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Sensory, chemical and ultrasonic measurements of the palatability attributes of bovine skeletal muscle

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Sensory, chemical and ultrasonic measurements of the palatability attributes of bovine skeletal muscle

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PAPER III

INTRODUCTION

The increasing concern by consumers towards fat in the diet has led meat producers to re-evaluate production strategies and even challenged them to efficiently produce lean beef that would command acceptable eating quality. Intact males ie. bulls, traditionally have been thought to produce tough beef and, due to the quality concerns and discrimination by the USDA grading system (Siedeman et al., 1982), the feeding of bulls in the United States has never been popular.

However, due to the superior performance characteristics,. leaness and higher yielding carcasses of bulls (Bailey et al., 1966; Arthaud et al., 1977), there may be an increasing possibility that young bulls under 15 months may replace steers of this age since they produce lean flesh which is now in demand and in greater quantities (Lawrie, 1985).

Data on the palatability of bulls versus steers has been variable. Reagan et al. (1971) and Gerrard et al. (1987) concluded that steers were definitely more tender, more flavorful and rated higher for overall satisfaction than bulls whereas Klastrup et al. (1983) and Vanderwert et al. (1985), found no significant differences in sensory evaluation of bulls and steers. Breed differences based on frame size have also revealed palatability differences (Smith et al., 1976; Adams et al., 1977; Cole et al., 1963). This effect is also compounded by time on feed trials showing increases in tenderness when cattle are grain fed for periods varying from 56 days (Miller et al., 1987), 100 days (Dolezal et al., 1982) and 180 days (Zinn et al., 1970).

In order to facilitate management practices, and reduce the incidence of dark cutting in beef, bulls have under normal circumstances been castrated. Dark cutting beef is a quality problem caused by chronic stress preslaughter depleting muscle glycogen reserves (Fisher and Augustini, 1979) and resulting in higher ultimate pH. The meat is dark in color (MacDougall and Rhodes, 1972), has reduced flavor (Dransfield, 1981) and is thus discriminated against by the consumer (Tarrant, 1981). However, high pH meat is unusually tender. Work by Yates et al. (1983) and Samejima and Wolfe (1976) using meat of various pHs (manipulated by acid/alkali) showed some troponin-T degradation. Troponin-T breakdown and the presence of a 30Kd component has been implicated in more tender longissimus muscles (MacBride and Parrish, 1977).

A number of attempts have been made to modify the USDA quality grading system. Even after such attempts, there have been many questions regarding its validity in terms of palatability. Tenderness is the most important palatability attribute (Lawrie, 1985) and any objective assessment that is to be introduced into the quality grading system must take cognizance of this fact.

The words fat and meat are synonymous in many peoples vocabulary. Due to the health concerns regarding fat and cholesterol in the diet, a certain segment of the population has chosen to restrict meat consumption. Serum cholesterol in normaiipidemic subjects is said to be unaffected by the nature and amount of dietary fat (Kestin et al., 1989; McNamara, 1991). In addition, the amount of saturated fat in meat is approximately 45% while the proportion of unsaturated fatty acids is approximately 55%. This may be further modified by increasing feed time (Hidiroglou, 1985).

The purpose of this research was to determine: (a) the difference in palatability between bulls and steers of three frame sizes fed to three different end-points; (b) cholesterol and fatty acid composition of bulls and steers based on lipid content and time on feed; (c) degradation of myofibrils isolated from dark cutting and normal pH beef and (d) utilization of ultrasound variables as they relate to sensory attributes of *longissimus thoracis* muscle.

Explanation of Thesis Format

This thesis consists of a general introduction, literature review, three publishable papers and a concluding summary. The papers represent the work done by the first author to fulfill requirements for the degree of Doctor of Philosophy. Co-authors were involved in the review for each paper. Each paper consists of a title page, abstract, introduction, materials and methods, results and discussion, conclusion and references. The format of the papers is in accordance with the Journal of Food Science style guide for research papers.

LITERATURE REVIEW

Considering the diversity and duration of the events which determine the nature of meat, it seems curious that the consumer's palate can only react to the commodity over a few minutes during mastication. Of the attributes of eating quality, color, water holding capacity and some of the odor of the meat are detected both before and after cooking and provide the consumer with a more prolonged sensation than do juiciness, texture, tenderness, taste and most of the odor which is detected during mastication (Lawrie, 1985). Intramuscular fat is an intrinsic component of meat accounting for some of the above parameters.

Clearly, whatever the scientific basis of the attributes of eating quality in meat, their significance will be determined by regional preferences and the views of the individual consumer. Some prefer markedly tough meat; others prefer excessively tender. A major study of the eating quality of grilled *longissimus dorsi* and *semimembranosus* muscle from Galloway and Charolais steers was undertaken by Dransfield et al. (1984). This study involved the assessment of beef steaks from the same animals in eight European Community countries. It was evident that the Irish and English panels tended to value flavor more highly than tenderness and juiciness, whereas the latter attributes were of predominant importance to Italian panelists. French and Belgian sensory panels showed preference for aged beef from older animals.

A sensory evaluation study (Savell et al., 1987) was conducted in a western, central and eastern US city (San Francisco, Kansas City and Philadelphia, respectively) using randomly selected consumers to assess the palatability of beef strip top loins having seven degrees of marbling. The results indicated that the

western and central city mean scores were consistently high independent of degrees of marbling. In contrast, the eastern city consumers produced comparable ratings with the other cities' mean scores, but the scores markedly declined with decreasing marbling.

Antemortem Factors Influencing Palatability

Plane of Nutrition

The type of diet and duration of feeding has a significant influence on the organoleptic characteristics of beef. The topic of feeding beef cattle various energy regimes is not new. An exhaustive amount of literature abounds in this field.

Differences in the plane of nutrition at any age from the late fetal stage to maturity not only alter growth generally but also affect the different skeletal regions, the different tissues and the various organs differentially. Thus, animals on different planes of nutrition, even if they are the same breed and weight will differ greatly in form and composition (Wallace, 1948).

Backus et al. (1967) and Bowling et al. (1978) found that high energy rations fed to cattle produced heavier carcasses, higher dressing percentages, higher marbling scores and higher carcass grades. Prior and coworkers (1977) and Ferrel et ai. (1978) stated that high energy diets resulted in an increased rate of gain, but most of the extra grain was a result of an increase in fat deposition. In addition, palatability characteristics of meat from these animals were not improved. Danner et al. (1980) substantiated these results and showed that increased dietary energy increased fat thickness, with no effect on quality grade. However, Bowling et al. (1979) and Crouse et al., (1978) suggest that an insulating fat layer would increase tenderness.

Cattle fed low energy diets need to be fed to heavier weights than cattle on high energy diets in order to reach the same carcass grade (Minish and Fox, 1979). It has been shown that a small amount of grain is necessary to ensure desirable palatability ratings of beef (Skelley et al., 1978). No grain in the diet produced carcasses of low marbling, low dressing percentage and low carcass grade. Taste panel scores for flavor were low when no grain was used in the diet. When comparing cattle raised on grain as opposed to pasture, grain fed cattle scored higher in quality, with improved carcass characteristics (Schroeder et al., 1980) and higher marbling scores (Reagan et al., 1977). Meat from grain fed cattle is higher in palatability, has less lean surface discoloration, higher muscle color, tenderness, flavor and palatability than grass fed cattle (Bowling et al., 1977).

Moody (1976) found that higher marbling scores were associated with juiciness and that flavor was also highly associated with intramuscular fat which is found in greater amounts in the meat of grain fed cattle than grass fed cattle.

Henrickson and coworkers (1965) concluded that feeding a moderate plane of nutrition at the beginning followed by a high level of nutrition appeared to be a good way to produce acceptable quality beef. Smith et al. (1977) reported that the placement of young cattle on moderate energy diets appears to be the best way to achieve the desired quality grade with a minimum of excess fat.

When viewing pasture versus grain feeding, Wanderstock and Matter (1948) and Meyer et al., (1960) found that cattle on pasture were poorer in palatability. Grain feeding is purported to contribute to overall eating quality and palatability of meat (Epley et al., 1968; Bowling et al., 1978; Skelley et al., 1978; Aberle et al., 1980; Dikeman et al., 1981). Beef produced by feeding grain is also

higher in marbling score (Reagan et al., 1977) and has higher sensory ratings for tenderness, flavor and overall satisfaction than meat from grass fed cattle (Bidner 1975; Bowling et al., 1977).

Results from the studies of Moody (1976) and Burson et al. (1980) claim that nutritional regime of cattle has little effect on beef palatability. However, these researchers agree that type of diet had a small influence on the organoleptic characteristics of beef.

Finally, the use of forage, either alone or supplemented with grain, allows the production of acceptable beef (Oltjen et al., 1976). It was demonstrated that cattle on high silage diets were as efficient converters of available energy to gain as were those fed high corn levels. Moreover the production of acceptable beef by using high forage diets is feasible if cattle are fed to a minimal carcass grade of Good or supplemented with grain (Reagan et al. 1977).

Time on Feed

Little et al. (1967) reported dressing percentages, marbling scores, ribeye area, internal fat, carcass weight and percentage fat trim all increased significantly as the feeding period increased. Palatabillty improved when feeding a high energy diet before slaughter, but the duration is short and little benefit is obtained from prolonged feeding. Dolezal et al. (1982) studied the feeding of steers for periods of 100 to 230 days and found few differences in palatability between rib steaks from carcasses of different USDA quality grades. These authors concluded that the extended time on feed beyond 100 days provided little additional palatabillty assurance. They also reported that the Choice grade could

be lowered with no appreciable loss in palatability if the stipulation was made that cattle had been fed a high concentrate diet for at least 90 days.

