TENDERNESS AND AGING RESPONSE OF BEEF MUSCLES OF DIFFERENT QUALITY GRADES BEFORE AND AFTER FREEZING

By

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CHAPTER I

INTRODUCTION

Tenderness is a quality of primary importance that is the least understood and the most variable, thus causing it to be the most studied quality of meat. In recent years, economic pressures have challenged the livestock and meat industries to seek ways of producing meat products that will enable consumers to receive maximum palatability benefits at the lowest cost. Postmortem aging of carcasses at refrigerated temperatures remains a major factor in improving tenderness and overall palatability of meat; however, many questions arise as to the length of aging to receive the maximum tenderness benefits. Tenderness differences occur between carcasses, between muscles within the same carcass, and within parts of the same muscle. Because of these differences, various muscles seem to react differently to postmortem storage. Smith et al. (1978) reported that aging of US Choice beef carcasses for 11 days will optimize tenderness, flavor, and overall palatability for the majority of muscles in steaks and roasts from the major cuts of the carcass. Tenderization during aging has been attributed to the proteolysis of myofibrillar proteins by calcium-dependent proteases (CDP) and cathespins. Calcium-dependent proteases have been associated with initial tenderness (within the first 24 hours); whereas, cathespins B and H improve the overall tenderness from 1-14 days of aging (Calkins and Seideman, 1988). The initial levels of CDP activities have also been related to the general aging response of muscles which improves the tenderness of meat. Koohmaraie (1986, 1987, 1988a,b) stated that the longissimus dorsi (ribeye) muscle had the highest CDP activity and the highest aging response, whereas, the psoas major (tenderloin) had the least CDP activity and the

lowest aging response. Olson et al. (1976) found the longissimus dorsi (ribeye) and semitendinosus (eye of round) muscles to progressively increase in tenderness over 14 days, while the psoas major (tenderloin) was unaffected during the aging period.

Postmortem aging remains an important procedure for producing tender meat; however, long aging periods are expensive as overhead increases, product loses yield due to evaporation, and microbial spoilage occurs. Packers are always looking for ways to reduce the holding time of carcasses without incurring a forfeit on quality. Understanding the causes of tenderness variation and potential minimization of aging requirements is of obvious economic importance. Currently, most packers are freezing and storing subprimals in warehouses, until further shipment, after three days of aging. Because of this trend, we questioned if freezing would affect the aging response of muscles. The fulfillment of this objective will help elucidate the practicality of aging subprimals or individual muscles after frozen storage.

CHAPTER II

REVIEW OF LITERATURE

Tenderness

Various factors contribute to the consumer acceptance of a meat product. Palatability, price, visual appearance, and availability of the product are a few; however, palatability is the most important to the acceptance of meat. The palatability of red meat has been a source for many research products and with the increased consumer demand for lean meat, palatability traits will be researched further. Tenderness, juiciness, and flavor are the main characteristics of a palatable product, but tenderness is the most closely monitored trait.

Tenderness of meat has been attributed to a multitude of different factors. Cattle breeds, postmortem aging, muscle type, quality grade, connective tissue, and cooking procedures are some traits commonly affecting meat tenderness. Because of the number of factors influencing tenderness, it is the most variable palatability trait in meat. It is essential that the meat industry learn to control the variation in products so it can provide a consistent retail product to the consumers who decide the future of this industry.

Postmortem Aging

Aging or "ripening" of beef has been a common practice for many years to assure a tender, acceptable retail product. Aging of beef is simply holding carcasses or wholesale cuts at refrigerated temperatures (32-40°F; 0-2°C) to allow "natural processes" to improve flavor and tenderness. An aging period of 2 weeks was

commonly used by packers to assure tenderness, however, today aging for extended time periods creates problems in merchandising and the use of storage facilities due to increased inventories of beef. Packers like Excel, Monfort, and IBP fabricate carcasses into subprimals after 3 days of aging. These subprimals are vacuum packaged, boxed, and stored in warehouses until exported or sold to retail chains. Morgan et al. (1991) conducted a national survey that summarized the time (d) for primal and subprimal cuts to arrive from warehouses to the various retail outlets. They found that the average post-fabrication time for all cuts was approximately 17 days. The minimum post-fabrication time was 3 days and the maximum time was 90 days. Because these subprimals are stored below freezing in the warehouse, the aging of beef is stalled, thus inhibiting the aging process. At retail outlets subprimals are stored at refrigerated temperatures that could provide an opportunity for the subprimals to age.

The correlation between increased tenderness and aging is well established (Davey and Gilbert, 1969; Minks and Stringer, 1972). Many studies in the past century have focused on aging beef at refrigerated temperatures to obtain the maximum tenderness within a short aging period. However, the actual aging period to achieve the maximum tenderness remains controversial and unresolved. A factor that may keep the controversy going is the response of muscles to aging. All muscles within a beef carcass seem to respond differently to aging thus affecting the overall tenderness of that muscle. Gothard et al. (1966) found the semimembranosus muscles of bulls, steers, and heifers to be consistently less tender than the longissimus dorsi muscles immediately after slaughter. However, these tenderness trends were normally reversed after 7 days. Semimembranosus muscles underwent considerable tenderization during aging, but final longissimus dorsi shear values differed very little from initial values. Field et al. (1971) found that the longissimus dorsi had a greater aging response than the biceps femoris which is similar to Semiek and Rileys (1974) findings for the longissimus dorsi, semitendinosus, and biceps femoris. It is evident that the longissimus benefits more

from aging than the biceps femoris and semitendinosus muscles. Not only do different muscles within the same carcass respond differently to the same aging period, but the same muscle from the same carcass reaches its maximum tenderness at different aging periods.

Martin et al. (1971) stated that a cooler aging of 6 days for young carcasses of all sizes, sexes, and degrees of fatness was sufficient to produce a consumer product of satisfactory tenderness. In agreement, Crouse et al. (1990) found the greatest improvement in shear force values (an objective measurement of tenderness) within the first 6 days of aging for the longissimus dorsi muscle from bulls and steers, whereas an additional 8 days of aging showed only marginal improvement. Minks and Stringer (1972) also indicated a slight improvement of the longissimus muscle aged from 7 to 15 days; however, they found a greater tenderization occurring within the first 7 days of aging. In contrast, a study conducted by Mitchell et al. (1991) evaluated the shear force values of the longissimus dorsi and found the steaks to be significantly more tender when aged 10 or 21 days compared to steaks aged for 3 days. They also observed a similar trend for the semimembranosus muscle; tenderness of the semimembranosus steaks improved with an increased aging period. Mitchell et al. (1991) concluded that there was little advantage in extending the aging period of the longissimus dorsi and semimembranosus steaks beyond 10 days of aging.

Smith et al. (1978) studied 20 muscles from USDA Choice carcasses and found 8 muscles to achieve minimal shear force after 5 to 8 days of aging; the other 12 muscles required 11 or more days of aging. The infraspinatus, longissimus dorsi, semimembranosus, and semitendinosus were a few of the major muscles requiring 11 days of aging to reach maximum tenderization; however, Culp et al. (1973) reported that tenderness did not improve beyond 8 days of aging for these 4 muscles. Parrish (1969) did not find a significant aging effect on shear force values of USDA Choice longissimus dorsi or semimembranosus steaks, but he observed shear force values for

longissimus dorsi steaks aged 11 days were 27.4% lower than those steaks aged for 4 days.

Prolonged aging beyond 14 days does not enhance tenderness according to Larmond et al. (1969), Martin et al. (1971), and Davis et al. (1975); however, Koohmaraie (1988a) reported that 14 days of aging greatly decreased shear force values for the longissimus dorsi but affected the psoas major muscle slightly. Olson et al. (1976) also found the psoas major to be characteristically different from the longissimus dorsi and semitendinosus muscles. The psoas major did not respond to aging to increase tenderness, but the longissimus dorsi and semitendinosus muscles progressively decreased in shear values over the aging period. The longissimus dorsi has also increased in tenderness, as indicated by shear values, from 2 to 21 days of aging (Field et al., 1971; Jennings, 1978); however, Culp et al. (1973) found shear force values to be lower for the longissimus dorsi, infraspinatus, semimembranosus, and semitendinosus muscles after 28 days of aging.

To improve tenderness more rapidly by the aging process, researchers have stored carcasses at higher temperatures (Parrish et al., 1969; 1973; Martin et al., 1971). Pierson and Fox (1976) found that the longissimus dorsi muscle aged for 3 to 5 days at 20°C was significantly more tender than the same muscle aged at 3°C for a similar period. Shear force values decreased linearly as aging time increased at 3°C; however, a significant quadratic effect between shear force and aging time was observed at 20°C with the lowest shear force occurring after aging for 5 days. Higher storage temperatures accelerate certain reactions in muscle cells which affects the tenderness of meat; however, bacterial growth and excessive shrinkage are major problems that may be encountered during high temperature storage.

Proteolytic Enzymes

Tenderization of meat during aging is largely attributed to the effects of proteolytic enzymes altering myofibrillar proteins (Locker, 1960; Parrish, 1973; Goll, 1974; Dutson, 1983; Goll et al., 1983; Tarrant, 1987). Numerous proteolytic enzymes are found in skeletal muscle, but only calcium-dependent protease (CDP) and certain cathepsins have been shown to degrade myofibrillar proteins. The individual CDPs, individual cathepsins, or the synergistic action of the two have been identified as being primarily responsible for the postmortem changes leading to meat tenderization. (Dutson, 1983; Pearson et al., 1983; Dutson and Pearson, 1985; Greaser, 1986; Asghar and Bhatti, 1987; Koohmaraie, 1988).

Calcium Dependent Proteases

Scientists use a variety of names to refer to calcium-dependent proteases (CDP). These names include: calcium-activated factor (Busch et al., 1972; Olson et al., 1977; Koohmaraie, 1984, 1986); calcium-dependent neutral proteinase (Vidalenc et al., 1983; Ducasting et al., 1985); calcium-activated protease (Suzuki et al., 1982); and calpain (Murachi, 1985).

Calcium-dependent protease were identified in skeletal muscle by Busch et al. (1972) and later purified by Dayton et al. (1976). Mellgren (1980) reported the existence of a second form of CDP; therefore, the two forms of the protease are referred to as CDP-I and CDP-II. A third component of the proteolytic system is an endogenous inhibitor (calpastatin or CDP inhibitor) that inhibits the activity of both CDP-I and CDP-II (Koohmaraie, 1988). These proteases and their inhibitor are located in the cytosol of the muscle cell and at the Z-disks where most changes occur in the muscle during postmortem storage (Dayton and Schollmeyer, 1981).

Four factors seem to influence the activity of muscle proteases - temperature, pH, free calcium concentration in the muscle cells, and the type of muscle. Temperature of the carcass falls at a rate depending on the size of the carcass and the holding environment. With forced chilling, enzyme activities decline rapidly, whereas for a slow cooling carcass enzymes maintain a higher activity.

A neutral pH keeps the calcium-dependent proteases active (Dayton et al., 1976), but their activity declines as the pH of muscle falls after slaughter (Etherington, 1984). Dayton et al. (1976) observed little activity of CDPs below muscle pH 6.0 and others have concluded that since muscle pH drops down to 5.5 within 24 hr postmortem, CDPs cannot be expected to bring about any marked changes in proteins (Etherington, 1984; Asghar and Bhatti, 1987). Koohmaraie et al. (1986) observed that CDP-I retained 24-28% of its activity under normal postmortem conditions (pH 5.5-5.8; 5°C) and he stated that this level of activity was sufficient to reproduce most of the known changes associated with the tenderization process during postmortem aging. In contrast, Calkins and Rhynalds (1989) found that the drop in muscle pH and temperature associated with aging reduces CDP-I activity by 93.6% from optimal pH and temperature conditions. The change from normal postmortem conditions does not affect CDP-II activity as Koohmaraie (1987) observed that CDP-II retained 80.2% of its original activity at 14 days of aging; however, calpastatin is susceptible to postmortem aging (Vidalenc et al., 1983; Koohmaraie, 1987) where only 20.7% of its original activity was present after 24 hr and by 6 days of aging its activity was practically eliminated. Ducasting et al. (1985) disagrees as their findings reported that 90% of the inhibitor's activity was present at 24 and 72 hr postmortem.

The third factor influencing the activity of CDPs is the free intracellular calcium concentration available to the muscle cells. The calcium ion concentration of the living muscle cell is normally maintained at about 10^{-8}M, with a transient rise to 10^{-5}M during a contraction (Etherington, 1984). After death, the calcium ion pump fails,

permitting the free ion concentration to be released from the sarcoplasmic reticulum (Calkins et al., 1987) and equilibrate through the tissue; thereby, activating the calcium requiring proteases in the muscle cell (10^{-4}M) (Etherington, 1984). The activation of CDP-I requires 50-70 µM calcium, whereas CDP-II requires 1-5 mM calcium for activation (Mellgren, 1980; Dayton et al., 1981; Szpacenko et al., 1981; Goll et al., 1983; Inomata et al., 1984; Karlsson et al., 1985). Intracellular free calcium concentration is thought to be only 1-10 µM but the calcium concentration increases gradually with increasing time of postmortem storage and may reach levels at which CDP-I can be activated (Goll et al., 1983). Calcium dependent protease-II, on the other hand, requires a higher level of calcium and the free intracellular calcium concentration would never reach this level, so CDP-II cannot be activated during postmortem storage. Koohmaraie (1987) concluded that improvement in tenderness resulting from postmortem storage must be derived from changes in the myofibrils and since CDP-I activities paralleled the myofibrillar changes, it was reasonable to suggest that CDP-I, not CDP-II, plays an important role in the fragmentation of myofibrils and consequently in improvement of meat tenderness resulting from postmortem storage.

