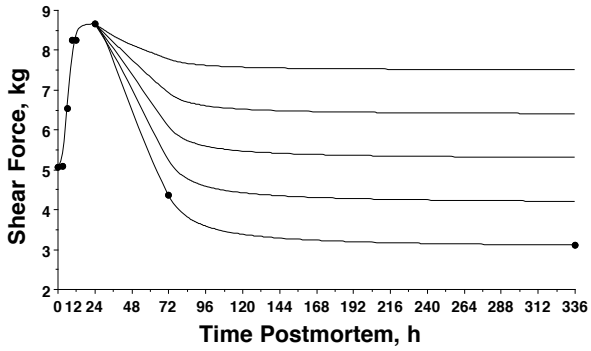


Figure 7



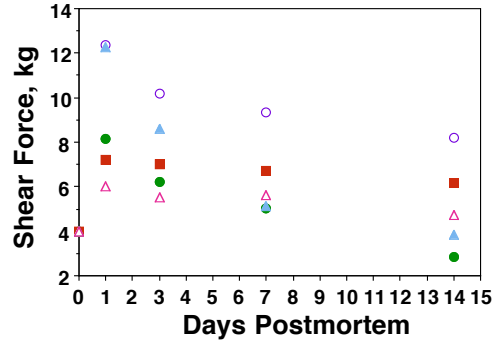


Figure 8

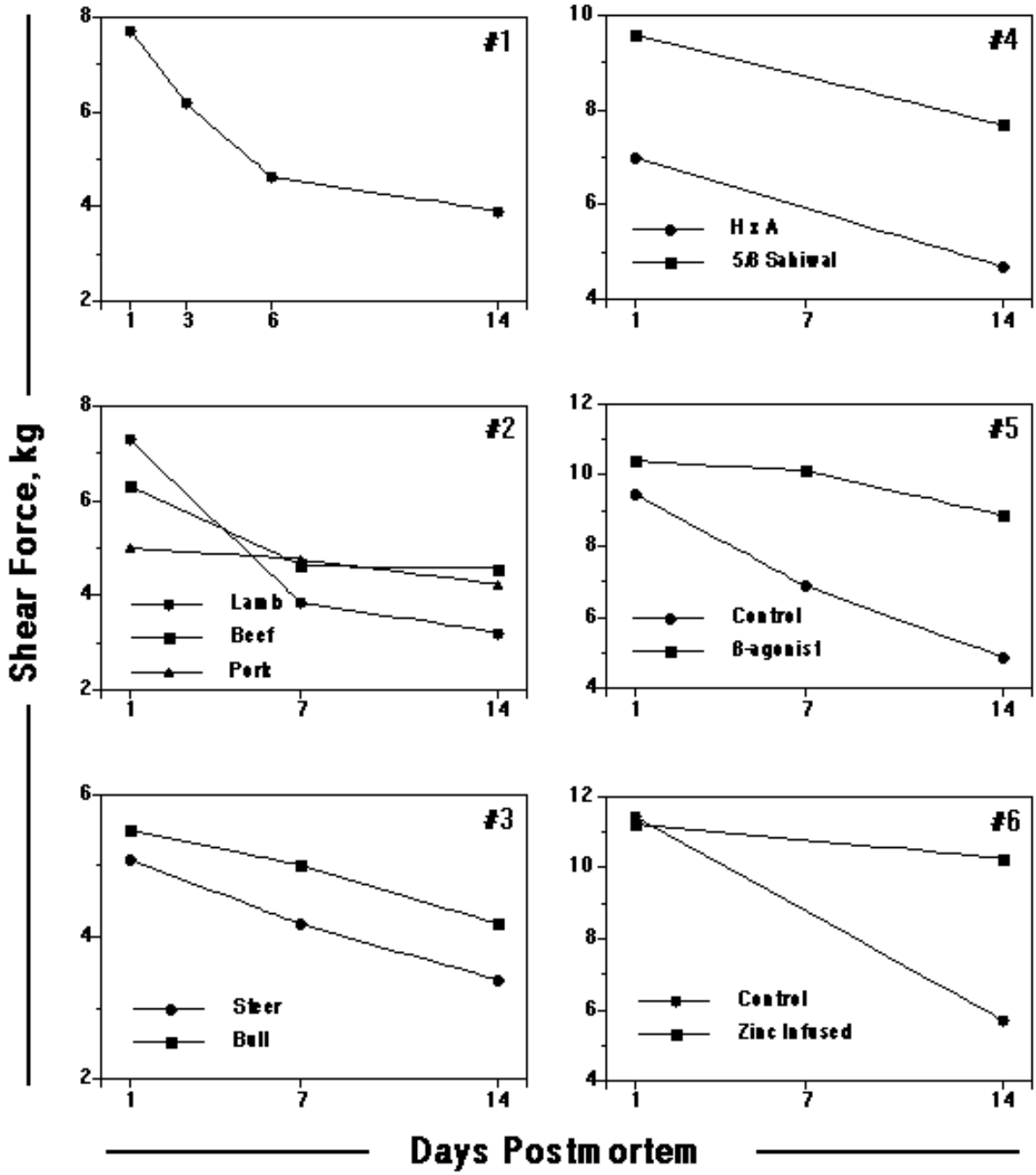


Figure 9

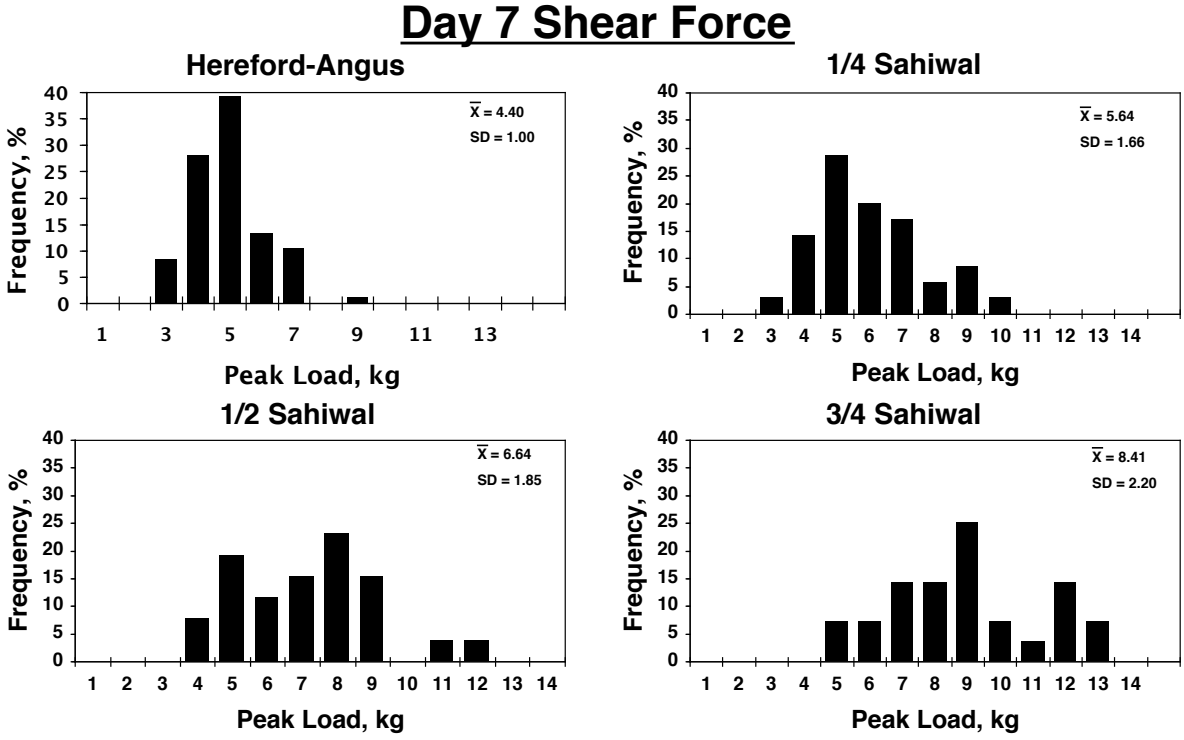

Day 7 Shear Force
Hereford-Angus
X̄ = 4.40
SD = 1.00
1/4 Sahiwal
X̄ = 5.64
SD = 1.66
1/2 Sahiwal
X̄ = 6.64
SD = 1.85
3/4 Sahiwal
X̄ = 8.41
SD = 2.20
Frequency, %
Peak Load, kg