A study by Zinn et al. (1970a) comprised 100 Hereford steers and 100 Hereford heifers of approximately 250 (± 45) days initial age that were slaughtered at 30 day intervals over 270 day feeding period. These researchers found shear force values for all muscles (*triceps brachii, longissimus* and *semitendinosis*) to be lower ($P<0.05$) at 150 and 180 days when compared to other time periods. There was an interaction between time on feed and animal age. The first 180 days had a beneficial effect on tenderness, but beyond this time frame, age exerted a significant negative influence. In addition, marbling score and carcass grade increased significantly up to 240 days on feed. The data indicated that steers and heifers deposited intramuscular fat at a similar rate, and that the deposition of intramuscular fat is not a continuous process but proceeds in a stepwise pattern at 60 to 90 day intervals.

In a similar study, Tatum et al. (1980) examined young steers from a commercial feedlot that were fed identical high concentrate finishing diets for 100, 130 and 160 days. Results indicate that increasing fed time from 100 to 160 days had a beneficial effect on flavor desirability but did not significantly effect juiciness, tenderness or overall palatability. However, palatability of steaks is generally increased with marbling although increase in palatability was not consistent with higher marbling scores, nor were these results statistically significant. Steaks from the highest grade (average to high Choice) were more juicy, more flavorful and more desirable in overall palatability ($P<0.05$) than steaks from the lowest grade (low Good and high Standard).

Miller and coworkers (1987) tested two pre-finishing dietary treatments (low energy or high energy) on 8 month old calves for 6 months and then assigned these steers to a finishing diet for 56, 112 or 168 days. Following slaughter at 20 months, steers backgrounded on high energy diets produced heavier ($P<0.01$) and fatter ($P<0.01$) carcasses with higher ($P<0.01$) marbling scores and quality grades. *Longissimus* steaks were lower ($P<0.05$) in ease of fragmentation, less ($P<0.05$) detectable in connective tissue and higher ($P<0.05$) in tenderness ratings as compared to steers on low energy diets.

Breeding

Differences among breeds have become very important in beef production systems not only from an economic standpoint but also from a palatability standpoint.

According to Gerrard (1951) the breed type Aberdeen Angus has been regarded as a premier breed for good quality meat. The carcass gives a high proportion of the cuts which are most in demand, there is usually a substantial amount of intramuscular fat and the eating quality of the flesh is excellent; on the other hand the carcass is relatively light. Because of the small carcass such meat is relatively expensive.

Breed type has an influence on the growth rate of various carcass tissues (Fortin et al., 1981). These scientists noted that Angus cattle had a growth rate for fat to muscle, higher than that for Holstein cattle when placed on high energy diet. Gregory and coworkers (1978) reported that there were important differences in carcass composition among breeds which appear to be due to an additive effect of genes independent of those breed effects on growth rate.

The comparison of palatability and muscle characteristics of steaks from steers of various percentages of Brahman and Angus breeding slaughtered at similar stages of subcutaneous fat deposition was conducted by Johnson et al. (1990). Their findings revealed that steaks from steers with 1/2 or higher percentage Brahman breeding were less tender than steaks from 1/4 or lower percentage Brahman. Percentage Brahman did not affect flavor, juiciness or incidence of off-flavor.

Quality and yield grade are also influenced by breed type. Bidner et al. (1976) studied growth and carcass traits of Angus, Brahman and backcrosses and found that quality grade and yield grade were higher for Angus, intermediate for Angus x Brahman crosses and low for straight bred Brahman animals. However, Marion et al. (1980) showed no quality grade differences between British and large type cattle. Knapp et al. (1989) slaughtered steers and heifers selected to represent the following cattle types: English steers and heifers as well as exotic steers and heifers (<50% Bos indicus steers and heifers and >50% Bos indicus steers). They found no differences in USDA quality grades among the cattle types and the differences in the mean scores were not large.

Cundiff and associates (1981) used animals of equal age, and found that breed groups differed significantly in marbling. They used Herefords, Angus, Charolais, Jersey, Limousin, Simmental, South Devon, Red Poll, Brown Swiss, Gelbveih, Chianina, Sahiwai and Brahman sires on Hereford and Angus dams. Their observations indicate that Jersey, Red Poll, Hereford, Angus and South Devon crosses had the most marbling (Small+ and above) whereas Chianina, Limousin, Brahman, Sahiwai and Simmental crosses had the least amount of marbling (Small- and below). Similar differences in marbling among breed

groups as reported by Cundiff et al., (1981) were also observed by Koch et al., (1976).

Numerous scientists have reported widely varying effects of breeding on palatability. Koch et al. (1979) stated that there were small differences in palatability of rib eye steaks among breed groups where animals were of similar age and had been raised under similar feeding and management conditions. No differences in tenderness among breeds using *longissimus dorsi* muscle were reported by Freeden et al. (1972) who worked with Hereford, Holstein and Angus crosses. Also, no differences in palatability of *semitendinosis* steaks were observed between biological types (Marion et al., 1980). Suess and associates (1966) reported no significant differences in *longissimus* muscle tenderness due to sires while working with Angus cattle. Furthermore, breed groups did not significantly affect taste panel palatability scores of rib steaks from Hereford, Limousin and Simmental crossbred cattle as observed by Dikeman and Crouse (1975).

Breed of sire did not affect tenderness, but significantly influenced juiciness (Cover et al., 1957). These researchers also observed that sires within breeds had no effect on juiciness, but did have significant effects on tenderness. Sire effects for tenderness has been reported by Wilson and coworkers (1969) in studies about the influence of sire upon carcass traits of beef cattle.

Breed differences in sensory quality of Charolais and Angus steers were detected for rib eye steaks from carcasses at light slaughter weight (Barber et al., 1981). Lightweight Charolais (516 kg) received lower flavor and juiciness scores than lightweight Angus (409 kg) steaks. Adams et al. (1977) also concluded that

steaks from Charolais crossbreds were less juicy and lower in overall acceptability than steaks from Angus crossbred steers.

A summary of differences existing due to breeding in terms of palatability is as follows: Angus purebreds have higher quality grade, marbling, kidney, pelvic and heart fat than Hereford straightbreds (Cole et al., 1963; Ramsey et al., 1963; Glimp et al., 1971). Hereford cattle gain faster (Glimp et al., 1971), have higher feed efficiency and larger loin eyes than Angus steers (Cole et al., 1963). Loin eye steaks from Hereford carcasses are higher in palatability than steaks from Angus carcasses (Ramsey et al., 1963), and the steaks from Angus carcasses received higher taste panel flavor and juiciness ratings than those from Charolais carcasses (Barber et al., 1981).

Brahman steers have to be fed longer, gain slower, and have lower carcass weight and quality grade than Brahman x British crosses (Cole et al., 1963). They also have lower marbling scores than British breeds (Young et al., 1978). Moreover, Brahman rib eye steaks are the least tender of all the breeds (Ramsey, et al., 1963).

Charolais, Simmental and Limousin crossbreds, when compared with Hereford and Angus crossbreds, have the following characteristics: faster gain (Smith et al., 1976b); lower fat cover, less trimmable fat, higher yield grade, lower quality grade and larger ribeye area (Adams et al., 1977; Maino et al., 1981). Charolais rib steaks are less juicy and lower in overall acceptability than steaks from Angus crossbred steers (Adams et al., 1977).

Hereford and Angus crossbreds exhibit the following characteristics: greater average daily gain and feed conversion than straightbred Angus (O'Mary et al.,

1979); greater fat cover, higher marbling and quality grade and lower yield grade than larger breeds (Young et al., 1978).

Jersey cattle, when compared to other British breeds, have the following characteristics: smaller size and slower growth (Smith et al., 1976b); the most fat cover (Young et al., 1978); the highest percentage of kidney, pelvic and heart fat and ribeye fat (Koch et al., 1976; Young et al., 1978); the smallest ribeye area (Koch et al., 1976; Young et al., 1978); the lowest yield grade (Young et al., 1978). Rib eye steaks from Jersey carcasses are the most tender of all the breeds, as detected by taste panel and Warner-Bratzler shear forces (Koch et al., 1976), and they have higher palatability than those from Angus carcasses (Ramsey et al., 1963).

Carcasses from Holstein cattle have the lowest quality grade, marbling and fat cover (Cole et al., 1963), the smallest percentage of internal fat and the highest cutability (Young et al., 1978), when compared with British breeds. Steaks from Holstein carcasses compare favorably with other breeds and are accepted by consumers from the palatability standpoint (Zeigler et al., 1971).

Seideman and Koohmaraie (1987) investigated *Bos Taurus* and *Bos Indicus* breeding among steers and the relationship of carcass and meat characteristics to tenderness. These cattle were of 16-17 months of age. Their findings indicate that sarcomere length and collagen content and solubility were not significantly related to shear force values or sensory tenderness ratings. The fragmentation index was highly correlated to sensory tenderness ratings ($r = 0.60$) and shear force values ($r = 0.53$).