The type of muscle also seems to be related to postmortem CDP activity. Olson et al. (1977) and Koohmaraie et al. (1988a) observed that the longissimus dorsi and semitendinosus muscles had a higher CDP-I activity compared to the psoas major which indicated a lower CDP-I activity. According to Koohmaraie (1986, 1987, 1988a,b,c) the higher initial CDP-I activities may determine the aging response of muscles as these studies found that CDP-I activities followed the same general pattern as the aging response of muscles; the longissimus dorsi had the highest CDP-I activity and also had the highest aging response, whereas the psoas major had the lowest aging response as reflected by the lowest CDP activity. Unlike Koohmaraie's results, Etherington et al. (1987) reported no relationships between muscle aging rate and the activities of calcium-dependent proteases.

The increase in tenderness associated with postmortem aging of meat has been attributed to the endogenous proteolytic enzymes in muscle. Calkins et al. (1987) found initial shear force values between the time of slaughter and day 1 postmortem correlated to CDP-I activity thus suggesting that CDP-I helps to establish initial (day 1) meat tenderness. A lower correlation between CDP-I and day 3 shear force values is probably a reflection of reduced CDP-I activity and suggests that other enzymes besides CDP-I are working after day 1. Johnson et al. (1990) found that CDPs had no relationship with Warner-Bratzler shear values; however, calpastatin was positively related to day 1 shear values. Whipple et al. (1990) reported a strong positive correlation for inhibitor activity at 24 hr postmortem and stated that 44% of the variation in Warner-Bratzler shear force values was explained by calpastatin activity at day 1. During the first 24 hr, the amount of inhibitor activity could play a major role in muscle proteolysis by regulating CDP action and limiting activity, thus inhibiting improvement in tenderness. Calpastatin activity at day 1 within the longissimus dorsi has been shown to be significantly related to the tenderness among breeds, gender subclasses, and within the gender subclasses of cattle (Whipple et al., 1990). Johnson et al. (1990) reported that calpastatin activity was greater in 3/4 Brahman than in Angus and 1/2 Brahman carcasses. A reduced rate of myofibrillar protein degradation, due to proteolysis, during postmortem storage is reported to be a major reason for reduced tenderness of meat from Bos indicus beef compared to Bos taurus breeds of beef (Wheeler et al., 1990; Whipple et al., 1990 a,b; Shackelford et al., 1991). Besides breed differences, calpastatin activity at 24 hr has been associated with higher shear force values of meat from bulls (Morgan et al., 1991) thus inhibiting CDP-I and CDP-II during aging to improve meat tenderness. However, in frozen storage calpastatin has been found to be unstable whereas CDP-I and CDP-II are not affected (Koohmaraie, 1990a; Whipple and Koohmaraie, 1991). Freezing meat from bulls and Bos indicus

breeds of cattle will inhibit calpastatin activity and may allow CDP-I and CDP-II to improve meat tenderness by aging meat after frozen storage.

Cathepsins

A second group of proteolytic enzymes that have been implicated in postmortem tenderization are lysosomal enzymes. Thirteen lysosomal enzymes have been reported, but only seven have been shown to exist in skeletal muscle (Goll et al., 1983). The main lysosomal enzymes involved in degrading myofibrillar proteins and enhancing meat tenderness are cathepsins B, H, and L.

Cathepsins B, H, and L have a pH optima in the acid range (3.0-6.0) and are found within the lysosome, (small vesicles located in the sarcroplasm,) of skeletal muscle. Because the cathepsins are bound or enclosed within the lysosome, their release is a prerequiste to initiate degradative changes in meat. Low pH and high temperature cause the lysosomal membrane to rupture (Sorimade, 1982) which releases cathepsins into the intermyofilament space of muscle to begin protein degradation on the myofibrillar proteins (Greaser, 1986); however, the cathepsin's ability to hydrolyze these proteins is dependent on their release which may not occur after three weeks of aging (LaCourt et al., 1986).

One of the first cathepsins observed was cathepsin B which was originally isolated from liver lysosome (Greenbaum and Fruton, 1957). Cathepsin B requires an optimum pH between 3.5-6.0 for activity (Asghar and Bhatti, 1987) to degrade myosin and actin. Bird (1977) found cathepsin B to maintain about 60-70% of its total activity at pH 5.5, which is the general ultimate pH of postmortem muscle. In contrast, cathepsin H exhibits activity at pH 7.0 to degrade myosin (Etherington, 1984). Because myosin, a major myofibrillar protein, is degraded by cathepsin B and H at an

acidic and neutral pH, these enzymes could enhance tenderness during postmortem storage.

Calkins et al. (1987) observed the shear force of the longissimus dorsi muscle from bull and steer carcasses as affected by the activities of cathepsin B and cathepsin H. They found that the overall change in shear force (day 1-14) was correlated to cathepsin B activity, but the change in shear force from day 3 to 6 was related to cathepsin B and H total acitivities. Cathepsins B and H increase tenderness of meat after one day of postmortem aging but are not the only enzymes to degrade muscle proteins during the aging process. Ouali et al. (1987) and Wheeler et al. (1990) stated that cathepsin B and L activities could be related to postmortem tenderization of meat since cathepsin L has maximum activity at pH 4.0-6.0 (Dutson, 1983), like cathepsin B. Johnson et al. (1990) reported that cathepsin B+L activity in the longissimus dorsi muscle was negatively related to Warner-Bratzer shear force values at day 10 implying that increasing levels of cathepsin B+L total activity is associated with increased tenderness in response to aging. Etherington et al. (1987), however, reported no relationships between muscle aging rate and the activities of several lysosomal enzymes.

Similar to calcium dependent protease activities, breeds of cattle differ in catheptic enzymatic activities which influences the rate of aging and improvement in tenderness. Johnson et al. (1990) found that the longissimus dorsi from Angus carcasses had a greater B+L activity and had a larger decrease in Warner-Bratzler shear force values in response to the 10 day aging period compared to carcasses with Brahman breeding. Unlike the calcium dependent proteases, the activity of lysosomal enzymes is basically similar in the longissimus dorsi, semitendinosus, and psoas major muscles regardless of the aging response (Koohmaraie, 1988a). Also, other research studies failed to find differences in catheptic enzymatic activities in muscle with varying levels of tenderness (Koohmaraie, 1988a,c; Whipple 1989a,b).

Muscle Location

Meat is not a homogeneous material and the variation between different muscles of the same animal and between the same muscles from different animals is of great importance when planning experiments. Many studies have shown much evidence in the considerable amount of variation in tenderness of muscles in different anatomical locations (Ramsbottom et al., 1945; Hiner and Hankins, 1950; Paul and Bratzler, 1955; Ginger and Wier, 1958; Kent, 1963; Zinn et al., 1970; McKeith et al., 1985; Morgan et al., 1991). Seideman et al. (1989) stated that various muscles within a carcass vary substantially in sensory properties as a result of postmortem aging and the various meat compostion and characteristic properties are related to ultimate tenderness. Their study found the psoas major, from bull and steer carcasses of various marbling scores, to be the most tender muscle as indicated by a sensory panel and shear force values; the least tender muscle for the sensory panel was the semimembranosus muscle, but the Warner-Bratzler shear (WBS) showed the longissimus dorsi to have the highest shear force values. A study conducted by Hiner and Hankins (1950) on steer carcasses within the USDA Good quality grade found the psoas major to be the most tender muscle, as indicated by WBS, while the wholesale round muscles (semimembranosus, semitendinosus, and biceps femoris) were the least tender. Ramsbottom and Strandine (1948) compared 50 muscles from 3 "US Good" heifer carcasses and also found the psoas major to be the most tender muscle while the semimembranosus and semitendinosus muscles were the least tender for sensory and shear values, agreeing with Morgan et al. (1991) who observed the same results. The infraspinatus (top blade steak) has been identified by some researchers as the most tender muscle within the wholesale chuck (Ramsbottom and Strandine, 1948; Smith et al., 1978; McKeith et al., 1985; Paterson and Parrish, 1986; Morgan et al., 1991). These studies also reported the infraspinatus to be lower in shear values than the

longissimus dorsi muscle from the rib and loin subprimals; however, the psoas major is the only muscle to be more tender than the infraspinatus.

A considerable amount of variation in tenderness of muscles from different locations within the carcass may be influenced by the connective tissue content in individual muscles. According to very early work by Lehmann (1907), the mechanical strength of a muscle is directly proportional to the amount of connective tissue present; the most active muscles which are subject to the greatest strains have the largest amounts of connective tissue and are the least tender. Individual muscles have been shown to differ in connective tissue by Ramsbottom et al. (1945), Ramsbottom and Strandine (1948), Ritchey and Hostetler (1964), Prost et al. (1975), and McKeith et al. (1985). All these studies showed that the level of connective tissue was lowest in the psoas major and the highest in the infraspinatus muscles. Furthermore, the muscles of the forequarter of the carcass contain more connective tissue than the muscles from the hindquarter.

Not only do muscles vary within anatomical location, studies also indicate that within a given muscle shear force values vary from end to end (Ginger and Wier, 1958; Paul and Bratzler, 1955) and from location to location (Alsmeyer et al., 1965; Hedrick et al., 1968). Ramsbottom et al. (1945) removed sections of the psoas major and longissimus dorsi and cut each section from either end and the middle of the representative muscle for the purpose of making direct comparisons on differences in tenderness. They found the longissimus dorsi to be less tender at the anterior ends of the muscle, but the psoas major was uniformly tender throughout the section. Unlike Ramsbottom's findings, Martin et al. (1971) found that samples from the rib had considerably lower average shear values than loin samples. This is in accord with reports by Martin et al. (1970) and Harrison et al. (1949) indicating an anterior to posterior falling gradient in tenderness of the longissimus dorsi muscle in bull and steer carcasses. For the wholesale round, Paul and Bratzler (1955) found the

semimembranosus muscle to be significantly different due to position. They found that the first and second steak removed from the anterior position of the semimembranosus to be more tender than the center portion, whereas the last three steaks removed from the posterior end were the least tender.

Muscles also vary within location of the cores or samples removed within the same steak. Tuma et al. (1962), Alsymeyer et al. (1965) and Walter et al. (1965) found cores removed from the medial and dorsal portion of the longissimus dorsi to be more tender than cores from lateral postions. McBee and Wiles (1967) removed steaks from the 3rd lumbar and found that the dorsal postion had a significantly lower mean shear force value than the other two locations. There was no significant difference in shear force values between the medial and lateral locations, although the medial location had the highest mean shear force value.

Tenderness variability within a muscle may also be due to the different heating rates and a considerable variation in internal meat temperature at the cessation of cooking (Shin et al.,1993). A particular internal temperature also differs for muscles within the same carcass (Bramblett et al., 1959; Cheng and Parrish, 1976; Locker and Daines, 1976) and may have a greater effect on palatabilty than marbling or aging (Cross et al., 1988). Meat becomes drier and less tender as the internal temperature of meat increases, thus decreasing its overall acceptance.

Marbling

Beef tenderness may be influenced by carcass quality grade, particularly marbling. USDA beef quality grades are designed to indicate expected palatability or acceptability of meat after cooking and are used to provide consumers a reliable guide for identifying beef quality levels (Smith, 1980). Quality of lean in beef is evaluated by considering its marbling and firmness in a cut muscle surface in relation to the

physiological maturity of the carcass (USDA, 1989). Marbling, or intramuscular fat, is evaluated for the amount and distribution within the longissimus dorsi (ribeye) muscle between the 12th and 13th ribs (USDA, 1989). The degrees of marbling, in descending order of amount, are: abundant (AB), moderately abundant (MA), slightly abundant (SA), moderate (MD), modest (MT), small (SM), slight (SL), traces (TR), practically devoid (PC), and devoid (D).

Marbling has traditionally been thought to affect palatability (Tatum et al., 1982), however, that theory has been difficult to prove. Many researchers have reported that tenderness, juiciness, and flavor increase with increasing degrees of marbling in a direct, linear relationship (McBee and Wiles, 1967; Jennings et al., 1978; Tatum et al., 1980; Dolezal et al., 1982), whereas others have reported very low or nonexistent associations (Carpenter et al., 1972; Parrish et al., 1973; Parrish, 1974; Dikeman and Crouse, 1975; Davis et al., 1979; Smith et al., 1984). Romans et al. (1965) found loin steaks containing moderate degrees of marbling to be juicier than steaks possessing slight marbling; although marbling level did not have a significant effect on tenderness as determined by the Warner-Bratzler shear. Breidenstein et al. (1968) also found that marbling level of the longissimus dorsi and semimembranosus muscles did not statistically affect shear force, but the abundant marbling level had the lowest shear force values. It is interesting to note that the modest marbling group of the longissimus dorsi muscle appeared to be "out of line" in that it showed a higher shear force and lower panel tenderness in comparison to the moderate and small marbling scores. For chuck muscles, Choi et al. (1987) found that quality grade did not significantly affect Warner-Bratzler shear force values, but chuck muscles from USDA Choice carcasses had lower shear values than those carcasses from USDA Good. Unlike the former results, many studies have shown that increases in marbling content of loin steaks were significantly associated with lower shear values (Jennings et al.,1978; Tatum et al., 1980; Dolezal et al., 1982; Berry et al., 1993). Smith et al.

(1984) stated that marbling is of very limited value in explaining differences in sensory panel ratings of round steaks compared to loin and rib steaks. For the variation in tenderness, marbling has been accounted for 6.9% (Alsmeyer et al., 1959), 10% (Cover et al., 1958), and 11% (Palmer, 1958). Jeremiah et al. (1970) concluded that marbling was associated with 2-16% of the variability in flavor, juiciness, tenderness, and overall palatability; whereas, Blumer (1963) found that marbling explained 6.8% of the variability in tenderness and about 16% of the variation in juiciness.