Figure 10

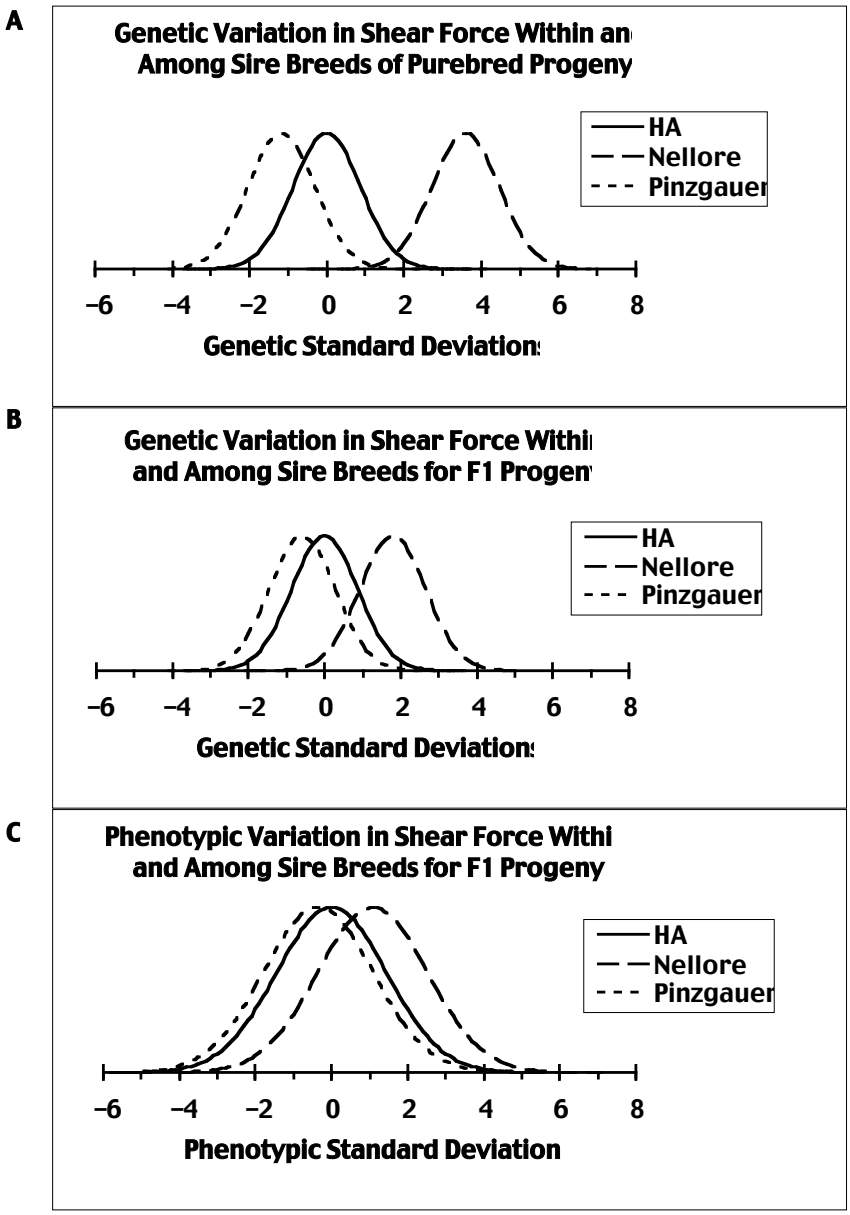

A
Genetic Variation in Shear Force Within an
Among Sire Breeds of Purebred Progeny
HA
Nellore
Pinzgauer
−6 −4 −2 0 2 4 6 8
Genetic Standard Deviation
B
Genetic Variation in Shear Force Withi
and Among Sire Breeds for F1 Progen
HA
Nellore
Pinzgauer
−6 −4 −2 0 2 4 6 8
Genetic Standard Deviation
C
Phenotypic Variation in Shear Force Withi
and Among Sire Breeds for F1 Progeny
HA
Nellore
Pinzgauer
−6 −4 −2 0 2 4 6 8
Phenotypic Standard Deviation