Wipple et al. (1990) evaluated attributes affecting *longissimus* muscle tenderness in *Bos indicus* and *Bos taurus* cattle. Steaks from Hereford x Angus

(HxA) had lower (P<0.05) shear force values and higher (P<0.05) sensory scores for tenderness at 1 and 14 days postmortem than steaks from 3/8 and 5/8 Sahiwal x Angus x Hereford (SAH) cattle. Correspondingly, 5/8 SAH had lower (P<0.05) myofibril fragmentation indices than HxA at 1, 3, 7 and 14 days postmortem. Breed cross effects were not significant for sarcomere length, fiber types, soluble and total collagen, cathepsin B and L, and calpain I and II activity and calpastatin activity. For HxA cattle, SDS-PAGE revealed that by day 1 desmin had been subjected to proteolysis and by day 14 could not be detected, but a 30,000 dalton component was clearly evident. Desmin remained visible in 5/8 SAH at 14 days postmortem and without a 30Kd component appearing. These researchers concluded that calpastatin activity in SAH did not decline as much by 24 hrs as it did in HxA. It has also been suggested that in the presence of sufficient Ca^{2+}, calpain I may undergo autolysis and therefore autolysis of calpain I may have been greater in SAH cattle (Crawford et al., 1987; Whipple et al., 1990).

Sex

Sex affects both live characteristics of animals and palatability. Marion et al. (1980) observed that steers were heavier than heifers at birth although no significant differences were noted on weaning weight gains. Live weight gain and feed conversion for steers and heifers were not significantly different (Hedrick et al., 1969). Smith et al. (1989) showed that feeding bulls at a constant energy diet produced low Choice beef that was quite palatable, although yield of edible product was reduced. Bulls fed by a two-phase regimen produced high Select, greater yield and similar palatable beef when implanted with both zeranol and estradiol.

Bulls have been shown to make markedly efficient gains than steers (Bailey et al., 1966, Arthaud et al., 1977) and show more efficient conversion of feed to lean (Hedrick et al., 1969). Steer carcasses had superior marbling scores and higher quality grades than bulls (Arthaud et al., 1977). Bull carcasses averaged middle and high Good whereas steers averaged low Choice because steers had more marbling, finer texture and a more desirable color of lean than bulls.

Hedrick et al. (1969) found no consistent differences in quality grade between steer and heifer carcasses. No differences in percentage retail yield due to sex were observed by Suess et al. (1966). Wilson et al. (1969) demonstrated that heifer carcasses had lower percent cutability and higher marbling scores than steer carcasses. Conversely, Thrift et al. (1970) found no sex differences in marbling scores.

Crouse et al. (1991) examined the relationship of muscle fiber size to tenderness of bulls and steers at 15 months of age. Animal gender was found to be non-significant. Muscle fiber size was said to play a key role in tenderness but prior to postmortem proteolysis. They concluded that postmortem proteolysis during storage eliminates the effect of fiber size on tenderness observed soon after slaughter.

Palatability of Bull and Steer Beef

It is a well documented fact that bulls have up to 15% advantage in growth rate, feed efficiency, and carcass leaness when compared to steers (non-implanted) at the same age or time on feed. (Seideman et al., 1982). There is approximately, a 50% reduction in growth rate etc. when this comparison is made with implanted steers (Brethour, 1982). Bull carcasses are leaner (Pearson, 1966)

with less fat than steer carcasses (Fredeen et al., 1971; Arthaud et al., 1977). They also ranked higher for ribeye area, dressing percentage and retail cut yield (Champagne et al., 1969).

Despite these major advantages, eating quality of bull beef has achieved a stigma with the immediate thought of "tough meat" as the over-riding factor in consumer selection. Indeed, this has been the case as reported by high Warner-Bratzler shear force measurements and lower sensory panel ratings for tenderness (Cahill, 1964; Field 1971; Forrest, 1975). In recent times, the trend to market younger animals has gained tremendous popularity. In addition, consumer demand for lean beef has opened a channel for marketing young lean bull beef.

Reviews by Field (1971) and Seideman et al. (1982) on the palatability traits of bulls versus steers indicate that rib eye steaks from young bull carcasses are generally acceptable even though their tenderness ratings may be slightly less than that of steers. Data on the palatability of rib eye steaks from young bulls versus steers has been variable though. Reagan et al. (1971) concluded that rib eye steaks from steers were more tender, more flavorful and rated higher for overall satisfaction. Arthaud et al. (1977) reported more desirable sensory evaluations for steer beef in terms of tenderness, juiciness and flavor when compared to bulls. On the contrary, Klastrup et al. (1983) and Vanderwert et al. (1985) found no significant difference in sensory evaluation between steaks from bulls versus steers. Scientists in the USA (Tatum, 1981 and Dikeman, 1987) have maintained that a value of approximately 3% ether-extractable lipid is necessary to provide juicy, tender beef. However, in a Danish study, high levels of tenderness, juiciness, and flavor were found in grilled steaks averaging only 1.6% extractable lipid

(Liboriussen et al., 1977). Rib steaks from young bulls of various breeds used in their study averaged between 7 and 8 (on a 10 point scale) for tenderness, juiciness and flavor. Dransfield (1984) showed only one score to be lower ($P<0.05$) between bulls and castrates—that of juiciness of roasted *M. longissimus* from bulls.

The reduced tenderness of bulls has been attributed to an increased amount of cross-linked collagen (Boccard et al., 1979; Gerrard et al., 1987) and lower amounts of fat cover (Crouse et al., 1983; Riley et al., 1983) the latter leading to a higher incidence of cold induced toughness. Rate of carcass chilling is considered to be the principal reason for tenderness differences between bulls and steers. However, following electrical stimulation (Crouse et al., 1983; Riley et al., 1983) and aging, bull beef was still found to be relatively tough, thus, cold shortening could not be attributed to the cause. Work by Boccard et al. (1979) claim that the concentration of collagen is higher in bulls while Hinch and Thwaites (1982) and Gerrard and coworkers (1987) mention that a difference in the type of collagen has been found; bulls having a lower concentration of heat soluble collagen (i.e. an increase in cross-linked collagen). The concensus view from many papers (Cross et al., 1973; 1974; Liboriussen et al., 1977; Hedrick et al., 1969; Jacobs et al., 1977) is that young entire males are of comparable eating quality to that of their castrated counterparts and that toughening due to collagen is only significant when extremes of age are compared. Aberle et al. (1981) suggested that a short feeding period of a high energy diet may improve tenderness by increasing the proportion of heat soluble collagen and elevate protein synthesis and turnover rates. This in turn would provide a layer of fat to serve as insulation against cold shortening.

Marbling and Maturity

Marbling and maturity are subjective evaluations used to determine USDA beef quality grades. The amount of marbling in the ribeye has always received primary importance in the grading system (Bray, 1964). Reviews by Blumer (1963), Bray (1964), and Parrish (1974, 1981) showed low correlations between marbling and palatability. Parrish (1981) reported that marbling was positively and significantly related to palatability but of a low magnitude. Variations in the tenderness ranged from 36% (Kropf and Graf, 1959; Doty and Pierce, 1961) to less than 1% (Goll et al., 1965) that could be accounted for by marbling. Variations in flavor accounted for by marbling range from 4% (Simone et al., 1959) to less than 1% (Dryden and Marchello, 1970).

Marbling is more strongly related to juiciness than tenderness. Variation attributes of marbling range from a high of 67% (Doty and Pierce,1961) to a low of less than 1% (Goll et al.,1965; Suess et al.,1966). Campion et al. (1975) and Crouse et al., (1978) indicated that marbling accounted for 6-9% of the variation in juiciness.

Results published by Gilpin et al. (1965) and Goll et al. (1965) indicate that marbling had no effect on tenderness, juiciness or flavor of rib steaks. McBee and Wiles (1967), however, showed that as marbling scores decreased, tenderness, juiciness and flavor of loin steaks also decreased.

Parrish et al. (1973a), used different end point-temperatures (60, 70 and 80°C) and different marbling degrees in relation to palatability of beef steaks. These researchers concluded that 'Slight', 'Modest' and 'Moderately Abundant' degrees had no significant effect on palatability attributes but that steaks cooked to higher degrees of doneness were less palatable. No significant difference in

panel tenderness and shear force was obtained between steaks with 'Slight' and Moderate amounts of marbling by Romans et al. (1965). Campion et al. (1975) suggest that 2.9% fat in the longissimus muscle was sufficient for desirable acceptability. This percentage is said to correspond to the Slight degree of marbling.

Two researchers (Smith and Carpenter, 1974) have stated that steaks with higher degrees of marbling provide "insurance" in maintaining tenderness when high temperature cookery is employed and when meat is cooked until it is medium done. It is also possible that marbling acts as a heat conductor resulting in higher internal cooked meat temperatures before the meat appears to be cooked to an advanced degree of doneness. Berry and Leddy (1990) also arrived at this conclusion stated that steaks cooked on electric broilers require longer cooking times (and subsequently more cooking loss) to reach 70°C when steaks had less marbling. Because steaks with less marbling have more moisture, the longer cooking time and additional cooking loss may be the result of evaporative cooling during cooking. There are four theories regarding the mechanism by which marbling influences tenderness (Savell and Cross, 1987): bite theory-marbling decreases density by replacing protein with lipid; strain theory-increased marbling results in connective tissue membranes becoming thinner and less resistant to fragmentation; lubrication theory-increased lubrication of muscle fibers increases juiciness and sensation of tenderness; and as mentioned above, insurance theory.