Composition

Different muscles have different biochemical and histological properties because of differences in physiological function. It is possible that the textural properties, like tenderness, of muscles is determined by the constituents and arrangement of tissue. Muscles are analyzed by the proximate analysis procedure to determine moisture, fat, and protein content. Seideman et al. (1989) stated that various meat composition and characteristic properties of different muscles within the same carcass are related to ultimate tenderness; however, Johnson et al. (1988) found that the relationship between Warner-Bratzler shear force values and the proximate analysis traits of percent moisture, fat, and protein for different chuck muscles were very low.

Tenderness of meat is partly influenced by the marbling score of carcasses and many studies have reported a strong relationship between marbling and percent fat within muscles (Cole et al., 1960; Tuma et al., 1962; Breidenstein et al., 1968; McBee and Wiles, 1967; Campion et al., 1975; Choi et al., 1987; Brackebusch et al., 1991b). As intramuscular fat content increases with increasing marbling scores, an inverse relationship occurs between marbling score and moisture content. Garrett and Hinman (1971) noted that as fat content increased, the water content decreased for the infraspinatus, longissimus dorsi, and semimembranosus steaks as marbling level

increased. Brackebusch et al. (1991b) found the longissimus dorsi marbling level linearly related to percent fat of the infraspinatus, supraspinatus, longissimus dorsi, psoas major, semimembranosus, and semitendinosus muscles; a similar relationship was observed between marbling score and percent moisture for the muscles. Cole et al. (1960), Tuma et al. (1962), McBee and Wiles (1967), and Brackebusch et al. (1991b) observed that the protein content of major muscles tends to decrease with inceasing marbling score, a similar response to moisture levels.

The composition of muscles vary, regardless of marbling, for proximate analysis traits. The psoas major tends to have the highest percent of ether extractable material compared to other major muscles (Ramsbottom and Strandine, 1948; Hunt and Hedrick, 1977; Seideman et al., 1989); however, McKeith et al. (1985) and Brackebusch et al. (1991b) showed the infraspinatus to contain a higher percentage of fat than the psoas major. For all chuck muscles evaluated, the infraspinatus rates the highest in fat content (McKeith et al., 1985; Choi et al., 1987; Cecchi, 1988; Johnson et al., 1988; Brackebusch et al., 1991b). The higher fat level in the psoas major and infraspinatus tends to improve the subjective and objective measurement of tenderness and overall palatability. The semimembranosus and semitendinosus muscles show the lowest fat content compared to chuck muscles and those muscles associated with posture. The moisture and protein content are lower in the psoas major and infraspinatus muscles compared to the longissimus dorsi, supraspinatus, and round muscles (Ramsbottom and Strandine, 1948; Hunt and Hedrick, 1977; McKeith et al., 1985; Brackebusch et al., 1991b); it is not suprising that the round muscles show the highest percentage of moisture and protein.

Freezing

Freezing is often necessary for meat products that must undergo transcontinental and transoceanic distribution or must be purchased far in advance of consumption as a result of price, supply, and demand. The effect of freezing on meat quality, mainly tenderness and moisture loss, has been studied extensively but results have been inconclusive. Much of the earlier research on freezing promoted the view that various freezing treatments increased the tenderness of meat (Hankins and Hiner, 1940; Hiner and Hankins, 1941; Hiner et al., 1945; Hiner et al., 1951, Hiner and Hankins, 1951). Hankins and Hiner (1938) and Hiner et al. (1945) reported that beef samples increased consistently in tenderness as freezing temperatures were lowered; however, Pearson et al. (1950) reported that the rate of freezing did not influence tenderness or palatability. Tenderness of beef samples aged for 5, 15, 25, and 35 days at 0.6 to 1.7°C became more tender and tenderness was further increased by subsequent freezing (Hiner and Hankins, 1941), but Bray et al. (1942) found that freezing had no effect on tenderness of beef aged for an unreported period of time before freezing. Locker et al. (1975) concluded that quick-freezing of meat before aging is responsible for pronounced toughness and recommended conditioning and aging the meat before freezing. Quick freezing, or fast freezing, causes the water inside the cells to be frozen into small ice crystals. The tiny ice centers are dispersed thoughout the muscle substance, being held apart by proteins and other cellular material, but does not damage the cell walls (Nord, 1936; Hiner, 1944; Hiner and Hankins, 1947) Hiner (1944) concluded that as the rate and degree of freezing increased, with decreased temperatures, it appeared that there was less time or opportunity for the transfer of water into the spaces between cells, thus causing less damage to the cell wall. Quick freezing increases the tenderness of meat (Hiner et al.,

1945; Hiner and Hankins, 1947) and reduces shear force values (Berry and Leddy, 1989).

Slow freezing promotes the formation of large ice crystals between muscle fibers which creates more structural damage to the cell walls (Kuprianoff, 1952; Drozdov, 1955; Bevilacqua et al., 1979). If damage has been done to the cell wall during freezing, less water will be reabsorbed during thawing and a greater amount of exudate is released. The amount of exudate arising during thawing is an important quality or detriment of frozen meat.

Beef muscle contains approximately 70-75% water, most of it being within the fibers which are closely held together by interstitial connective tissue. A loss of moisture and water-soluble components such as protein, vitamins, minerals, or flavor components are of great economic importance. Freezing causes an extra loss of water in comparison with corresponding unfrozen samples, however the latter depends on the physical conditions during postmortem storage (Kuprianoff, 1952; Hicks et al., 1956). During postmortem storage meat can lose considerable weight due to evaporation, but as meat ages for long periods of time, less "drip" is observed and the meat has a greater capacity of absorbing water, or a greater water holding capacity. Hamm (1960) stated that to avoid a loss of water holding capacity during freezing and thawing, the meat should be frozen in a state of high hydration which is after postmortem aging. Unsuprisingly, some reports have observed that the drip losses of frozen aged meat are much less than those of frozen rigor muscles (Wierbicki et al., 1957; Bouton et al., 1958; Sleeth et al., 1957, 1958) because the aging of meat results in an increase of its water holding capacity. Deatherage and Hamm (1960) found that slow freezing (-15°C) causes a significant decrease in water holding capacity of thawed meat, whereas quick freezing results in a small but significant increase of meat hydration.

The influence of frozen storage on the tenderness of meat is very controversial, but there is reasonably good agreement that drip loss tends to increase and yield to

decrease with frozen storage. Mitchell et al. (1991) found that the frozen semimembranosus steaks had higher shear force values and that the thawed longissimus dorsi and semimembranosus steaks had an increased thaw loss which was reflected in lower juiciness ratings for these steaks. Cooking loss, another form of moisture loss, is a result of denaturation of the proteins and is influenced by the temperature, time of cooking, and water holding capacity of the meat (Locker and Daines, 1974). Postmortem glycolysis lowers the water holding capacity (Lawrie, 1968) and aging improves it (Parrish et al., 1969). Cohen (1984) assumed that meat after aging will have less cooking loss; however, his findings indicated that changes induced by freezing probably have more influence on cooking loss than the extent of aging, as observed by myofibrillar fragmentation index.

Warner-Bratzler Values

The current US beef population varies extremely in tenderness. A trained panel identifies tenderness subjectively, which can differ widely with in the group. Warner-Bratzler shear (WBS) force values, however, provide an objective assessment of tenderness. The relationship of WBS force to consumer acceptability has not been researched thoroughly and the value of research to improve meat tenderness hinges upon establishing the relationship of tenderness with consumer purchasing decisions. Shackelford et al. (1991) reported that WBS values of top loin steaks should not exceed 3.9 kg for a 68% confidence level and 4.6 kg for a 50% confidence level to assure overall tenderness ratings of "slightly tender" or greater from a trained sensory panel. A single WBS threshold cannot be applied to all types of retail cuts or consumer markets (retail vs. foodservice). Morgan et al. (1991) compared chuck, rib, and loin cuts to the 68% confidence level for tenderness. Interestingly, the top blade steak and

chuck eye from the chuck primal received no overall tenderness ratings below "slightly tender" providing evidence that some chuck steaks that are marketed as "convenience, marinate and grill" items can be successfully merchandised at the retail level. The 50% confidence level was applied to cuts from the round because of increased toughness and lower consumer expectations. Shear force values obtained in the Morgan et al. (1991) study indicated that a high percentage of retail cuts from the chuck and round would receive overall tenderness rating scores of less than "slightly tender." All segments of the beef industry should strive for 100% of all retail cuts to receive an overall tenderness rating of "slightly tender" within a 95% confidence level (Morgan et al., 1991).

CHAPTER III

TENDERNESS AND AGING RESPONSE OF BEEF MUSCLES OF DIFFERENT QUALITY GRADES BEFORE AND AFTER FREEZING

ABSTRACT

This study was designed to determine the aging response and tenderness of six different beef muscles from different quality grades before and after freezing. The infraspinatus, supraspinatus, longissimus dorsi, psoas major, semimembranosus, and semitendinosus were cut into 2.5 cm thick steaks from the right and left sides of 28 beef carcasses representing the USDA quality grades of average Choice, low Choice, high Select, and low Select. Two steaks/muscle/side were assigned to aging periods of 2, 5, 7, 14, or 21 days. One steak from each aging period was aged prior to freezing and the second steak was frozen prior to aging. Steaks frozen before aging had higher ($P<.05$) purge and cook losses than steaks frozen after aging. Warner-Bratzler shear force values were similar ($P>.05$) for both treatments. The infraspinatus, longissimus dorsi, and semitendinosus muscles showed no decrease ($P>.05$) in shear values beyond 14 days of aging. The psoas major, supraspinatus, and semimembranosus muscles did not change ($P>.05$) in shear value over the aging period. Except for the infraspinatus, all muscles from low Choice carcasses had lower shear values ($P<.05$) when compared to carcasses from other quality grades. No difference ($P>.05$) was observed for shear values of the infraspinatus, longissimus dorsi, psoas major, and semimembranosus muscles from Select carcasses. Muscles from average Choice carcasses were higher ($P<.05$) in fat content than muscles within the low Choice and Select grades. This

study indicates that freezing will not significantly affect the overall aging response and tenderness of muscles.

(Key Words : Beef, Tenderness, Aging, Freezing.)

Introduction

Tenderness is the major palatability trait that affects consumer acceptance of beef. Tenderness differences occur between carcasses, between muscles within the same carcass, and between regions of the same muscle. Smith et al. (1978) reported that aging of U.S. Choice beef carcasses for 11 days optimized the tenderness of muscles from major cuts of the carcass; however, muscles differed in their response to aging. Olson et al. (1977) found a progressive decrease in shear values in the longissimus and semitendinosus muscles, but the psoas major was unaffected by postmortem storage. Differences in the aging response of muscles could be attributed to the activities of calcium-dependent proteases (CDPs). Koohmaraie et al. (1988) found the longissimus muscle to have high CDP-I activity and aging response, whereas, the psoas major had low CDP-I activity and aging response.

Postmortem aging of meat improves tenderness; however, long aging periods are impractical as packers incur a major expense in merchandising. Packers seek ways to reduce the holding time of carcasses without incurring a forfeit on quality. Therefore, the objective of this study was to examine the aging response and tenderness of different beef muscles before and after freezing.

Materials and Methods

Sample Preparation. Twenty-eight steers, within the USDA average Choice, low Choice, high Select, and low Select grades, were slaughtered at two commercial beef operations. The carcasses were fabricated at 48 hr to obtain the infraspinatus (IF), supraspinatus (SS), longissimus dorsi (LD), psoas major (PM), semimembranosus (SM), and semitendinosus (ST) muscles from each side. A 1.3 cm thick steak was removed at the anterior and center sections of each muscle to obtain pH and proximate analysis data. Steaks cut to 2.5 cm thick were assigned to an aging period of 2, 5, 7, 14, or 21 days. Due to the inability of the SS and ST to yield enough steaks for all aging periods, the 5 day treatment was eliminated for these muscles.

Steaks were weighed, vacuum packaged, and assigned to treatment. The first 2.5 cm steak from each side and muscle was refrigerated (4^{o}C) for the assigned aging period and then frozen (T1). The second steak was immediately frozen (-20^{o}C), allowed to thaw (4^{o}C) for 18 hr, and then aged for the assigned periods (T2). The project was designed so that steaks from each treatment were frozen and thawed one time and steaks were frozen approximately for 4 weeks.

Cooking Preparation. All steaks from a given muscle and aging period representing both treatments were cooked on the same day to reduce variability. Steaks frozen after being aged (T1) were thawed at 4^{o}C for 18 h prior to cooking. Steaks were cooked at 177^{o}C in an impingement oven to an internal temperature of 70^{o}C as measured by an iron constantan thermocouple (Model #39658-J, Atkins Technical Inc., Gainesville, Fla.). Steaks were weighed prior to and after cooking to determine purge/thaw loss and cook loss.

Warner-Bratzler Analysis. Six to eight 12.5 mm cores were removed parallel to the muscle fibers after the steaks cooled for two hours at room temperature (20°C). Warner-Bratzler shear analysis was conducted using an attachment to the Instron Universal Testing Machine (Model #4502, Instron, Canton, Mass). A 1 kN load cell detected the force required to shear through the sample core as the crosshead moved at 200 mm/min. The peak force (kg) was recorded by an IBM PS2 (Model 55 SX) using software provided by Instron Corporation and analyzed as an objective measurement for tenderness.