Figure 11

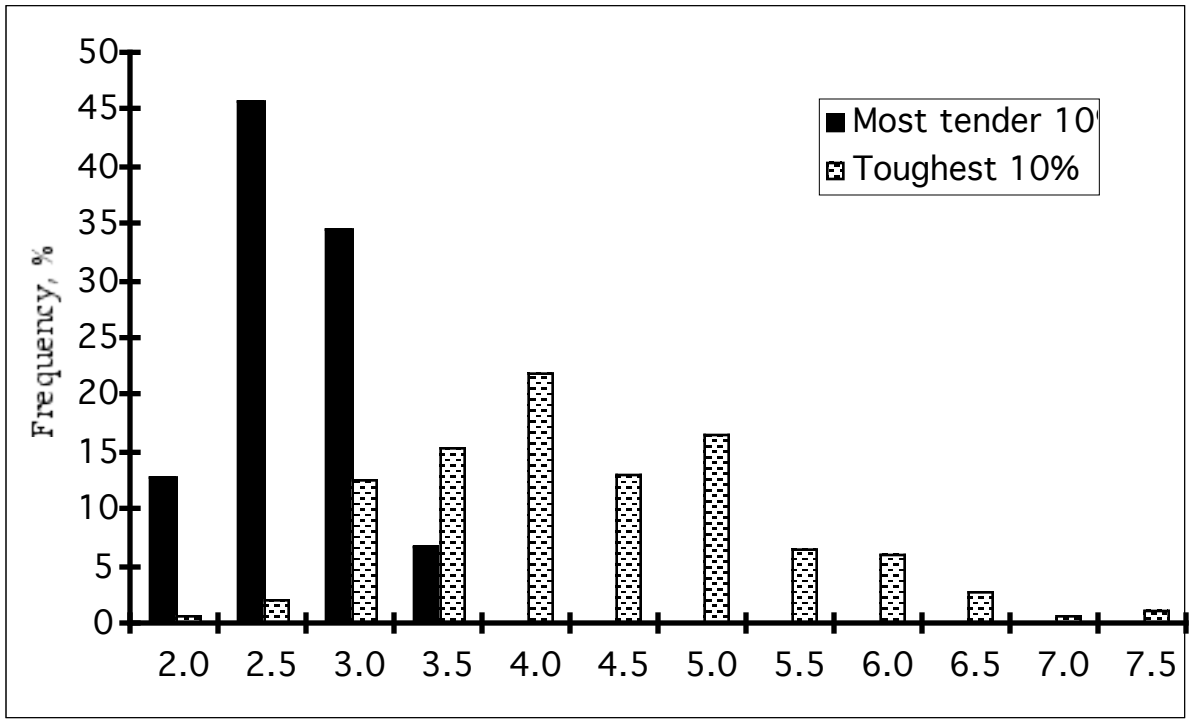


Figure 12

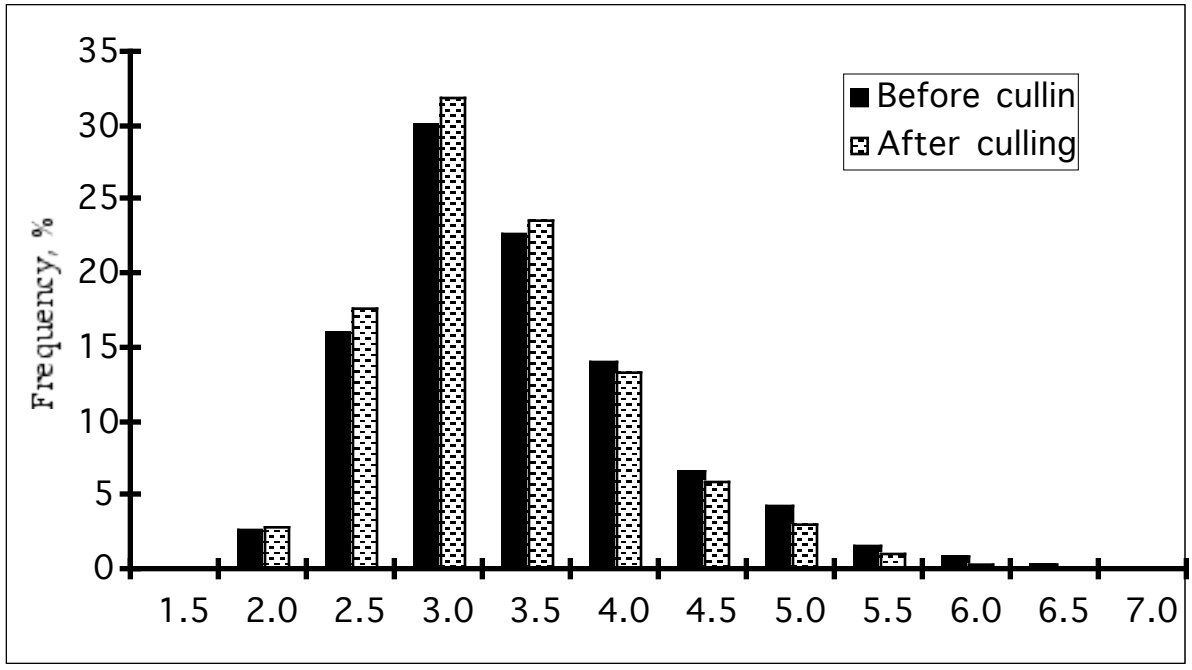


Figure 13

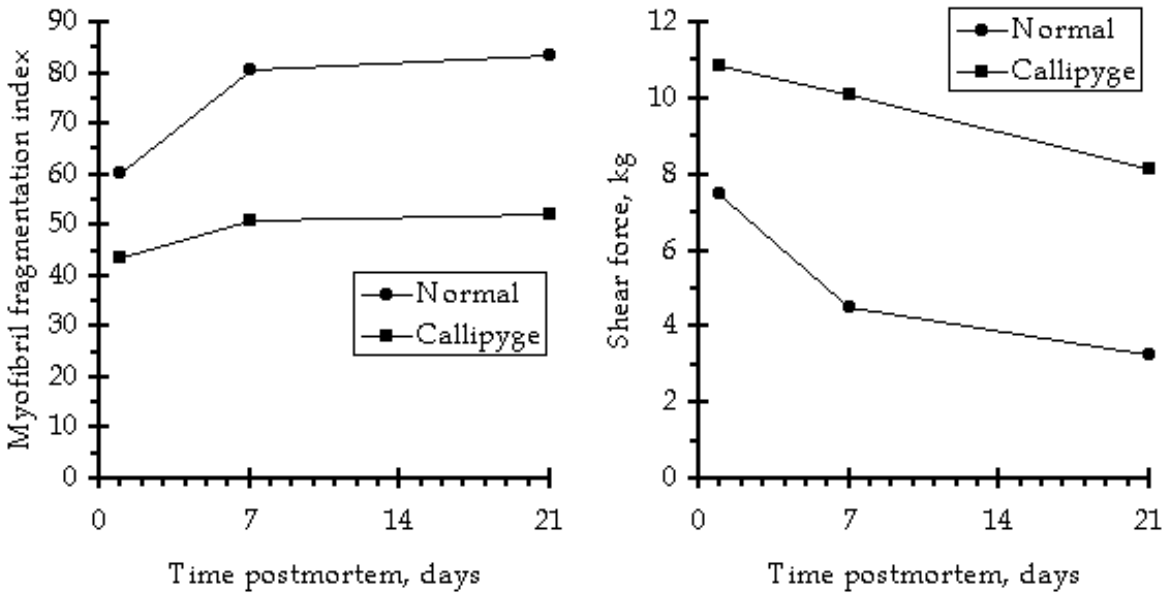


Figure 14

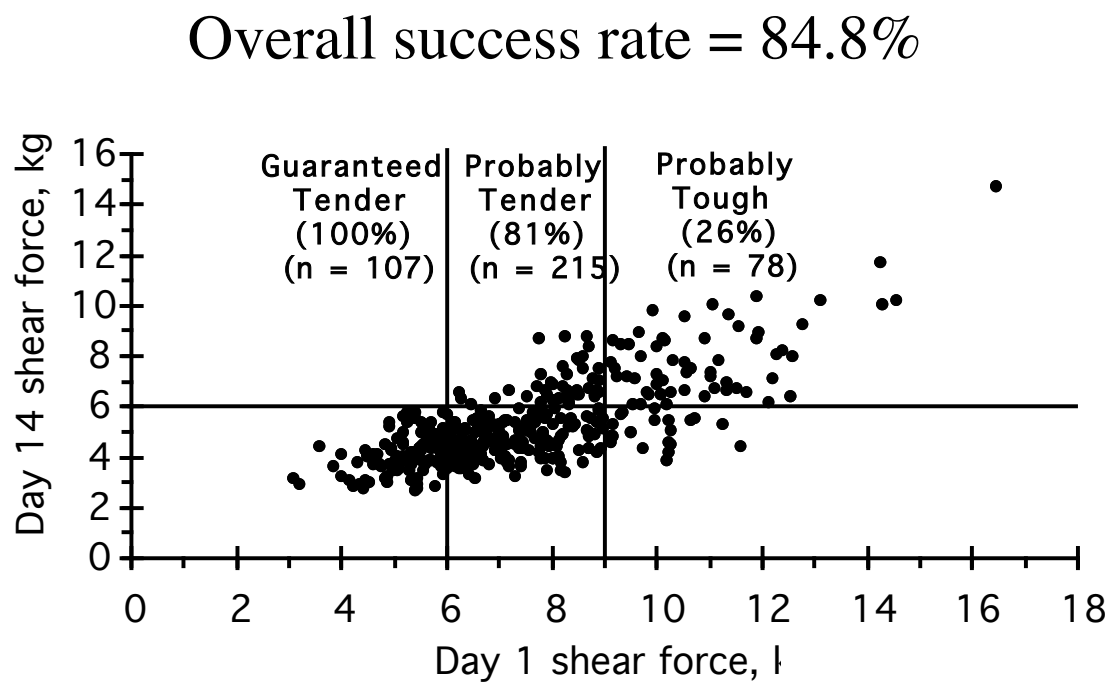