Carcass maturity has also been used to determine beef carcass quality grades. Rib steaks from young animals have been reported to be more tender than most mature carcasses (Goll et al., 1965; Berry et al., 1974). The decrease in

tenderness of older animals has been attributed to a decrease in collagen solubility (Ritchey and Hoststler, 1964), the adhesion properties of muscle fibers (Berry et al., 1974) and the MFI as indicated by the significant difference observed between carcass of A maturity and all other maturity carcasses (Parrish et al.,1979).

Tuma and coworkers (1963) reported that the greatest difference in tenderness of rib steaks was between 18 and 42 months of age. Goll et al. (1965) studied rib steaks from A, B and F maturity carcasses and found that A and B maturity were more tender than those from F maturity carcasses. The above research workers did not find any significant differences in juiciness and flavor due to maturity. Therefore, it appears that maturity of carcasses does not have any significant effect on either juiciness or flavor but does affect tenderness.

In the study by Berry and Leddy (1990), sensory, shear and cooking properties of steaks varying widely in marbling that resulted from the use of a rapid restaurant method of broiling were compared to those properties obtained with a standard research broiling method. Loins from the bottom third of six marbling groups, Moderately Abundant, Slightly Abundant, Moderate, Modest, Small and Slight were rapidly broiled (restaurant type-charbroiled) to a visual end-point of medium to medium-rare degree of doneness and compared with broiling on an electric broiler to a final internal temperature of 70°C. Differences between broiling methods were generally more pronounced in steaks of lower marbling. Rib steaks with Slight marbling and broiled by charbroiling had tenderness, juiciness and flavor scores equivalent to or higher than those of steaks with Moderate marbling broiled by electric broiling procedure.

An extensive study was carried out by Smith et al. (1984) on the relationship of USDA marbling groups to palatability of cooked beef. As marbling increased from practically devoid to moderately abundant, loin steaks were more palatable about 2/3 of the time, round steaks were more palatable about 1/8 of the time and loin steaks were more likely to be assigned high panel ratings and to have low shear force values. However, increases in marbling from Slight to Moderately Abundant (A maturity) and from Small to Moderately Abundant (A and B maturity) had little or no effect on percentage incidence of loin or round steaks with panel ratings <2.99 or >4.00 or with shear values >6.35kg or <4.99kg. Differences in marbling explained 33% (loin) and 7% (top round) of the variation in overall palatability ratings in A, B, C, and A and B maturity carcasses, respectively.

Smith et al. (1987) in yet another study determined the relationship of USDA quality grades to palatability of cooked beef. Prime carcasses produced loin and round steaks that were more palatable ($P<0.05$) than were those from carcasses of Choice through Canner (7 grades) in 85.7% of the comparisons and from carcasses of Choice through Standard (3 grades) in 69.0% of comparisons; comparable percentages were 71.4% (6 grades) and 42.9% (2 grades) for Choice and 74.3% (5 grades) and 35.7% (1 grade) for Good. Among Prime through Standard carcasses, grade predicted flavor, tenderness and overall palatability of loin steaks with 30-38% accuracy, but could explain no more than 8% of the variation in panel ratings or shear force values of round steak.

De Pulgar (1982) found that the palatability of rib steaks between Slight $^{0-49}$ and Slight$^{50-100}$ marbling, and Slight marbling with equal, greater than or less than 0.76cm of fat cover showed very little difference in palatability. Steaks with

Small marbling were only slightly, but more significantly tender, flavorful and more desirable than steaks with Slight marbling.

Post Mortem Changes in Skeletal Muscle

It is now pertinent to review myofibrillar protein degradation with reference to tenderness. Tenderness is probably the most important attribute rated by the average consumer when judging eating quality (Lawrie, 1985).

An obvious change which occurs in skeletal muscle postmortem is the development of rigor mortis, i.e., the stiffening of the musculature after death. The factors which influence the time course of rigor have been described by Bendall (1980). At least four definitions of rigor have been used. The most commonly accepted definition of rigor is based on ATP content. Thus, a muscle devoid of ATP is said to be in rigor. British researchers favored extensibility of muscle postmortem as an indicator of rigor. As a muscle begins to lose its extensibility, it is said to be in the onset phase while full rigor is characterized by total absence of any extensibility. This definition however, precludes the possibility of the resolution of rigor, because, once lost, extensibility cannot be restored. Busch et al. (1972) associated the phase of rigor with the ability of isolated muscle strips to develop and maintain isometric tension. At 2°C, isometric tension developed at the same time postmortem that other researchers had observed a decrease in extensibility. The ability to maintain isometric tension was gradually lost and interpreted as the resolution of rigor. No ability to maintain isometric tension was observed if muscle strips were stored at 16° or 37°C. Goll et al. (1974) have described the relationship between postmortem tenderness of muscle (meat) and rigor. Excised bovine muscle stored at 2°C at first became less tender and then

became more tender. The resolution of rigor is probably one of the first changes noticed influencing tenderness.

Another change that occurs in the myofibril during postmortem aging is the degradation of the Z-line. Also noted is the myofibril fragmentation in the structural region of the Z-disc (Parrish et al., 1973b; Olson et al., 1976) and smaller myofibril fragments in tender than tough meat samples. Olson and Parrish (1977) were able to find a strong relationship between myofibril fragmentation index (MFI), a term based on the premise that the amount of light absorbed by a myofibril suspension will be proportional to the relative size of myofibrils and fiber pieces in the suspension and tenderness. The effect of postmortem storage and temperature on MFI and Warner-Bratzler shear values on different muscles are also presented by Parrish (1977). The MFI was found to account for 50% of the variation in tenderness of *longissimus* muscle from A-maturity carcasses.

Subsequently, Olson et al. (1977) were able to demonstrate by means of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) limited and specific proteolysis in bovine muscle undergoing postmortem storage. There was no change in the major myofibrillar proteins except for a very subtle change, the simultaneous disappearance of troponin-T and the concomitant appearance of a 30,000-dalton component was observed. This intriguing finding was shown to occur only in tender and not tough bovine *longissimus* (MacBride and Parrish, 1977). Ouali et al. (1983) noted the resolution of new bands of unknown origin between tropomyosin and myosin light chain 1 in bovine *sternomandibularus* stored for 6 days at 15°C. Their molecular weights ranged from 27,000 to 33,500. The main band corresponded to the 30,000 component reported by Olson et al. (1977).

The relatively recent discovered myofibrillar/cytoskeletal proteins have also been implicated in meat tenderness. Evidence has brought to light the existence of a well-defined cytoskeletal framework in the muscle cell (Lazarides, 1982; Robson et al., 1981). This filamentous cytoskeleton is largely made up of titin, nebulin and desmin. An increasing variety of other proteins seemingly associated with the cytoskeleton are being discovered and characterized (Greaser et al., 1981).

Desmin, sometimes referred to as skeletin or 10nm filament protein, is a constituent of the salt-insoluble fraction of muscle. These filaments *in vivo* are thought to have a structural role in skeletal muscle. Immunological evidence (Lazarides and Hubbard, 1976) showed that desmin is present in the periphery of each Z-disc, forming a honeycomb network of collars within the Z-plane that presumably maintains the alignment of adjacent sarcomeres and unifies the contractile actions of all separate myofibrils.

Wang and co-workers (Wang et al., 1979) identified three very large polypeptides in striated muscle samples by SDS-PAGE. The largest two migrated as a closely spaced doublet, titin-1 (T_1) and titin-2 (T_2), of similar molecular weight, 1.4×10^6. Third band, later given the name nebulin, had a molecular weight of 5.5×10^5 and was shown to be associated with the N_2 line of the sarcomere. Before titin was discovered, Maruyama and associates (Maruyama et al., 1976) isolated a very insoluble, elastic-like protein from muscle and called it "connectin". Connectin was of a highly heterogenous composition containing differing amounts of several proteins, including titin, nebulin, myosin, desmin, actin and some connective tissue. Maruyama et al. (1981) were able to show that some of their high molecular weight polypeptides (with specific reference to the doublet)

were actually titin. Maruyama et al. (1977) also found that a large portion of this protein did not penetrate into a 10% gel indicating that it was extremely large and insoluble due to the exhaustive extraction procedure with strong alkali, urea and hot phenol.

Due to the sensitive nature of the protein titin, Wang (1982a) found it a difficult task to produce reproducible results. Titin was susceptible to rapid degradation both with and without SDS and due to its large size, extremely immobile on SDS gels.

Monospecific antibody localization suggest that within the sarcomere, titin is located at the junction of the A-I bands, M-line, Z-line and possibly throughout the entire A-band. Wang (1982b) proposed that the transverse structures contained titin and that these structures were attached to the ends of the thick filaments. Titin is said to connect Z-line to Z-line and are elastic along the entire length, except where they interact with inelastic structure, e.g., M-line or thick filaments. Titin may also form the scaffolding within the sarcomere for other proteins to be assembled.

Nebulin is a high molecular weight ($6x10^5$), highly insoluble myofibrillar protein. Immunoflourescence studies indicate that nebulin is located in the I-band of the myofibril at the level of the N_2-line. It may also be attached to longitudinal, elastic filaments in the sarcomere, e.g., titin. It has been shown that nebulin binds to α-actinin (Nave et al., 1990) and to F-actin (Jin and Wang, 1991). Thus the possibility of nebulin attaching or anchoring the actin filament to the Z-line does exist (Robson et al., 1991).