Proximate Analysis. A 1.3 cm steak removed from the center portion of right and left sides of each muscle was evaluated for composition. Right side samples were aged prior to being frozen (T1), whereas left side samples were frozen before aging (T2). Steaks were assigned to a seven day aging period. Samples were pulverized with liquid nitrogen and analyzed in duplicate. Percentage moisture was determined by oven drying method and fat was determined according to the modified Soxhlet Extraction procedure (AOAC, 1984). Protein was determined by complete combustion using a Nitrogen and Food Protein Determinator (LECO FP-428, St. Joseph, MI).

pH Analysis. A 1.3 cm steak from the anterior portion of the right and left sides of each muscle was used for pH analysis. These samples were frozen at 48 hr postmortem. Five grams of pulverized sample were diluted with 50 ml of distilled water. The pH value for analysis represents average over 4 samples. Analysis was conducted on the pH meter (Model #130, Corning Glass, Corning, NY).

Statistical Analysis. Statistical analysis was performed using the General Linear Models procedure (SAS, 1988). The model included the effects of aging, treatment, grade, muscle, and all interactions. When appropriate, means were separated using

Fischer's least significant difference and contrasts. Regression analysis was performed to predict Warner-Bratzler shear force values over days of aging. Significant differences were reported at the P<.05 level.

Results and Discussion

Population means, standard errors, and range for carcass traits are shown in Table 1. Selection was limited to "A" maturity carcasses within the slight, small, and modest degrees of marbling. Neither the genetic history nor the previous feeding regimens of the animals were known.

Table 2 shows the two day pH values of each muscle. Steaks frozen before aging (T2) were similar (P>.05) in initial (2 day) pH values to steaks aged before frozen storage (T1); however, muscles did vary in pH values. The supraspinatus had the highest pH (P<.05), while the longissimus and semimembranosus muscles had the lowest (P<.05) values.

An age by treatment by muscle interaction was observed for total moisture loss. The percentage of total moisture loss is a combination of the muscles losing moisture during purge, thawing, and cooking with muscles losing the greatest percentage of moisture during cooking. Figure 1 presents the effect of treatment on percentage of total moisture loss for each muscle. In general, muscles frozen prior to aging (T2) tended to have a greater (P<.05) total moisture loss than muscles aged before frozen storage (T1). Freezing may have affected the muscles by creating ice crystals between the muscle fibers, causing structural damage to the cell walls of the muscles and increasing the amount of moisture lost during the aging period. A quadratic effect was observed for the SS and ST muscles. Steaks aged before freezing (T1) had a decrease in moisture loss from 14 to 21 days of aging. Changes in protein structure may give these muscles a greater water holding capacity rusulting in greater moisture retention

after a long aging period. For steaks frozen prior to aging (T2), the SS continued to show a quadratic effect, with a decrease in moisture loss from 14 to 21 days; however, the ST showed a 6-7% increase in moisture loss from 14 to 21 days. The reason for this increase is not completely clear but may be due to the differences in the aging process and the changes induced by freezing for the ST thus causing a greater moisture loss during cooking. Cohen (1984) assumed that after aging, meat would have less cooking loss; however, his findings indicated that changes induced by freezing probably have more influence on cooking loss than the extent of aging.

Thaw and cook losses of muscles as affected by quality grade are shown in Table 3. Except for the LD and PM, muscles within the low Choice grade had the lowest ($P<.05$) thaw loss among grades and, except for the SM, muscles within the low Select grade had a lower ($P<.05$) thaw loss than in the high Select grade. The high Select grade indicated the highest ($P<.05$) thaw loss for the IF, SS, LD, PM, and ST muscles; however, the SS and ST within the high Select grade were similar to the average Choice grade for thaw loss. Thaw loss was greatest ($P<.05$) for the SM in the average Choice grade when compared to other grades. Cook loss was lower ($P<.05$) for the SS, ST, and SM in the low Choice grade than in the average Choice and Select grades. The IF, LD, and PM indicated a decrease ($P<.05$) in cook loss as the quality grade increased.

Tables 4 through 6 reflect compostional properties of each muscle as affected by quality grade. Muscles within the average Choice grade had a lower ($P<.05$) moisture content and a higher ($P<.05$) intramuscular fat content compared to the other quality grades. The SS, LD, and ST muscles were not different ($P>.05$) for moisture content within the low Choice and Select grades, but the IF and SM had a higher ($P<.05$) moisture content in the low Select grade and the PM showed a higher ($P<.05$) moisture content within the Select grade compared to the Choice grade. No difference ($P<.05$) was observed for intramuscular fat in the IF, SS, LD, SM, and ST muscles

between the low Choice and Select grades, but the PM had the lowest (P<.05) fat content in the low Select grade. Generally, as fat content increases with increasing marbling scores, an inverse relationship occurs between marbling scores and moisture content. Brackebusch et al. (1991) observed that the protein content of muscles tends to decrease with increasing marbling levels, but these effects were not always significant, which agrees with our results. We found a lower (P<.05) protein content for the IF within the average Choice grade and a lower (P<.05) protein content for the LD and PM muscles within the Choice grade compared to select. The SS, SM, and ST muscles were similar (P>.05) in protein content for all quality grades.

Quality grades of muscles affected shear values as shown in Figure 2. The SS, LD, PM, SM, and ST muscles within the average Choice grade had higher (P<.05) shear values compared to the other quality grades; these muscles had the lowest (P<.05) shear values within the low Choice grade. The IF had the lowest (P<.05) shear values within the average Choice grade. The reason the majority of the muscles had higher shear values in the average Choice grade is unclear, as past research (Jennings et al., 1978; Tatum et al., 1980; Dolezal et al., 1982; Berry et al. 1993) indicate that shear values for the longissimus decrease as marbling levels increase. The genetic background, feeding regimen, and maintenance of these animals may have influenced the overall shear values for these muscles in our study. Results of Breidenstein et al. (1968) agrees with our results. they found the modest marbling group (or average Choice quality grade) "out of line" as it showed a higher shear value and a lower panel tenderness for the LD and SM muscles compared to the small and moderate marbling levels. For the Select grades, the LD and PM did not have diferent (P>.05) shear values, but the SS and ST had lower (P<.05) shear values for the low Select grade.

Calcium-dependent proteases (CDP-I and CDP-II) and cathepsins have been identified as having activity during the aging process. Calcium-dependent proteases

establish initial tenderness within 2 days (Calkins and Seideman, 1988), while the cathepsins B and H influence the tenderization of beef over a long period of time (Koohmaraie, 1990). Koohmaraie et al. (1988) has also shown that calpastatin or the CDP inhibitor is unstable in frozen storage thus suggesting that meat will be able to tenderize more fully after frozen storage. In our study, shear values for muscles frozen prior to aging (T2) were not different (P>.05) from muscles aged prior to freezing (T1). Regression lines for average shear values over aging time were developed for each muscle (Figure 3). Aging resulted in a decrease (P<.05) in shear values for the IF, LD, and ST muscles up to 14 days of aging. The SS, PM, and SM muscles were unaffected (P>.05) by aging time for shear values. According to Shackelford et al. (1990), the window of acceptability requires shear values to be below 4.5 kg for consumers to identify beef as tender. In this study, the IF and PM did not require aging to have a shear value below 4.5 kg. The LD and ST required 6 and 15 days of aging, respectively, to reach 4.5 kg with the SS and SM not reaching 4.5 kg in this study. Freezing will not affect the aging response of muscles, but some muscles will require a longer aging period or will need mechanical tenderization to ensure consumer acceptability.

Variation (reported as plus or minus two standard deviations) is shown for the shear force values of each quality grade type stratified by aging period for the infraspinatus, supraspinatus, and psoas major muscles (Figure 4 through 7). Because of the inability of the SS to yield enough steaks for the aging periods, we selected to omit the 14 day aging period, in addition to the 5 day period, for this muscle in the low Choice and low Select carcasses. The IF shear force variance decreased (P<.05) as postmortem aging increased for the average Choice and Select grades. The PM variation decreased (P<.05) as aging time increased within the average Choice and high Select carcasses, but the variation in low Select carcasses for the PM increased (P<.05) as the aging period increased. The SS shear force variance decreased (P<.05) in low

Select carcasses as the aging period increased, but the shear force variation did not change (P>.05) for the SS in the Choice quality grades regardless of the aging period.

Figure 8 shows the variation for shear force values of the longissimus, semimembranosus, and semitendinosus muscles stratified by postmortem aging within all quality grades. The longissimus and semitendinosus muscles decreased (P<.05) in variability as the aging period increased, whereas the semimembranosus numerically increased in variation as aging period increased. The semimembranosus muscle variation follows along the same path as its aging response over time where it remains fairly constant; it is also interesting to note that the longissimus variation shows a numeric increase from 14 to 21 days which may suggest that aging beyond 14 days is impractical for this muscle for all quality grades.

Implications

In this study, freezing muscles prior to aging resulted in a greater total moisture loss, but there was no significant effect on the aging response or tenderness of muscles. The infraspinatus, longissimus, and semitendinosus muscles had maximum improvement in tenderness at 14 days, whereas the supraspinatus, psoas major, and semimembranosus muscles had no response to aging. Since aging periods are long and expensive for packers, it may be possible for retail outlets to age meat for a few days after frozen storage to increase tenderness. However, more extensive research is needed to address the enzymatic activities of beef muscles after frozen storage and its relationship to the tenderness of beef.

Table 1. Carcass characteristics.

Trait	Mean	SE	Minimum	Maximum
HCW,kg[a]	334.44	4.38	280.32	- 377.84
Actual fat thickness,mm	11.81	.76	3.05	- 19.81
Adj. fat thickness,mm	12.19	.66	5.08	- 19.30
LD muscle area,cm^2[b]	79.40	1.24	65.16	- 96.13
KPH fat,%[c]	1.76	.07	1.00	- 2.50
USDA Yield Grade	2.92	.10	2.02	- 3.97
USDA Overall Maturity Score[d]	143.75	1.30	135.00	- 160.00
USDA Marbling Score[e]	414.82	16.41	305.00	- 580.00

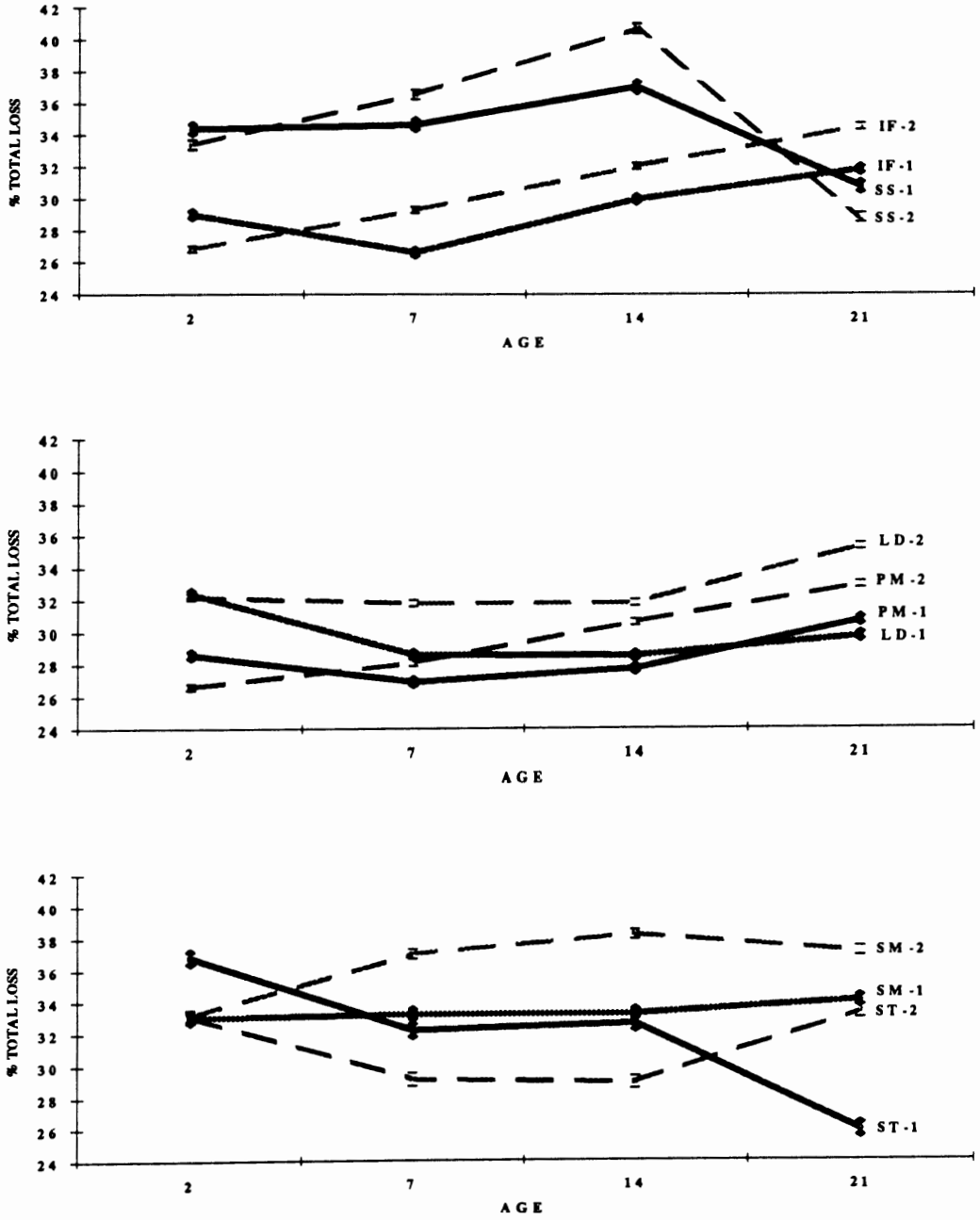
[a]Hot Carcass weight
[b]Longissimus Dorsi
[c]Kidney, Pelvic, and Heart Fat
[d]Maturity Score: 100=A^{00}, 200=B^{00}
[e]Marbling Score: 300=Slight^{00}, 400=Small^{00}, 500=Modest^{00}

Table 2. pH values stratified by muscle.