Figure 15

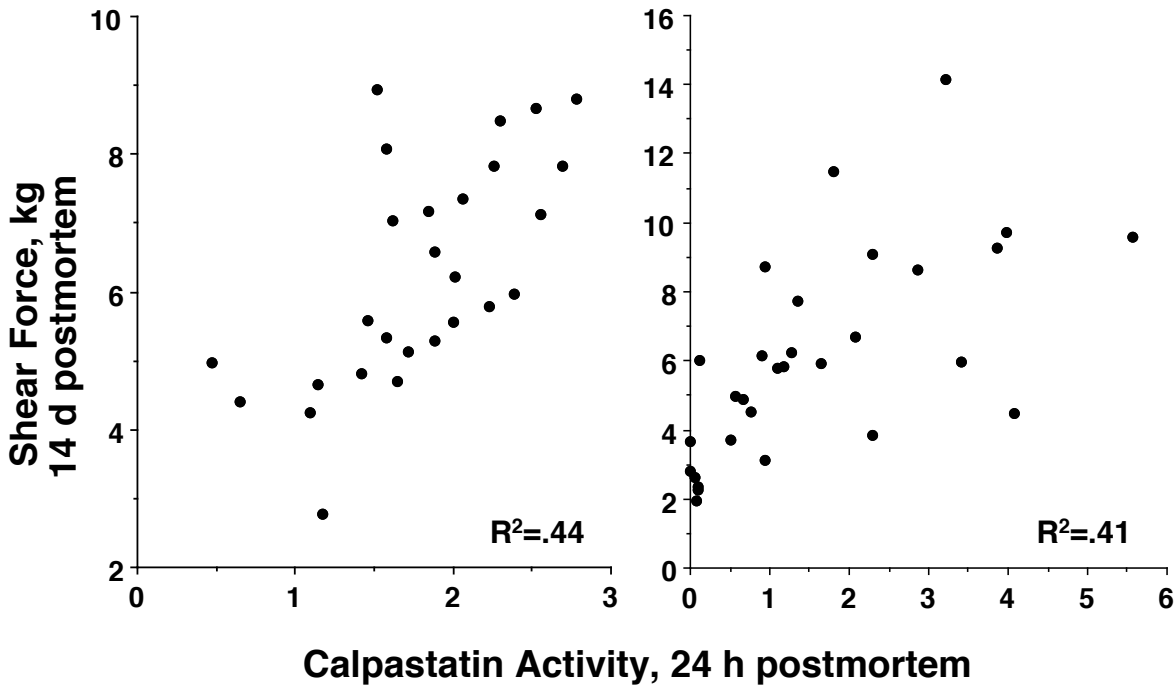


Figure 16

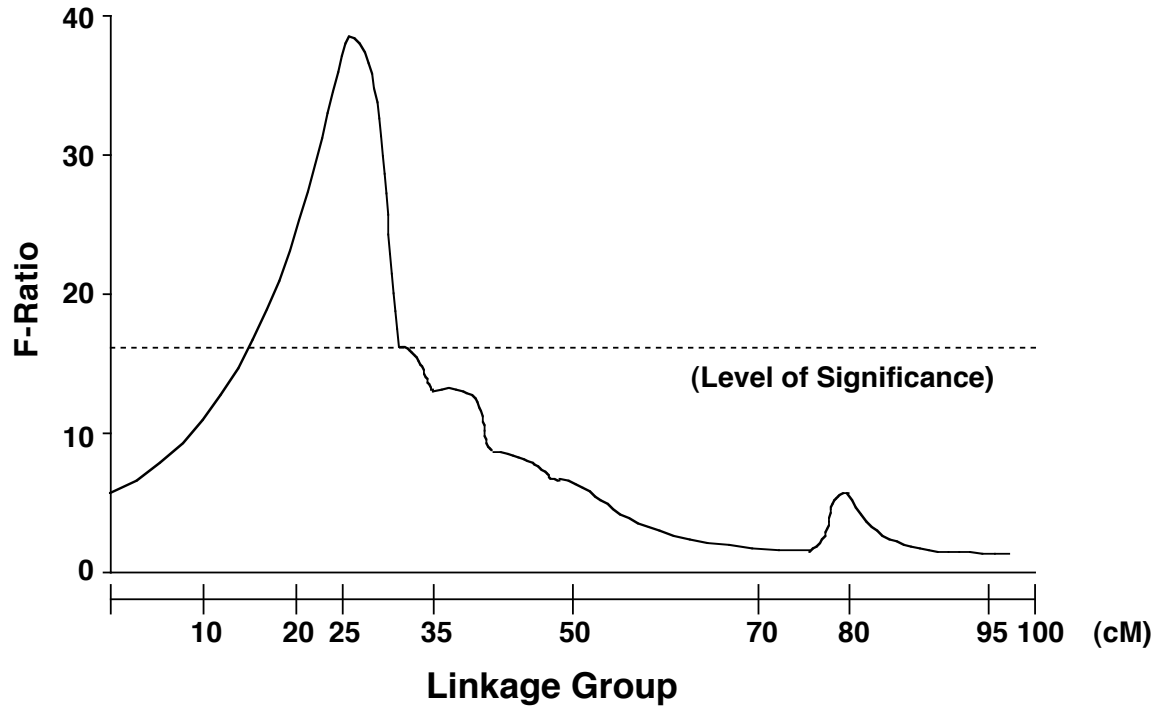


Table 1. Means and variation in palatability attributes of beef rib steaks at seven days postmortem

n = 1,667	Mean	Standard deviation	Coefficient of variation
Tenderness	4.77	.80	16.8
Juiciness	5.07	.41	8.1
Flavor intensity	4.82	.32	6.6

Table 2. Effect of postrigor calcium chloride injection on consumer evaluation of beef steak palatability

n = 1,001	Control	$CaCl_2$
Tenderness	5.1[b]	5.8[a]
Juiciness	5.8	5.9
Flavor desirability	5.8	6.0

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