Both titin and nebulin are susceptible to degradation by proteases including calcium activated factor (CAF), (Wang and Ramirez-Mitchell, 1983). Lusby et al. (1983) have shown that titin is degraded in postmortem bovine *longissimus*

muscle. Wang et al. (1979) observed that the proteolytic degradation of titin is always accompanied by the conversion of T_1 and T_2. Paterson and Parrish (1986) reported that the degradation of titin coincides with increased tenderness scores and decreased Warner-Bratzler shear values in beef *infraspinatus* muscle. Their results also indicate that tender muscle contains less titin and nebulin. Using bovine *longissimus* muscle, Anderson and Parrish (1989) detected differences in titin content between tough and tender steaks. Fritz and Greaser (1991) reported *psoas major* muscle to have less than 1% of the four anti-titin bands per sarcomere at 45 minutes postmortem but increased to 65% at 48 hrs after death.

It is interesting to note that Locker (1976) views the gap filaments and their destruction of prime importance when considering tenderness. It was Locker and Wild (1984) who postulated that nebulin, rather than being the protein of the "N" line, is present in the gap filaments together with connectin and that it is the breakdown of nebulin which accounts for the lability of the gap filaments in conditioning.

Dark Cutting Beef

Dark cutting beef, also referred to as DFD (dark, firm, dry) beef is a meat quality problem caused by chronic stress preslaughter which depletes muscle glycogen stores (Fisher and Augustini, 1979; Warriss et al., 1984). The meat is dark in color (MacDougall and Rhodes, 1972), more prone to bacterial spoilage (Newton and Gill, 1980), has reduced flavor (Dransfield, 1981) and is therefore discriminated against by the retail trade and consumer (Tarrant, 1981).

Extent of pH Change in Muscle

Glycogen metabolism in beef muscle is similar to that in monogastric species, despite differences in the nature of dietary supply of energy (McVeigh and Tarrant, 1982). Glycogen breakdown in muscle is under dual control by the sympathetic and somatomotor nervous systems which exercise their control by increasing contractions by means of the key intracellular messengers, cyclic AMP and Ca^{2+}, respectively. Cyclic AMP activates protein kinase and Ca^{2+} activates phosphorylase kinase, two enzymes that regulate sequential steps in the so called 'glycogenolytic cascade'. Thus there is an inverse relationship between the amount of glycogen at death and the final pH of muscle.

In beef, physical exercise before slaughter is by far the most common cause of dark cutting (Tarrant, 1987). The strenuous activity associated with handling, regrouping, transport, mounting, etc, greatly accelerates glycogen breakdown, which is activated by the rise in free Ca^{2+} concentration in the sarcoplasm that triggers muscle contraction. In ruminants, the preferred substrate for energy is free fatty acids (FFA). Therefore under normal circumstances FFA are utilized for energy and when work load is demanding, the ATP/ADP ratio drops such that glycogenolysis is stimulated through the action of the sympathetic nervous system. Where an increase in energy demand cannot be met by fat metabolism, the required increase in glucose would need to be met by local stores of glucose/glycogen and ultimately, lead to muscle fatigue and depletion of these stores (Lister et. al., 1981). Chatecholamines are integrants of the sympathetic nervous system. Tarrant (1981) reported that not only do stress susceptible animals release more chatecholamines but also react more readily to them when compared to normal animals.

As expected, it is the working muscles that are depleted of glycogen and become dark cutting. These are principally M. *longissimus dorsi* and M. *semitendinosus*, with other major hindlimb muscles being affected to a lesser degree (Kenny and Tarrant, 1984). In these working muscles it is the fast twitch fibers that lose their glycogen more rapidly, suggesting that they are recruited in preference to slow twitch fibers, which undergo a more gradual loss of glycogen. In contrast, the reverse situation prevails when the andrenergic mechanism is activated. Adrenaline injection demonstrated that slow fibers rapidly and completely exhaust glycogen reserves, whereas the fast fibers are only partially depleted. Zereola and Stickland (1991) concluded that slow twitch oxidative fibers were proportionately greater in DFD bulls than DFD steers. Zereola and Stickland (1991) showed that normal muscles with a high proportion of red fibers (assessed by relative area on histochemical slides) tend to have higher ultimate pH, although differences in pH between muscles were small in their study. These authors observed a higher percentage of αR (fast twitch red fibers with high oxidative activity) in their muscles. This relationship between αR fibers and DFD does not hold true in all instances, since the outer part of the *semitendinosis*, which contains less αR fiber than the *gluteus medius* muscle shows a high pH in dark cutting carcasses.

Howard and Lawrie (1956) found it most difficult to deplete the glycogen reserves in cattle, even after pre-slaughter exercise and fasting for 14 days were combined. Yet such depletion occurred, without fasting, if enforced exercise took place immediately after travel. Howard and Lawrie (1957) also undertook a study whereby cattle were injected with neopyrithiamin after fasting. Neopyrithiamin inhibits thiamin pyrophosphate and, thereby, fat oxidation. Whereas fasting *per se*

had little effect on lowering the glycogen level of muscle, its combination with neopyrithiamin lowered it markedly, leading to an average ultimate level of pH 6.1. Using this same train of thought, Lister and Spencer (1981) induced dark cutting characteristics in cattle by administering antilipolytic agents such as nicotinic acid, after exposure to isoprenaline (a β-adrenergic antagonist which promotes lipolysis and glycolysis). The above effect is nullified by simultaneous administration of caffeine (which stimulates lipolysis and spares glycogen). Therefore, caffeine administration may be potentially used as a prophylactic to conserve muscle glycogen in animals exposed to stress.

Factors other than fatigue and inanition controlling the level of muscle glycogen have been suggested by Petaja (1983). Well fed and rested steers, yielded meat of high pH. It appears that some animals are of an excitable temperament. In such animals, short range muscular tension, not manifested by external movement, reduce glycogen reserves to a chronically low level (Howard and Lawrie, 1956). Muscles also differ in their susceptibility to pre-slaughter glycogen depletion by stress. Warris et al. (1984) demonstrated that the ultimate pH of *psoas major* tended to be lower than that of *longissimus dorsi*; whereas Howard and Lawrie (1956) reported the reverse.

Consequences of DFD

When the ultimate color pH of meat is high, the surviving activity of the cytochrome enzymes is greater (Lawrie 1956). Muscle proteins are considerably above their iso-electric point, and thus, water in the muscle will be associated with them causing the fiber to be tightly packed and present a barrier to diffusion. As a result, the layer of bright red oxymyoglobin becomes vanishingly small and the

purple-red myoglobin pigment predominates. The high pH alters the absorption characteristics of the myoglobin and such a 'closed' structure causes the surface to appear dark.

Sensory Changes

Eating quality, judged as tenderness, juiciness and flavor, is affected by many production factors and by variations in rigor development and proteolysis in meat.

Tenderness

Tenderness of meat depends mainly on variations in myofibrillar proteins, connective tissue proteins and water and their interactions with cooking conditions (Dransfield, 1981). Alterations of pH by postmortem injections of lactic acid or ammonia (Winkler, 1939) produced minimum tenderness at pH 6.0 while pre-slaughter injections of epinephrine (Penny et al., 1963) increased ultimated pH and tenderness. More recently increases in tenderness associated with high ultimate pH have been shown by (Freeden et al., 1974; Yu and Lee 1986; Purchas, 1990). In beef *M. semitendinosis* heated to 80°C (MacDougall et al., 1979), toughening decreased linearly with increase in pH. However, the relationship varies between muscles and cooking conditions. Topside roast, sirloin roast and grilled rump revealed minimum tenderness occurred in the pH range of 5.8-6.1 (Bouton et al., 1957). A curvilinear relation for tenderness (Bouton et al., 1973; Dransfield, 1981; Purchas, 1990), has shown that toughness is exhibited at about pH 6.1.

Scientists such as Bouton et al. (1973) and Fjelkner-Modig and Ruderus, (1983) claim that a tenderizing effect is also experienced with high pH meat depending on the degree of muscle shortening and ageing. Bouton et al. (1973)

demonstrated increases in tenderness based on sensory and shear force measurements for *longissimus dorsi* muscle at pH 5.5-6.0 meat. It has been suggested by Purchas (1990) that increase in shear force values up to pH 6.2 were at least partly due to decreased sarcomere length. Evidence produced by Yu and Lee (1986) showed no significant difference in sarcomere length and shear force measurements for both hot and cold sides for three pH groups (5.5-5.7, 5.8-6.3, and 6.4-7.0). Although high pH meat was more tender than the intermediate group, these authors concluded that factors other than sarcomere length contributed to the tenderizatlon of meat.

Myofibrillar protein degradation patterns of meat based on pH and temperature have been studied by Yu and Lee (1986), Yates et al. (1983) and Samejima and Wolfe (1976). The above studies involved manipulation of pH either by acid or alkali, or, injection of epinephrine prior to slaughter in order to achieve a wide pH range. Yates et al. (1983) reported that alterations in troponin-T and α-actinin at pH 7.0 were the most noticeable. More troponin-T degradation occurred at pH 5.4 and 37°C than at pH 7.0 and 4°C. Authors Yates et al. (1983) also found a significantly lower amount of M and C-protein, 50-100Kd and 28-32Kd at pH 7 when compared to pH 5.4 at 37°C. Yu and Lee (1986) found the degradation of troponin-C, tropomyosin and α–Actinin, troponin-T and troponin-I at high pH accounted for enhancing tenderness of such meat (Penny, 1974; Suzuki et al., 1982). Drabikowski et al. (1977) measured the production of amino nitrogen in beef homogenates. Overall proteolytic activity was highest at pH 3.5 with little activity at pH 7.0.