MUSCLE	pH[a]	SE[b]
Infraspinatus	5.70[d]	.017
Supraspinatus	5.80[c]	.014
Longissimus	5.40[f]	.009
Psoas Major	5.70[d]	.018
Semimembranosus	5.40[f]	.013
Semitendinosus	5.50[e]	.015

[a]pH values evaluated on 2 day aging period of right and left sides of muscles
[b]Standard error
[c,d,e,f]Means in same column with different superscripts differ (P<.05)

Figure 1. Age length and treatment effect on percentage total moisture loss within muscles.



MUSCLES: IF=infraspinatus, SS=supraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus, ST=semitendinosus

TREATMENTS: 1 (Solid line)=aged before frozen
2 (Dashed line)=frozen before aged

Table 3. Effect of USDA quality grade on percentage thaw and cook loss within muscles.

	USDA Quality Grade[a]									
	LS		HS		LC		AC		SE[c]	
Muscle[b]	Thaw	Cook	Thaw	Cook	Thaw	Cook	Thaw	Cook	Thaw	Cook
IF	5.3[e]	26.1[d]	6.3[d]	25.1[e]	4.5[f]	23.4[f]	5.3[e]	24.2[f]	.10	.18
SS	6.8[e]	25.2[e]	8.8[d]	26.5[e]	5.8[f]	23.6[f]	8.6[d]	28.1[d]	.15	.26
LD	6.4[f]	25.0[d]	7.6[d]	25.0[d]	6.1[f]	24.6[e]	7.0[e]	23.2[f]	.14	.15
PM	4.8[f]	23.9[d]	7.1[d]	23.8[d]	4.6[f]	22.9[e]	5.9[e]	22.9[e]	.12	.15
SM	9.7[e]	24.5[e]	10.2[e]	25.2[e]	8.3[f]	23.1[f]	11.2[d]	27.2[d]	.15	.17
ST	8.9[e]	20.3[f]	10.4[d]	22.5[e]	8.0[f]	18.3g	10.0[d]	27.6[d]	.17	.26

[a]LS=low Select, HS=high Select, LC=low Choice, AC=average Choice

[b]IF=infraspinatus, SS=supraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus, ST=semitendinosus

[c]Standard error

[d,e,f,g]Means in the same row within same dependent variable with different superscripts differ (P<.05)

Table 4. USDA quality grade on percentage moisture within muscles.

	USDA Quality Grade[a]				
MUSCLE[b]	LS	HS	LC	AC	SE[c]
IF	73.6[d]	71.5[e]	71.7[e]	66.8[f]	.33
SS	76.4[d]	75.0[d]	75.6[d]	70.7[e]	.30
LD	72.2[d]	71.1[d]	71.1[d]	67.9[e]	.22
PM	72.5[d]	71.4[d]	70.8[e]	70.8[e]	.26
SM	75.0[d]	72.9[f]	73.6[e]	71.2[g]	.23
ST	75.0[d]	74.5[d]	74.5[d]	73.1[e]	.16

[a]LS=low Select, HS=high Select, LC=low Choice, AC=average Choice

[b]IF=infraspinatus, SS=supraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus, ST=semitendinosus

[c]Standard error

[d,e,f,g] Means in same row with different superscripts differ (P<.05)

Table 5. USDA quality grade on percentage intramuscular fat within muscles.

	USDA Quality Grade[a]				
MUSCLE[b]	LS	HS	LC	AC	SE[c]
IF	6.7[e]	8.2[e]	7.3[e]	14.1[d]	.43
SS	1.9[e]	2.8[e]	2.2[e]	7.0[d]	.28
LD	4.3[e]	4.9[e]	5.4[e]	9.2[d]	.26
PM	4.0[e]	5.2[e]	6.2[d]	7.0[d]	.29
SM	1.7[e]	2.8[e]	2.4[e]	4.3[d]	.23
ST	2.2[e]	2.4[e]	2.5[e]	4.0[d]	.12

[a]LS=low Select, HS=high Select, LC=low Choice, AC=average Choice

[b]IF=infraspinatus, SS=supraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus, ST=semitendinosus

[c]Standard error

[d,e,]Means in same row with different superscripts differ (P<.05)

Table 6. USDA quality grade on percentage protein within muscles.

	USDA Quality Grade[a]				
MUSCLE[b]	LS	HS	LC	AC	SE[c]
IF	19.8[d]	19.9[d]	20.2[d]	18.8[e]	.18
SS	20.7[e]	20.1	21.0	21.6	.23
LD	22.1[e]	23.0[d]	22.1[e]	22.1[e]	.10
PM	22.1[d]	21.6[d]	21.2[e]	21.2[e]	.14
SM	22.6	22.8	22.7	22.5	.21
ST	21.8	22.5	22.1	21.9	.14

[a]LS=low Select, HS=high Select, LC=low Choice, AC=average Choice

[b]IF=infraspinatus, SS=supraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus, ST=semitendinosus

[c]Standard error

[d,e,f] Means in same row with different superscripts differ (P<.05)

Figure 2. USDA quality grade on average Warner-Bratzler shear values within muscles.

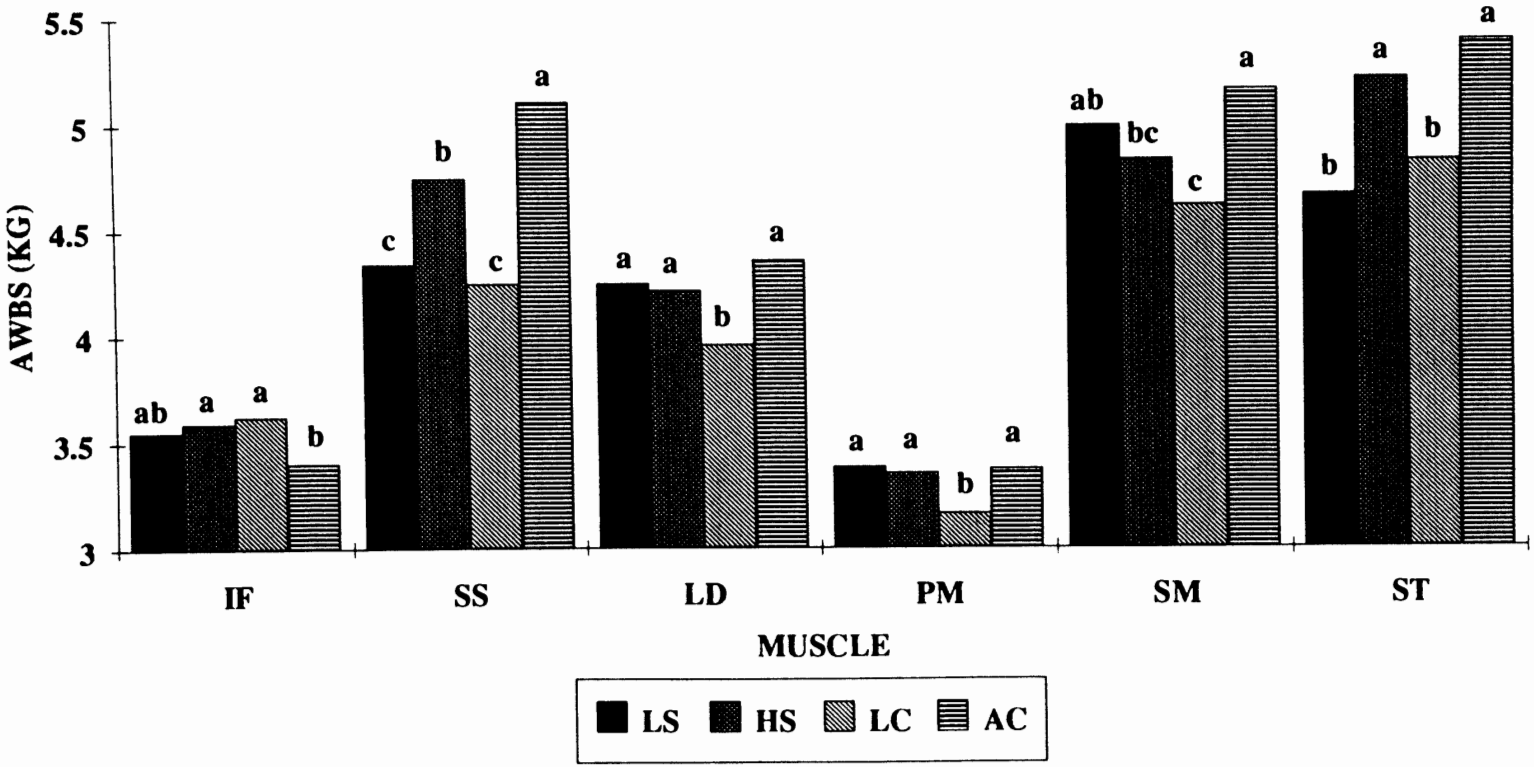
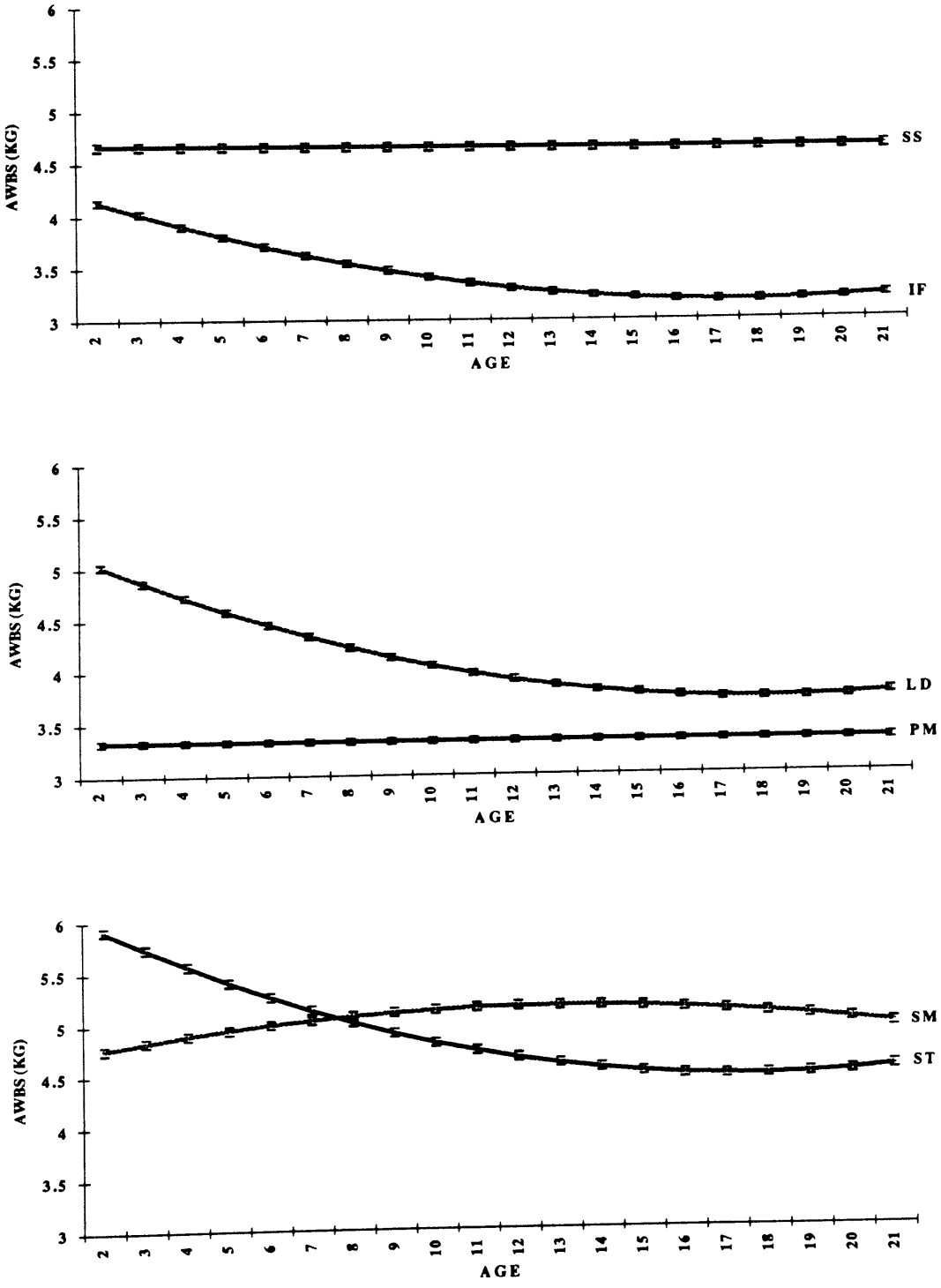
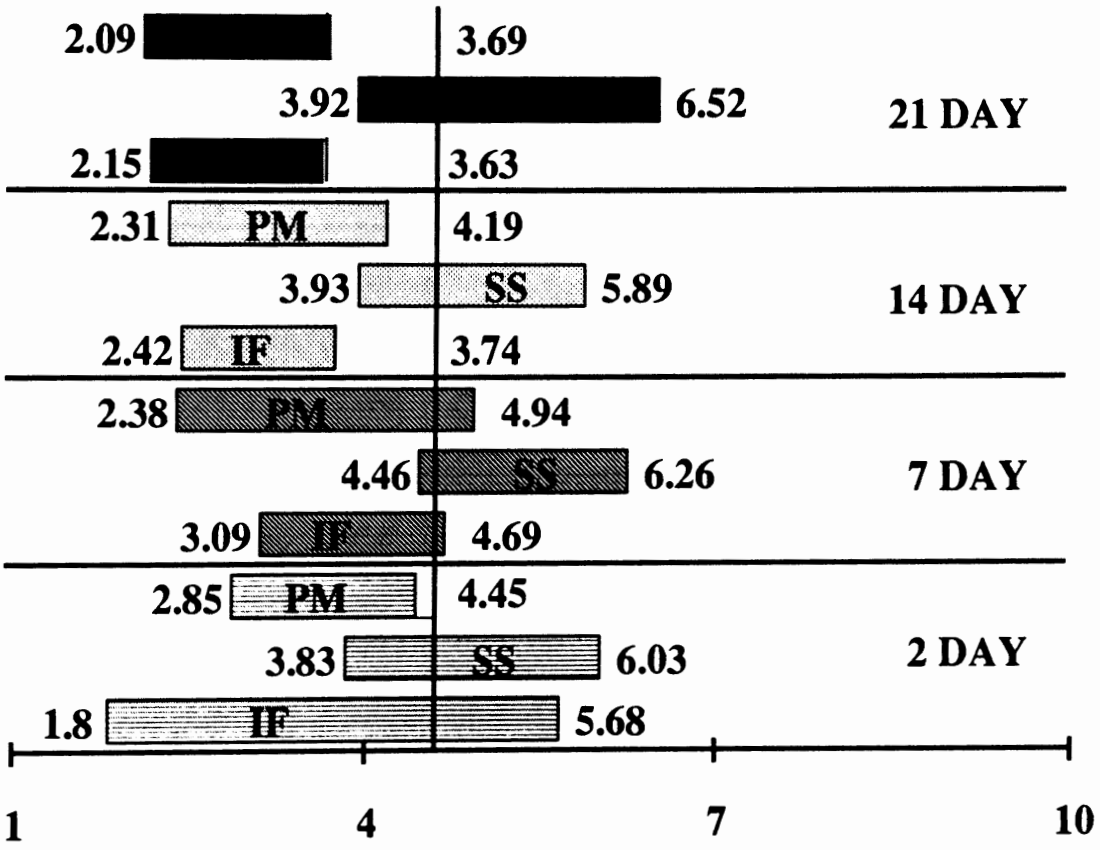