One of the most striking microscopical changes that occur in postmortem muscle during aging is the weakening and subsequent disappearance of the Z-

lines. In 1972, Busch and coworkers described studies in which they observed the complete removal of Z-lines from rabbit skeletal muscle that had been incubated in a saline solution containing 1mM Ca^{2+} and 5mM Mg^{2+} for 9 hours at 37°C/pH 7.1. They also noted no other changes at the microstructural level of the myofibril. Later, a protein fraction was isolated which produced the described changes in the Z-line at calcium concentrations greater than 0.1mM and a pH of 7.0. This fraction was coined the name CASF (calcium activated sarcoplasmic factor) and a variety of other names including CAF and Calpain (Goli et al., 1985).

Two forms of Calpain exist. These forms differ in their requirement for calcium activation (μM quantaties required by Calpain I and mM quanties required by Calpain II). An inhibitor to CAF has been described by Shannon and Goll (1985) and Murachi (1989). One molecule of the inhibitor has been shown to inhibit six moles of CAF. Larger quantities of the enzyme hydrolyze the inhibitor.

Penny (1974) demonstrated that the proteinase used tropomyosin, α-actinin and troponin as substrates. Their results also showed that actin and actomyosin were not digested, but actomyosin was shown to have reduced Mg^{2+} activated ATPase activity after treatment with the enzyme, while Ca^{2+} activated ATPase activity appeared to be unaffected. Penny reported that the degradation of the Z-line results from the digestion of α-actinin, a structural protein of the Z-line. However, several studies have shown that CAF does not appear to actually degrade α-actinin (Goli et al., 1983; Goll et al., 1985).

Two other proteins that are removed at about he same rate as the Z-line are Troponin-T and desmin. It is unlikely that Troponin-T, as it is not located at the Z-line, would play a structural role in maintaining the the integrity of the Z-line.

Desmin, on the other hand, is located at the periphery of the Z-disc and its degradation may play a role in the removal of the Z-line (Goll et al., 1983).

Juiciness

Juiciness may be described as two organoleptic components--an initial impression of wetness during the first few chews and sustained juiciness, largely due to the stimulatory effect of fat on salivation. Such a distinction has not been made in studying DFD beef (Dransfield, 1981). Consumer trials in England (Dransfield, 1981) revealed that bull beef of normal pH was equal in juiciness to steer beef of normal pH and DFD bull beef and normal pH steer meat were equally juicy.

There is some suggestion that juiciness reaches a minimum when the pH level of meat is about 6 (Howard and Lawrie, 1956); this possibly reflects the greater ability of muscle proteins to bind water in this pH region; but, if this were the entire explanation, juiciness would be expected to decrease even further with higher pH levels. Purchas (1990) reported a curvilinear relationship for expressed juice based on pH.

Flavor

Bouton et al. (1957) first reported that the higher the ultimate pH, the lower the flavor intensity as determined by taste panel. A suggestion put forward by Lawrie (1981) for this reduced flavor is that swollen structures of high pH interfere with the access of the palate to flavor substances. This phenomenon may play some role in reducing the flavor of DFD beef but it is by no means the only cause.

The weak flavor of DFD beef may result from the lack of carbohydrate expected in meat, producing little interaction with amino acids or proteins. The Maillard reaction, which forms the base of certain meat flavor patents, is thus limited.

Microbial Spoilage

DFD meat is characterized by a high ultimate pH (>6.0) but it also contains little or no glucose. In normal meat, glucose is preferentially utilized by meat bacteria.

The rapid spoilage of DFD meat has been thought to be due to accelerated growth of spoilage microflora at the higher ultimate pH (Tarrant, 1976). This simple explanation can be at best only partly correct; as the growth rates of the major aerobic spoilage organisms of fresh meats; the pseudomonads, are unaffected by pH changes in the range occurring in meat. Fromm and Monroe (1965), Rey et al. (1976) and Newton and Gill (1978) claim that the only effect of elevated pH on growth of these organisms is a reduction in the lag phase, which has no significant effect upon the time required for spoilage to develop. However, certain other psychotrophic bacteria which are inhibited by the pH of normal meat are able to grow on meat at high pH. These include species of *Acinetobacter* and *Acteromonas putrifaciens.* In addition, the growth of *Enterobacter liquefaciens* and *Yersinia enterocolitica* in vacuum packaged meat is faster on DFD meat than on normal pH meat (McMeekin, 1977).

Under aerobic conditions, pseudomonads will degrade amino acids (glucose not being present) without delay. *Acinetobacter* spp. are unusual in that they

cannot utilize hexoses, so when growing on meat they will attack amino acids regardless of the presence of glucose.

Anaerobic conditions produced by vacuum packaging allows the growth of three facultative anerobes to dominate. These are *Yersinia enterocolitica*, *Enterobacter liquefaciens* and *Altermonas putrefaciens* (Seelye and Yearbury, 1979). *Yersinia enterocolitica* exhibits an accelerated growth on vacuum-packaged DFD meat but does not seem to cause any particular spoilage problems even though certain strains are known to be pathogenic to man and animals (Newton, 1979).

E. liquifactens produces odors at low cell densities on DFD meat. This only occurs when amino acids are metabolized and can be prevented by the addition of glucose. *A. putrifaciens* is responsible for the characteristic green discoloration limiting the storage life of vacuum-packaged DFD meat. Greening is a result of hydrogen sulphide production (Taylor and Shaw, 1977) from cysteine or glutathione and the reaction with myoglobin to form sulphmyoglobin.

Measures of Marbling

Intramuscular Fat

As in other fat depots, lipid in muscle tissue is present in groups of identifiable fat cells generally considered to be similar in origin to connective tissue cells (fibroblasts). They are situated mainly in the perimysium and also in the endomysium (Fjelkner-Modig, 1985). Lipid is also present in the muscle as intracellular lipid droplets (at higher concentrations in red than white fibres) and as an integral constituent of cell membranes (mainly phospholipids).

The major component of marbling fat is triglyceride, located in groups of fat cells in the perimysium of muscle. Triglycerides can be extracted with a solvent such as diethyl ether or hexane and indeed most published studies on muscle lipid have used this standard procedure. If analysis of total lipid is required i.e. including phospholipids in cell membranes, a more polar solvent is used such as chloroform-methanol (2:1) (Sahasrabudhe and Smallbone, 1983). The fat content of the *longissimus* muscle is determined as the weight loss from the dried sample after continuous extraction in diethyl ether for 8 hours and reported on a whole tissue basis. This standard method is an AOAC approved method.

Marbling is intermingling or dispersion of fat within lean. It is subjectively estimated on the lean cut surface of the ribeye muscle at the twelfth rib muscle surface. The grade standards specify more marbling for the higher grades and lower amounts for the lower grades. The USDA quality grades, from highest to lowest, are Prime, Choice, Select (previously Good), Standard, Commercial, Utility and Cutter (Boggs and Merkel, 1979). The amount of marbling in the rib eye muscle is divided into 10 degrees. These degrees from lowest to highest are (1) Devoid, (2) Practically Devoid, (3) Traces, (4) Slight, (5) Small, (6) Modest, (7) Moderate, (8) Slightly Abundant (9) Moderately Abundant (10) Abundant. The Standard chategory encompasses marbling from Practically Devoid to Traces; Select chategory, the Slight amount of marbling; Choice chategory, from Small to Moderate marbling,. and Prime to include Slightly Abundant and Abundant degrees of marbling. Photographic representations of these degrees of marbling to depict standard requirements are used as a base for such measurements. Thus, the measurement of marbling is subjective.

In actual practice, the Federal Meat Graders subdivide each degree of marbling into percentages in increments of 10 from 0-100% and the percentages are written as superscripts following the degree of marbling, e.g. Small^{10}, Small^{20} etc.

The quality grades for A and B maturity carcasses are USDA Prime, Choice, Select and Standard. For C and older maturities, the quality grades are Commercial, Utility and Cutter. Standard information with regard to texture and distribution of marbling is lacking. The distribution of marbling is reflected in terms of the uniformity or lack of uniformity of the intramuscular fat within the ribeye surface. Texture of marbling is indicated by the size of the dispersed fat pieces; the larger being considered coarse while minute pieces are considered fine textured. No scales have been reported to date with regard to the above mentioned factors. There is obviously scope for the development of such criteria to provide a more consistent means of assessing marbling distribution and texture.

Cross (1981), reported that it was impossible to define how the human evaluates marbling due to the complexity of the mind.. In his suggested scheme for the determination of quality and yield grade, the following factors are recommended for assessing marbling: (1) ribeye area, (2) number of pieces of intramuscular fat in the ribeye, (3) total area of the pieces of intramuscular fat in the ribeye and (4) distribution of intramuscular fat in the ribeye following subdivision of the muscle into 16 regions.

Instrumental Methods for Measuring Marbling

According to Brethour (1990), ultrasound has been used for over 30 years to estimate backfat thickness (Hazel and Kline, 1959) and ribeye area (Stouffer,

1959). The smooth boundary between tissues such as the layer between subcutaneous fat and muscle provides for ultrasonic reflections that can be monitored. By means of a transducer, ultrasonic signals are passed through tissues whereby material density reflections are scanned. A summary of the principles of ultrasound and measurement of intramuscular fat is given by Whittaker et al. (1992).