Figure 3. Regression of Warner-Bratzler shear values over aging.



MUSCLES: IF=infraspinatus, SS=supraspinatus, LD=longissimus,
PM=psoas major, SM=semimembranous, ST=semitendinous
AWBS=average Warner-Bratzler shear values

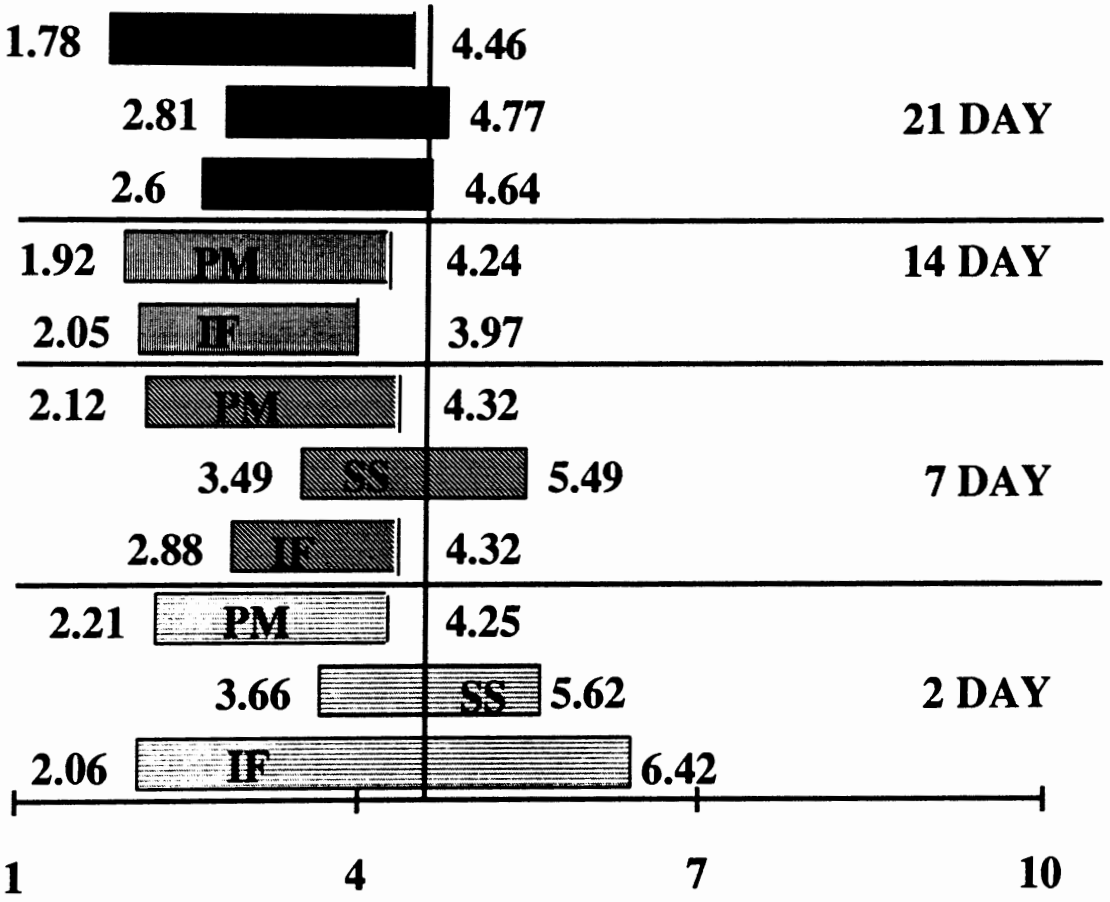
Figure 4. Variation (reported as the mean ± two standard deviations) in shear force (kg) within muscles over aging periods from average Choice carcasses.



	Variance			
Muscles	2 day	7 day	14 day	21 day
IF=Infraspinatus	.94[a]	.16[b]	.11[b]	.14[b]
SS=Supraspinatus	.30	.21	.24	.42
PM=Psoas Major	.16[ab]	.41[a]	.27[ab]	.16[b]

[a,b]Means in same row with different superscripts differ (P<.05).

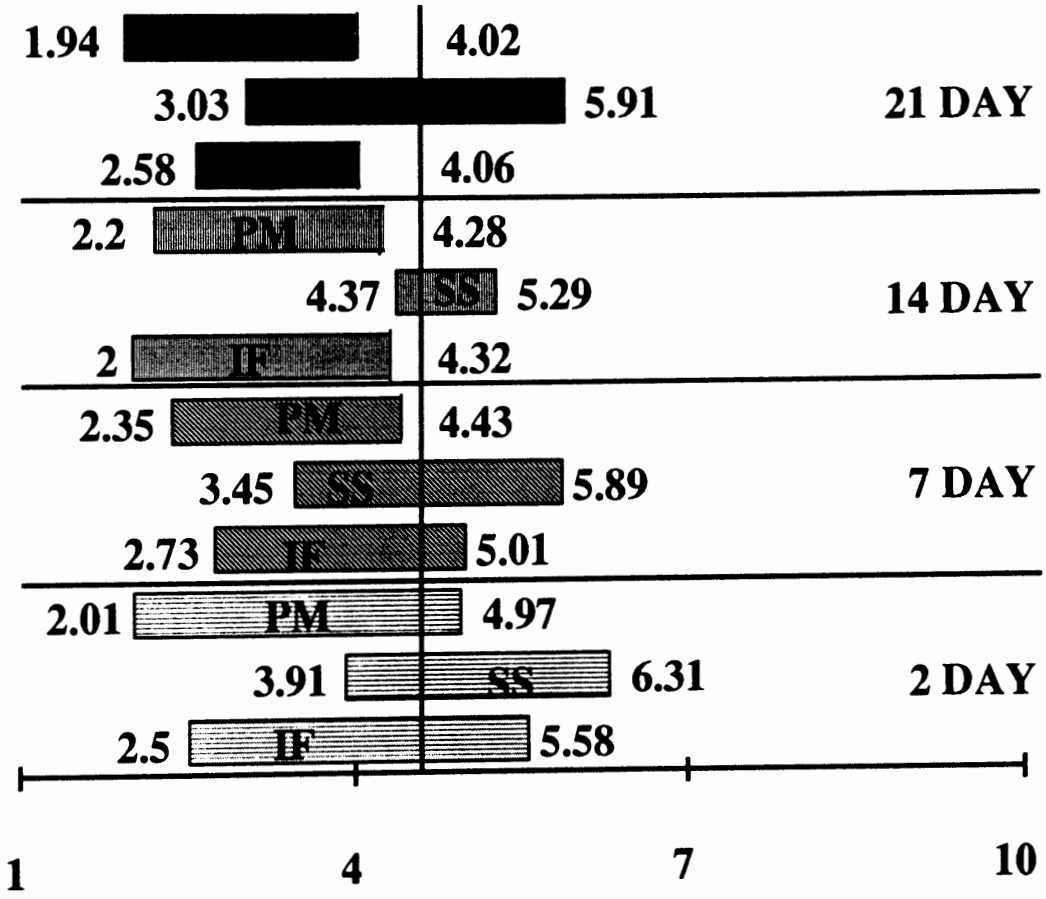
Figure 5. Variation (reported as the mean ± two standard deviations) in shear force (kg) within muscles over aging periods from low Choice carcasses.



	Variance			
Muscles	2 day	7 day	14 day	21 day
IF=Infraspinatus	1.19[a]	.13[b]	.23[b]	.06[b]
SS=Supraspinatus	.24	.25	---	.24
PM=Psoas Major	.26	.30	.59	.45

[a,b]Means in same row with different superscripts differ (P<.05).

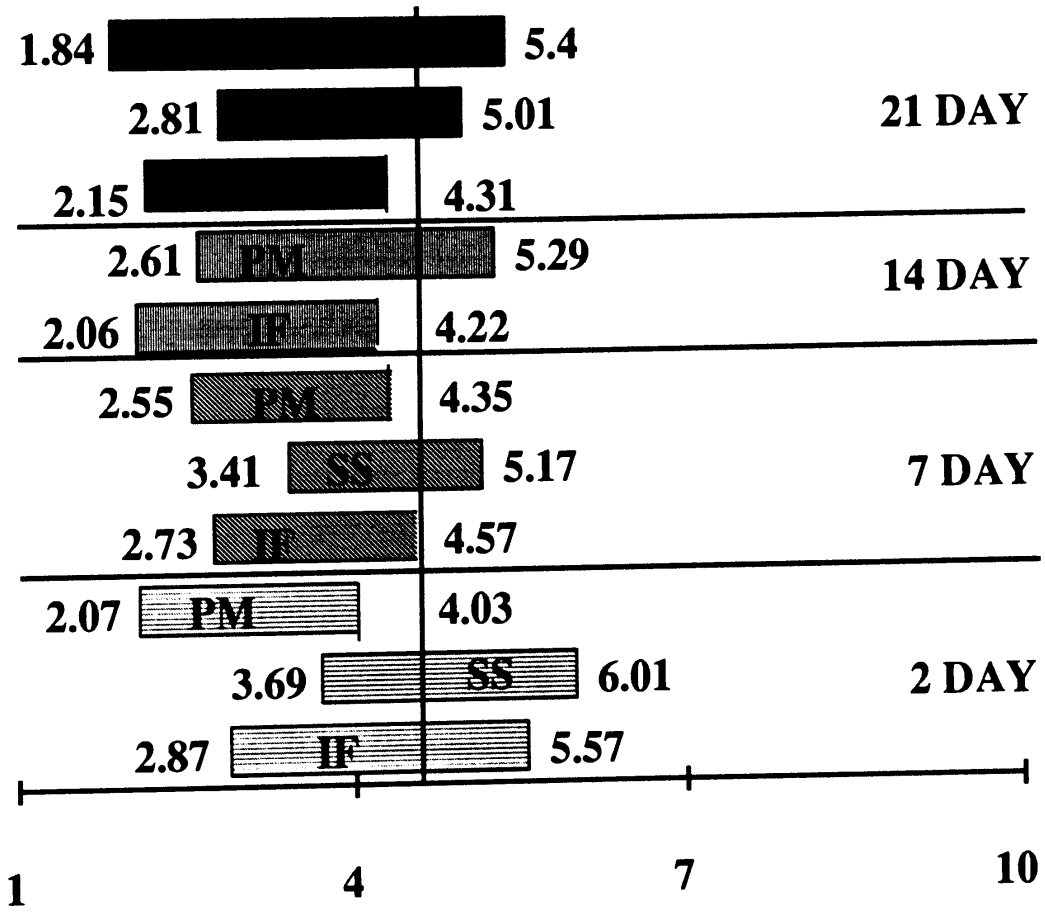
Figure 6. Variation (reported as the mean ± two standard deviations) in shear force (kg) within muscles over aging periods from high Select carcasses.



	Variance			
Muscles	2 day	7 day	14 day	21 day
IF=Infraspinatus	.59[a]	.32[ab]	.34[ab]	.14[b]
SS=Supraspinatus	.36[ab]	.37[ab]	.21[b]	.52[a]
PM=Psoas Major	.55[a]	.27[b]	.14[b]	.45[ab]

[a,b]Means in same row with different superscipts differ (P<.05).