Very little published information exists on objective methods of measuring marbling. Haumschild and Carlson (1983), conducted an *in vitro* textural differentiation of marbling in beef using an ultrasonic Bragg scattering technique. They used a statistical analysis of the Fourier determined frequencies in a multivariate analysis of variance. These frequencies relate to the marbling spatial domain. Haumschild and Carlson (1983) concluded that Select (then Good) grade of marbling is the most statistically predictable, and that this particular grade is easier to grade ultrasonically is evident upon visual examination of the tissue. They also claim that marbling becomes more evenly distributed as the quality grade decreases. Therefore, with less signal attenuation, uniform marbling may be easier to classify. The 'Standard' grade may be less predictable for two reasons. There may be too little information in the fat that is available ultrasonically, or the ultrasonic window and pulse length may not be wide enough to incorporate a representative sample of this particular grade.

Most of the work using ultrasound scanning has focused on predicting subcutaneous fat depth and carcass fat percentage across a range of animals. Hopkins (1990) observed very high correlations of fat depth measured at the eye muscle of lamb with coefficients of 0.93-0.95. It has been reported by Bass et al. (1982) that the accuracy of the instrument is over a limited range of fatness.

Brethour (1990) claimed that the use of ultrasound speckle is related to marbling score in cattle. Speckle patterns (i.e. wave fronts from several scatters producing a random graininess or mottling) produced by coherent pulses are affected by scatter size and density as well as by interrogation frequency. Speckle scores in 11 of 14 groups of cattle (N=619) were highly correlated (P<0.001) with carcass marbling scores. Live animal speckle scores classified carcasses as Select or Choice with 77% accuracy. Similar accuracy was achieved as much as 148 days before slaughter by adjusting regression speckle scores with days on feed. Brethour (1990), further claimed that the result of the mathematical analysis of the ultrasound radio-frequency signal or pixal map statistics might reveal parameters that could improve accuracy and precision of the method and predict specific attributes of beef quality e.g. tenderness.

Duello et al. (1990) claimed that marbling correlations with ultrasound were generally low but significant for bulls and steers. They also mentioned that ribeye area and fat cover can be accurately measured with current equipment but refinement of the tissue characterization function is needed to measure intramuscular fat. On the other hand, Perry et al. (1990) used attenuation values to assure carcass marbling. Attenuation values estimated marbling scores within 1.5 marbling scores in 94% of the cattle and correctly identified cattle with a Small amount of marbling 80% of the time. Attenuation values were claimed to be able to determine when cattle would reach Choice marbling.

R^2-values have been generated by a number of writers. The use of ultrasound speckle by Brethour (1990) was found to be highly correlated (r=0.67) to marbling, but generally accounted for 25-30% of the marbling score variance. An attempt by Brethour (1990) to classify carcasses by grade led to a classification

accuracy ranging from 53-94%. Park and Whittaker (1990) reported R^2-values for ether extractable fat and ultrasound frequency analysis of Fourier spectra to be 0.76. Image processing algorithms developed by Chen et al. (1989) revealed that image processing data did not give direct measurements of percent fat in the ribeye. One apparent reason is that these methods measured the amount of fat on a two-dimensional surface, while the chemical method is a three-dimensional measurement. Rouse et al. (1992) reported R^2-values accounting for 41% and 46% of the variation in marbling score following quantification of histogram functions. On the other hand, Bullock et al. (1991) found high R^2-values (0.74) for marbling using carcass variables that included hot carcass weight, backfat thickness, round fat, ribeye area and kidney, heart and pelvic fat percentage estimates.

The only previous attempt to estimate intramuscular fat objectively in live cattle has been from ether extracts of muscle biopsies (Rouse et al., 1989). The percent ether extract data were used in a regression analysis that fit percent ether extract as a linear and quadratic function of days on feed. Their results indicate a significant ($P<0.02$) quadratic coefficient affecting fitting the linear effect. The model accounted for 41% of the variation observed in percent ether extract among steers.

Whittaker et al. (1992) report that the velocity of ultrasonic waves is dependant on several variables, including temperature, density and acoustic impedence. Velocity of ultrasound through beef fat and muscle is approximately 1,540 and 1580 meters per second, respectively. In non-homogeneous materials such as muscle, ultrasound velocity is not accurately determined due to the variable composition of beef muscle (Whittaker et al.,1992) and the variability of

marbling deposits (Blumer et al., 1962). Ultrasonic waves posses two types of velocity. The first, longitudinal velocity, is the velocity of the wave as it travels away from the transducer. The second, shear velocity, is the sideways motion of the wave. Whittaker et al. (1992) reported that shear velocity is important for characterizing the elasticity of a material e.g., shear modulus can be calculated from shear velocity. Shear modulus is an indication of tenderness in beef. This suggests that ultrasound may be used to predict tenderness non-intrusively.

Instrumental Evaluations of Tenderness

A number of instruments including penetrometers, cutting devices, masticatometers, extruders and shear devices have been used to determine tenderness of meat. The interest in these instruments was obviously to produce high reproducible data in order to quantify differences in the sensory properties of food. Despite the abundance in instrumentation, research to try and define exactly which parameter is being measured has led to a certain amount of confusion. The Armour Tenderometer did not prove to be a reliable indicator of tenderness (Dikeman et al., 1972; Parrish et al., 1973). Szczesniak (1973) pointed out that since poor correlations have been reported between instrumental and sensory evaluations, one should keep in mind the fact that instruments measure only a small portion of the entire realm of parameters being evaluated by human beings.

The publication by Szczesniak (1973) reviews some of the above mentioned instruments. Clearly, the method of choice for tenderness measurement is the Warner-Bratzler (W-B) shear device. The mechanism consists of a blade 1 mm thick containing a triangular opening which is pulled through a slot approximately 1.2mm wide (Voisey, 1966). A cylindrical core of meat sample is sheared by the

force of the V-shaped blade passing through the sample at 90° to the meat fibers. The maximum force exerted on the blade is detected by the dynameter spring and read on a graduated dial scale. Most research today utilizes the aforementioned attachment adapted to an Instron Universal Testing Machine. Computerization has led to numerous advantages such as constant crosshead speed, calibration of the instrument etc.

Bouton et al. (1975) analyzed shear force deformation curves and noted that the curve contained two segments. They postulated that the first curve represented the force (initial yield force) required to compress and initiate shear fracture planes through the myofibrillar structure and was primarily dependent on myofibrillar strength. The second curve, the difference between the initial yield force and the peak force, could be an indication of the strength of the connective tissue and other remaining structures. It was found that the initial yield force values increased with cooking temperature (i.e. greater than 60°C), decreased with postmortem aging and were not significantly affected by animal age. On the other hand, the second peak was not significantly affected by postmortem aging of young or old animals, significantly increased with animal age and was significantly reduced by cooking to 90°C.

In a similar study by Moller (1981), movement of the crosshead was stopped after the first yield force value was recorded. He observed that nearly all muscle fibers were sheared through, while the connective tissue elements continued to hold the sample together. On resumption of the movement of the crosshead, the final yield value corresponded to typical deformation curves. Further investigations by Moller (1981) on heated samples to end points of 60-80°C revealed myofibrillar shear (initial yield) values increased while the connective

tissue component (final yield value) decreased, lending further proof to the relationship between yield values and muscle tissue components. W-B myofibrillar force and W-B connective tissue force were also found to be more significant estimators of sensory evaluations of tenderness and collagen solubility than the usual peak force values.

Cholesterol and Fatty Acids

Cholesterol (cholest-5-en-3-β-ol) is the principal sterol of the higher animals (Merk, 1983). It is found in all body tissues especially the brain, spinal cord and in animal fats or oils. The amount of information available on cholesterol and its affects with regard to health is overwhelming. The layperson (and scientist) is left staggered and confused by the controversy about the role of cholesterol. It is a word much bandied about, with little understanding of what it is. It has 'bad' connotations and many people suppose that foods containing it should be avoided at ail costs.

It is by no means the intention of this author to provide an account of the specific technical and medical parameters regarding cholesterol and health. However, it is appropriate that some of the controversies be put into perspective not only for the layperson, but also for the scientist.

Cholesterol is a prime example of a natural metabolite, but not an essential nutrient. It is an essential metabolite because without it biological membranes would not function properly. No closely related steroid can, apparently, substitute entirely for cholesterol and still maintain essential functions (Gurr, 1984). It is also important in so far as it is converted into a variety of steroid hormones with important regulatory functions and into bile acids that are crucial to the absorption

of fat (Vergroesen, 1975). It is not an essential nutrient in so far as the body can manufacture its own supply if none is present in the diet.

Oliver (1981) and Marx (1976) provide excellent accounts for the non specialist; helping to bring some of the controversies into perspective. Among the reasons for its "bad publicity" is the build up of plaques in the inner walls of the arteries (atherosclerosis), cholesterol and its esters being implicated. The cholesterol in the plaques has been shown (Kotala and Marx, 1976) to originate from cholesterol carried in the bloodstream by the low density lipoproteins (LDL). Exceptionally high concentrations (hyperlipoproteinaemia) are indicative of a metabolic disorder (sometimes inherited) that results in a failure to remove LDL at a sufficiently rapid rate, an overproduction of lipoproteins by one of the tissues or a combination of the two. Thus, although it is possible to influence the blood cholesterol concentration (Gurr, 1985) by dietary means, it does not follow that this will have any significant effect on the underlying disease if blood cholesterol concentration is only a secondary manifestation of a more deepseated disorder.