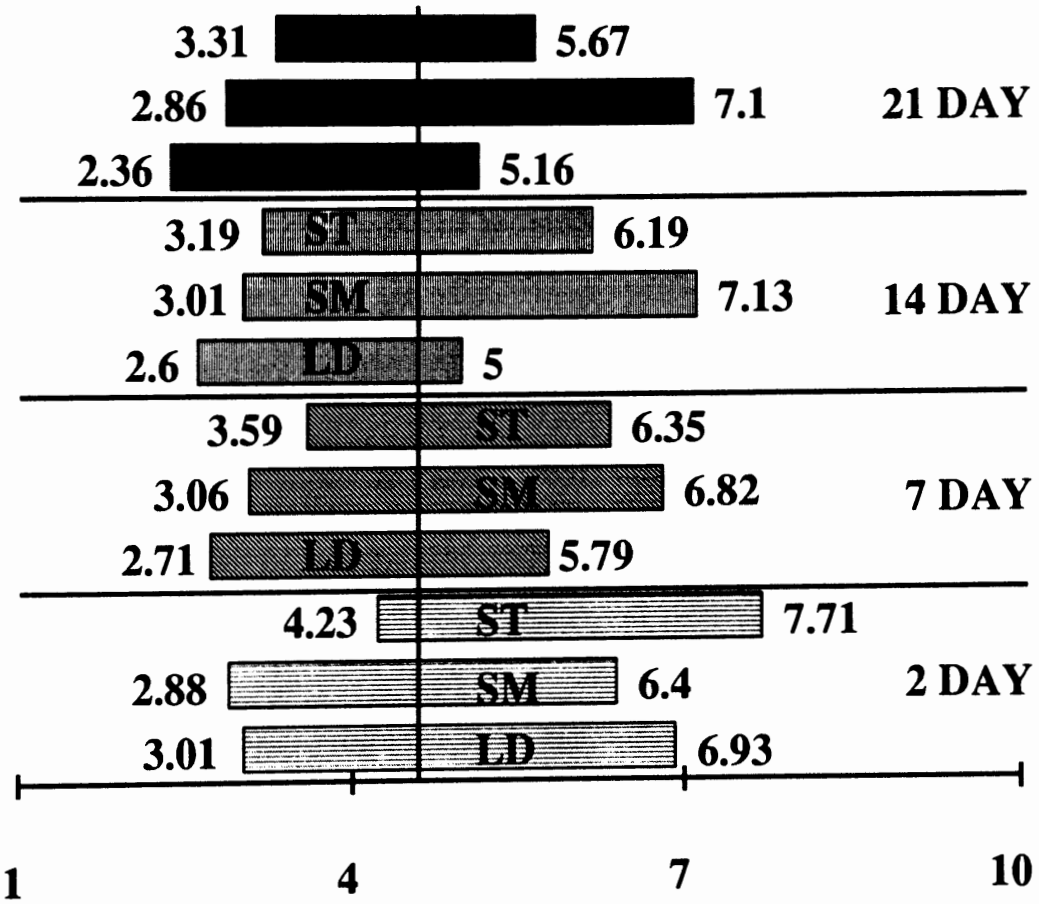
Figure 7. Variation (reported as the mean ± two standard deviations) in shear force (kg) within muscles over aging periods from low Select carcasses.



	Variance			
Muscles	2 day	7 day	14 day	21 day
IF=Infraspinatus	.44	.21	.29	.08
SS=Supraspinatus	.34	.19	----	.30
PM=Psoas Major	$.24^{b}$	$.21^{b}$	$.26^{ab}$	$.79^{a}$

^{a,b}Means in same row with different superscripts differ (P<.05).

Figure 8. Variation (reported as the mean ± two standard deviations) in shear force (kg) within muscles over aging periods.



	Variance			
Muscles	2 day	7 day	14 day	21 day
LD=Longissimus	.96[a]	.59[b]	.36[b]	.29[b]
SM=Semimembranosus	.77	.88	1.06	1.12
ST=Semitendinosus	.76[a]	.48[b]	.56[b]	.35[b]

[a,b]Means in same row with different superscripts differ (P<.05).

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APPENDIX

Table 1. ANOVA table of dependent variable - pH of muscles.

Source	DF[a]	Sum of Squares
Model	11	7.42*
Side	1	.02
Muscle	5	7.37*
Side*Muscle	5	.02

[a]Degrees of freedom
*P<.01

Table 2. ANOVA table of dependent variable - total loss sorted by muscle for means.

Source	DF[a]	Sum of Squares					
		IF[b]	SS[b]	LD[b]	PM[b]	SM[b]	ST[b]
Model	22	4153.87*	8106.95*	2643.77*	2640.55*	5482.92*	13,557.47*
Age	3	2300.32*	2375.43*	494.47*	1341.40*	552.50*	1837.27*
Treatment	1	199.34*	1.21	977.78*	119.19*	1007.91*	67.93
Age*Treatment	3	457.52*	257.17*	470.55*	382.95*	372.54*	2488.44*
Grade	3	1012.99*	2302.51*	370.62*	656.42*	2881.19*	7967.68*
Age*Grade	9	131.15	2161.12*	230.09	131.43	604.19*	832.67*
Treatment*Grade	3	53.82	357.22*	95.87	38.62	204.49**	462.05*

[a]Degrees of freedom
[b]IF=infraspinatus, SS=supraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus, ST=semitendinosus
*P<.01
**P<.05

Table 3. ANOVA table of dependent variable - total loss sorted by muscle for contrasts.

Source	DF[a]	Sum of Squares					
		IF[b]	SS[b]	LD[b]	PM[b]	SM[b]	ST[b]
Model	31	4251.44*	8347.00*	2738.50*	2761.47*	6058.81*	14,387.93*
Age-Treatment	7	2970.20*	2689.62*	1948.32*	1839.52*	1938.83*	4361.74*
Grade	3	1016.35*	2302.51*	371.32*	656.39*	2880.50*	7963.64*
Age-Treatment*Grade	21	282.51	2758.39*	419.02	290.46	1385.11*	2124.09*

[a]Degrees of freedom

[b]IF=infraspinatus, SS=supraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus, ST=semitendinosus

*P<.01

Table 4. ANOVA table of dependent variable - average Warner-Bratzler shear values sorted by muscle for contrasts.

Source	DF[a]	Sum of Squares					
		IF[b]	SS[b]	LD[b]	PM[b]	SM[b]	ST[b]
Model	22	80.47*	78.64*	131.57*	25.93*	56.07*	190.04*
Age	3	64.37*	16.69*	106.51*	3.03**	11.63*	143.32*
Treatment	1	.00	.53	.18	.10	4.20**	1.08
Age*Treatment	3	1.56	.71	2.05	1.32	3.61	2.41
Grade	3	3.24**	38.46*	9.69*	3.57**	17.74*	37.01*
Age*Grade	9	10.69*	16.90*	9.48	14.45*	13.33	3.74
Treatment*Grade	3	.88	2.55**	3.67	3.65**	5.12	1.27

[a]Degrees of freedom[b]IF=infraspinatus, SS=supraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus, ST=semitendinosus

*P<.01

**P<.05

Table 5. Time required for steaks to reach 70°C internal temperature on an impingement oven set at 177°C.

MUSCLE[a]	TIME (MINUTES)[b]	MEAN[c]
IF	10:35	4.8 sec/gm
SS	10:15	3.7 sec/gm
LD	13:00	2.3 sec/gm
PM	10:00	5.2 sec/gm
SM	12:00	4.0 sec/gm
ST	11:00	5.9 sec/gm

[a]IF=infraspinatus, SS=supraspinatus, LD=longissimus, PM=psoas major SM=semimembranosus, ST=semitendinosus

[b]Time required for steaks to reach 70°C internal temperature at the geometric center of the steak

[c]Seconds required per gram of each muscle

Table 6. Effect of aging period and treatment[a] on percentage cook loss.

	Aging Period											
	2			7			14			21		
Muscle[b]	A	B	SE[d]	A	B	SE[d]	A	B	SE[d]	A	B	SE[d]
IF	24.3[e]	22.7[f]	.43	22.6	23.8	.58	25.4	25.5	.41	26.7	26.6	.48
SS	27.9	26.7	.39	28.4	28.1	.34	28.2	28.5	.62	23.8[e]	19.9[f]	.86
LD	26.5	26.1	.42	22.8	23.4	.33	24.0	23.5[e]	.46	24.2	24.9	.38
PM	23.4[e]	21.9[f]	.35	22.7	23.3	.34	22.6[f]	24.3[e]	.45	24.3	24.6	.47
SM	25.1	24.6	.43	24.6	24.7	.47	25.0	25.4	.53	25.5	25.1	.62
ST	25.1[e]	22.6[f]	.72	23.9[e]	18.0[f]	.85	24.4[e]	18.7[f]	.83	19.8[f]	24.6[e]	.63

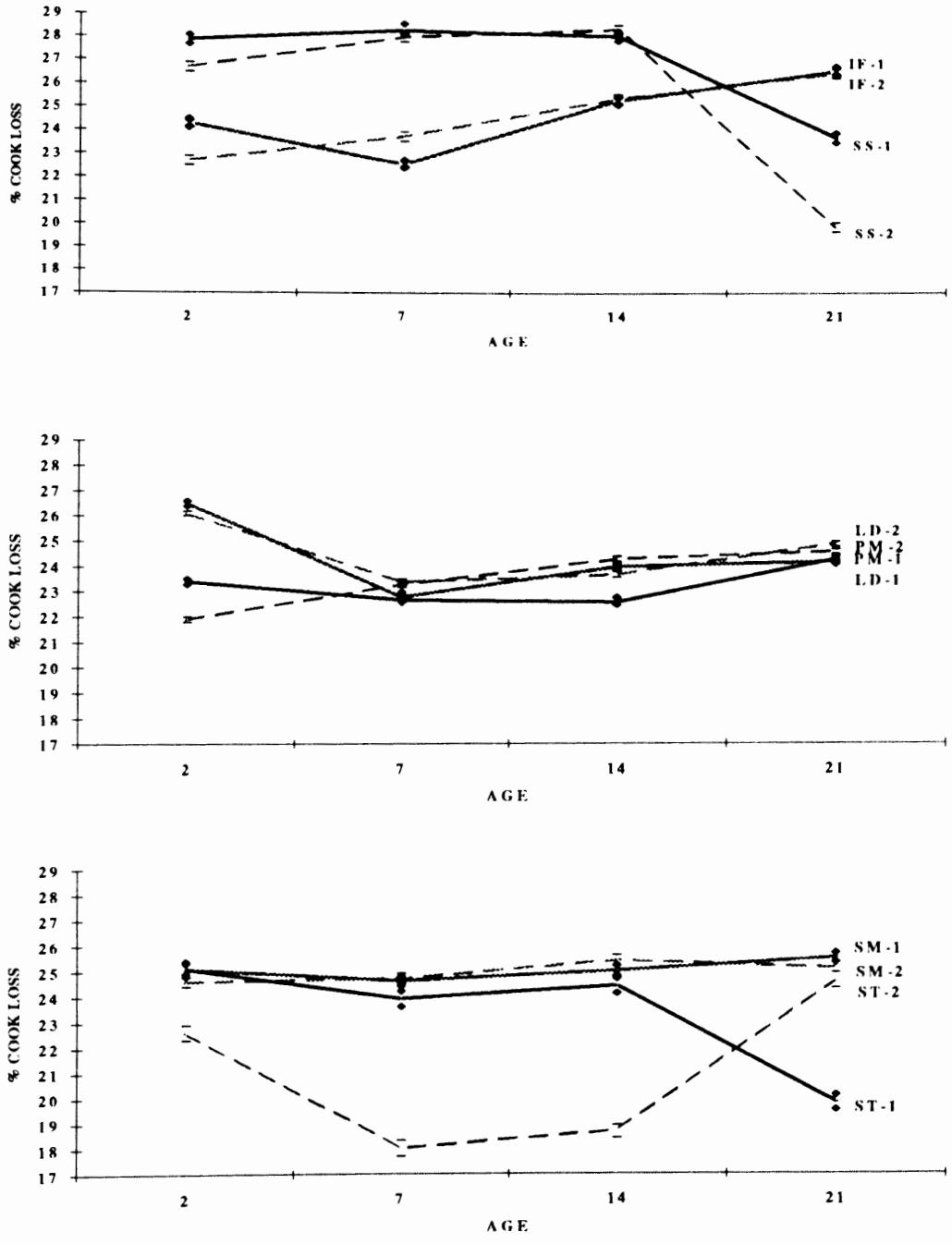
[a]Treatment A=aged before frozen; B=frozen before aged

[b]IF=infraspinatus, SS=supraspinatus, LD=longissimus; PM=psoas major; SM=semimembranosus, ST=semitendinosus

[d]Standard error

[e,f]Means in the same row within same aging period with different superscripts differ (P<.05)

Figure 1. Age and treatment on percentage cook loss within muscles.



MUSCLES: IF=infraspinatus, SS=supraspinatus, LD=longissimus,
PM=psoas major, SM=semimembranosus, ST=semitendinosus

TREATMENTS: 1 (Solid line)=aged before frozen
2 (Dashed line)=frozen before aged

Table 7. Effect of aging period and treatment[a] on percentage total moisture loss[b].

	Aging Period											
	2			7			14			21		
Muscle[c]	A	B	SE[d]	A	B	SE[d]	A	B	SE[d]	A	B	SE[d]
IF	29.0[e]	26.8[f]	.54	26.6[f]	29.3[e]	.63	30.0[f]	32.1[e]	.54	31.9[f]	34.6[e]	.64
SS	34.4	33.4	.61	34.7	36.6	.51	32.1	40.8	.98	30.8	28.8	1.08
LD	32.4	32.2	.47	28.6[f]	31.8[e]	.49	28.5[f]	31.8[e]	.61	29.7[f]	35.3[e]	.54
PM	28.6[e]	26.6[f]	.48	26.9	28.1	.49	27.7[f]	30.6[e]	.65	30.7[f]	32.9[e]	.64
SM	33.0	33.1	.60	33.2[f]	37.0[e]	.64	33.2[f]	38.2[e]	.79	34.0[f]	37.1[e]	.89
ST	36.8[e]	33.1[f]	1.01	32.2[e]	29.1[f]	1.01	32.6[e]	28.9[f]	1.07	25.9[f]	33.3[e]	.90

[a]Treatment A=aged before frozen; B=frozen before aged.

[b]Combination of purge/thaw and cook loss

[c]IF=infraspinatus, SS=supraspinatus, LD=longissimus; PM=psoas major; SM=semimembranosus, ST=semitendinosus

[d]Standard error

[e,f]Means in the same row within same aging period with different superscripts differ (P<.05)

Figure 2. USDA quality grade on percentage moisture within muscles.

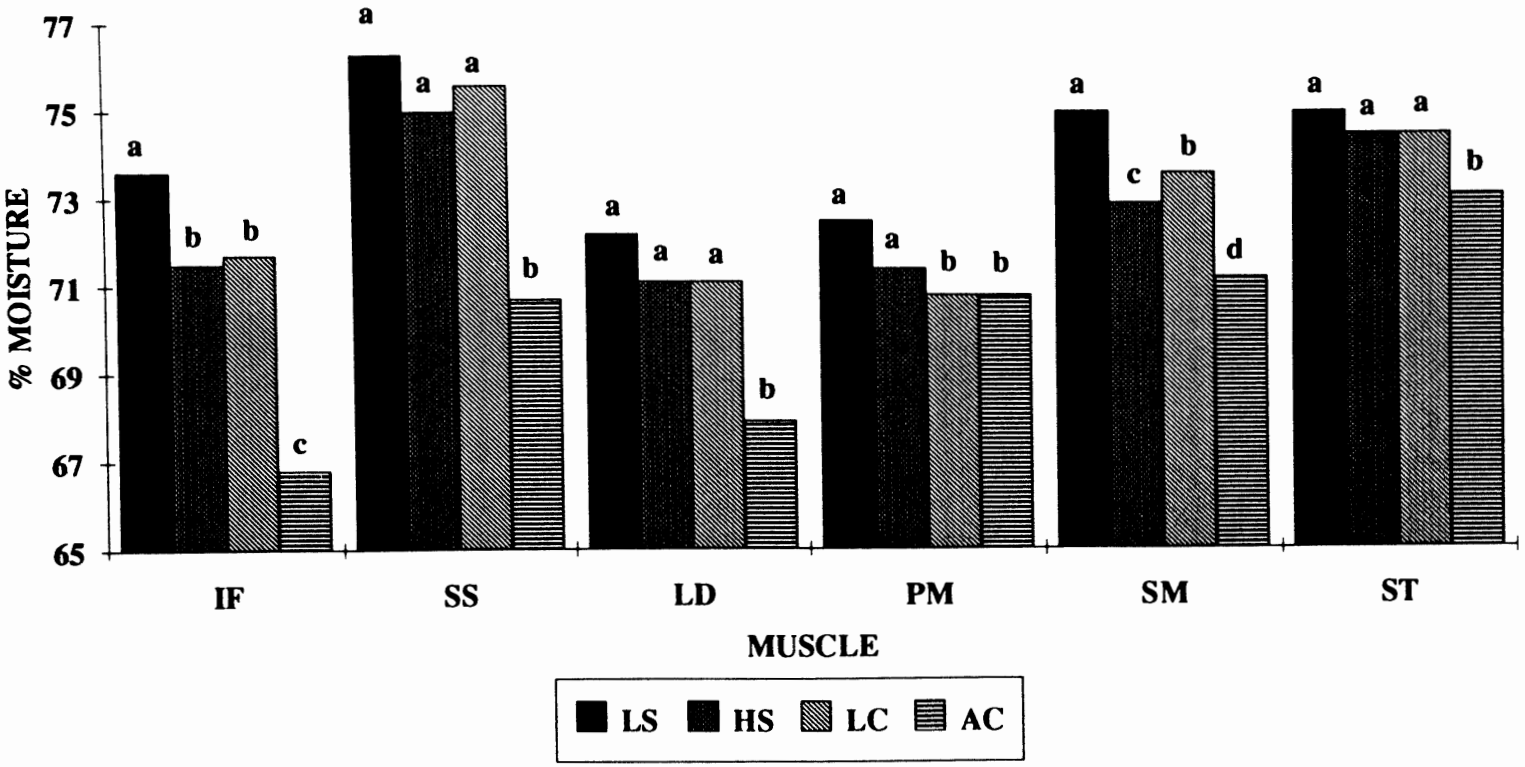


Figure 3. USDA quality grade on percentage intramuscular fat within muscles.

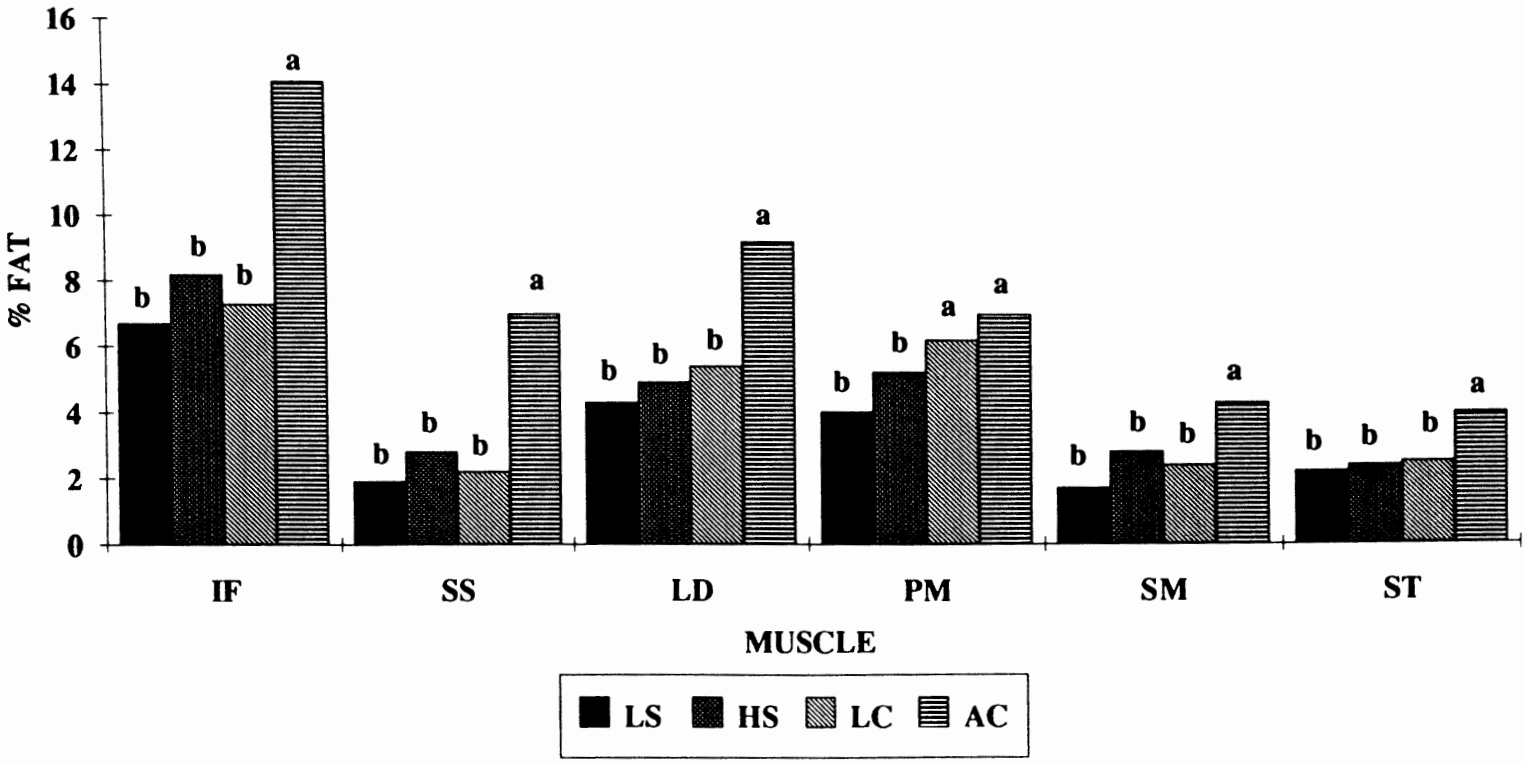


Figure 4. USDA quality grade on percentage protein within muscles.

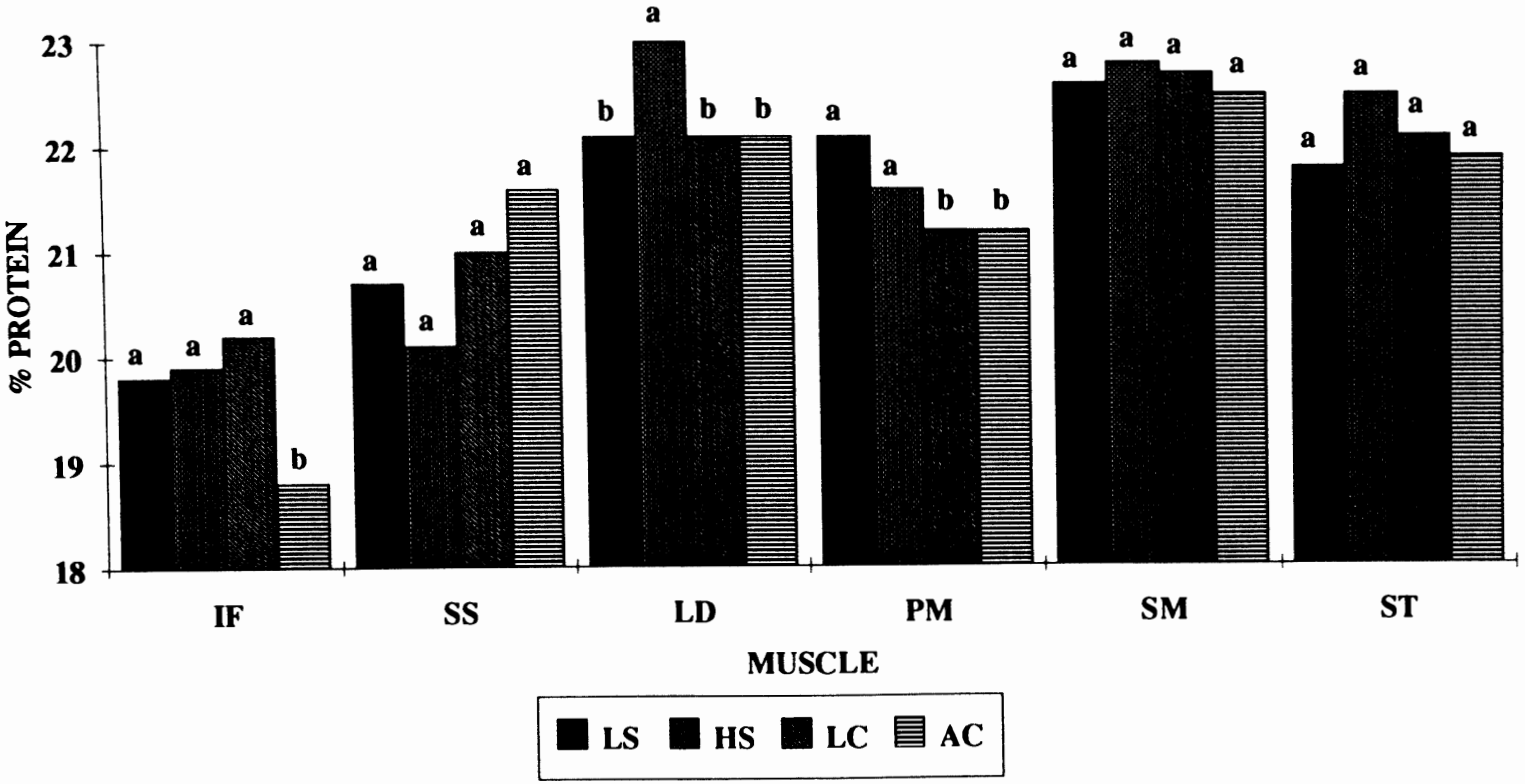


Table 8. USDA quality grade on Warner-Bratzler Shear values within muscles.

	USDA Quality Grade[a]				
MUSCLE[b]	LS	HS	LC	AC	SE[c]
IF	3.6[d]	3.6[d]	3.6[d]	3.4[e]	.03
SS	4.3[f]	4.7[e]	4.3[f]	5.1[d]	.03
LD	4.3[d]	4.2[d]	4.0[e]	4.4[d]	.04
PM	3.4[d]	3.4[d]	3.2[e]	3.4[d]	.03
SM	5.0[de]	4.8[ef]	4.6[f]	5.2[d]	.05
ST	4.7[e]	5.2[d]	4.8[e]	5.4[d]	.03

[a]LS=low Select, HS=high Select, LC=low Choice, AC=average Choice

[b]IF=infraspinatus, SS=supraspinatus, LD=longissimus, PM=psoas major, SM=semimembranosus, ST=semitendinosus

[c]Standard error

[d,e,f]Means in same row with different superscripts differ (P<.05)

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