Contrary to widespread belief, changing the amount of cholesterol in the diet has only a minor influence on blood cholesterol concentration (Coates, 1983; Oliver, 1981; Kestin et al., 1989). Firstly, there is a limit to the amount of cholesterol that can be efficiently absorbed (Brown et al., 1981). Secondly, a control mechanism ensures that blood concentrations are maintained within certain limits by regulating the amounts taken into tissues and at which cholesterol is synthesized in the body. Some individuals may however, be more 'sensitive' to dietary cholesterol, because of the way in which control mechanisms are 'tuned'.

The relative proportions of polyunsaturated to saturated fatty acids exerts a more potent dietary influence on blood cholesterol concentration (Malenka et al.,

1989; Kestin et al., 1989). Experimentally, it has been shown that saturated fatty acids are about twice as potent in raising blood cholesterol concentration as polyunsaturated fatty acids are in lowering it. Benfante and Reed (1990) reported that a ratio of *cis*-polyunsaturated to saturated fatty acids of about 0.5 (P/S ratio) would lower it and a ratio of less than 0.5 would increase it by a calculable amount. To compound the problem further ,it is now known (Gurr, 1985; McNamara, 1991) that the way in which the fatty acids are distributed among the three positions in the triacglycerol molecule as well as the nature of those fatty acids also have an influence on blood cholesterol concentration. Normally a diet in which butter contributes a large portion of the fat would result for many individuals in a rise in blood cholesterol concentration. The fatty acids in butter and in many other food fats are not randomly distributed among the three positions. When these three fatty acids are randomized (evenly distributed among the three positions) by interestification, the fat no longer exerts its hypercholesterolaemic effect when fed to human beings. This is said to be due to the increased amount of unsaturation within the triacylglycerol molecule.

It is worth mentioning here that during the last few years, several researchers have expressed the view that arterial damage observed as a result of feeding cholesterol rich diets to animals should be attributed to oxidation products of cholesterol and not to cholesterol itself. Cholesterol is normally a rather stable compound and would not be expected to oxidize rapidly on storage. Nevertheless, some oxygenated derivatives may be detected in commercial preparations of cholesterol. When these are purified and fed to animals, they more readily give rise to pathological lesions than purified cholesterol (Gurr, 1985). There is also a possibility that oxygenated metabolites of cholesterol

known to be formed in the body could also act as toxic agents. Much research is required on this subject before its practical nutritional importance can be assessed. McNamara (1991) cited additional heart disease risk factors—blood pressure, cigarette smoking, gender, life style, age and genetics to name but a few.

A few investigations relatively recently have demonstrated that not all saturated fatty acids are hypercholesterolaemic and that monosaturated fats are not neutral. Clinical studies have shown that the intake of stearic acid, a fatty acid found in meats and cocoa butter, actually lowers plasma cholesterol levels when compared to palmitic acid (Bonanome and Grundy, 1988). Reiser et al. (1985) reported that as yet our understanding of the full impact of saturated fatty acid chain length on plasma cholesterol levels is not well understood. McNamara (1991) concluded that only two major fatty acids in the American diet raise plasma cholesterol levels—myristic and palmitic acids.

Estimation of Cholesterol in Foods

Sweeney and Weihrauch (1976), provide a useful review article on the determination of cholesterol as well as relevant data. Cholesterol may be determined by means of three steps: extraction of lipids, separation of cholesterol from lipids and interfering compounds, and finally detection and measurement of isolated cholesterol.

Lipid Extraction

Organic solvents such as acetone (Tamura et al., 1964), ethyl ether-ethanol 1:3 (Hunter et al., 1945), chloroform (Wulfert, 1947), isopropanol (Levine et al.,

1964), benzene or hexane (Schwartz et al., 1967), chloroform-methanol (2:1) (Bondjens and Bjorkerud, 1971) and combinations thereof have been recommended. The sample may be blended with the solvent (Kelsey, 1939), refluxed (Roberts, 1968) or immersed for up to 24 hours (Bondjens and Bjorkerud, 1971). In the extraction process, the requirements are that the solvent will not disrupt the complexes with protein or with carbohydrate and that no chemical change in the lipid will be produced by oxidation or enzyme action (Cook and Rattray, 1958). A mixture of polar and nonpolar solvents provides better cholesterol extraction from any food in which part of the cholesterol may be bound to lipoprotein or other substance.

Separation of Cholesterol

It is possible to directly measure cholesterol at this stage, however, sterols other than cholesterol and other interfering materials that may be present react with the color-producing reagent providing erroneous color-complexes (Sweeney and Meihrauch, 1976). Part of the cholesterol at this stage may be esterified and also react with the color reagent.

Digitonin (a steroid saponin) precipitation of the free sterol, to form an insoluble molecular complex, cholesterol digitonide (Bladon et al., 1952) may be used. Other saponins such as tigonin and gitonin and the alkaloid tomatine also give precipitates with cholesterol. Gitonin has been recommended as an alternative to digitonin producing a more insoluble derivative (Delsal, 1943). Cholesterol can then be split from its digitonide by treatment with benzene, xylene, pyridine or dimethyl sulphonide. Total cholesterol is thus measured by saponification of cholesterol esters. Mild saponification must be used since

Sperry and Brand (1943) observed extraneous colors when strong saponification followed by neutralization with HCl was conducted.

Cholesterol may also be isolated by use of chromatographic procedures. These include column, thin layer chromatrography (TLC) high performance liquid chromatography (HPLC) and gas liquid chromatography (GLC).

Cholesterol Measurement

Gravimetric Determination

Methodology by Sperry and Webb (1950) and Szent Gyorgi (1923) are based on the formation of digitonide and its gravimetric determination. The weight of the cholesterol digitomide is 4.18 x that of cholesterol so that relatively small amounts of cholesterol may be estimated. Aluminum ions facilitate the precipitation within 5 minutes (Brown et al., 1954). Unfortunately, the process is slow, cumbersome and lacks sensitivity and specificity.

Titration

Small amounts of digitonide estimation may be made by oxidative methods using chromic acid. Unused chromic acid is titrated against thiosulphate (Okey, 1930) or potassium iodide (Haenni, 1941). This procedure is not specific since any compound could be oxidized and interfere with the reaction.

Colorimetric Determination

The Liebermann-Burchard method consists of the addition of acetic anhydride and a small amount of concentrated sulphuric acid to a solution of the sterol in a suitable organic solvent (e.g. chloroform). A sequence of colors is

produced which range from transient permanganate color through shades of green-blue and fading to shades of green-brown (Kenney, 1952). Tremendous criticism about this method exists. Certain sterols e.g. stigmasterol, sitosterol and even other lipids react with the color reagent. Cholesterol esters are said to react more rapidly with the Liebermann-Burchard reagent than does free cholesterol, and the reaction is heat and light labile (Sperry and Brand, 1943; Crawford, 1958).

The oxidation of cholesterol to cholest-4-en-3-one by use of the enzyme cholesterol oxidase was reported by Bergmeyer et al. (1972), and Allain et al., (1974). Use of 0-phthalaldehyde in glacial acetic acid provides a resulting color measured at 550 nm after 90 min. Commercial enzyme kits for the quantification of cholesterol have taken the above procedure one step further. Cholesterol esterase is used to free the cholesterol esters while cholesterol oxidase oxidises cholesterol to the ketone and hydrogen peroxide. The hydrogen peroxide is coupled with a chromagen, 4-aminoantipyrine and p-hydroxybenzene-sulphonate in the presence of peroxidase [EC 1.11.1.7] to yield a quinoneimine dye.

Thin Layer Chromatography

It is normal practice to separate and identify sterols by TLC rather than to determine them quantitatively. When quantitative procedures are used, color producing reagents are sprayed on the sheet and the size and color of the spot developed are determined by densitometry or eluted and determined colorimetrically.

Gas Liquid Chromatography

Quantitative determinations are made by comparison of peak height or peak area with that of standards of known concentration (Punwar, 1975). Boucrot (1971) claims that radio labelled isotopes are essential for microquantities since behavior of standards may differ from that of the substance being measured.

High Performance Liquid Chromatography

Jiang et al. (1991) compared four different methods for cholesterol determination and found that HPLC, GC and enzymatic methods were not significantly different from each other. Jaing et al. (1991) found that cholesterol in egg yolk was 23% higher than the average of the enzymatic, GC and HPLC methods.

Determination of Fatty Acid Composition of Lipids

Conventional techniques for the determination of fatty acid composition of lipids require solvent extraction, purification, hydrolysis and derivatization.

The method of choice for the analyst when individual lipid components are to be analyzed is the chloroform-methanol extraction procedure (Gurr, 1984). The sample is exhaustively extracted with a chloroform-methanol mixture (Osborne and Voogt, 1978) and the extract separated into two phases. The lower phase constitutes the chloroform layer containing extractable fat while the upper phase, aqueous methanol contains water soluble compounds. The chloroform layer may be dried and weighed after evaporation and then redissolved for further analysis.

TLC has been employed to separate constituent fatty acids and their positions are detected with a reagent spray to produce colored compounds. More

sophisticated separations can be achieved by incorporation into silica plates a substance that interacts with specific chemical groupings. Thus, the incorporation of silver nitrate (argentation TLC) allows the separation of fatty acids according to the number of configuration (*cis/trans*) of double bonds in fatty acid chains (Christie, 1983).

High performance liquid chromatography (HPLC) has been used to separate individual fatty acids, although GLC is the most widely used method. Excellent separations of complex mixtures according to carbon chain length and degree of unsaturation can be achieved using methylated esters. Internal standards permit quantification by means of an appropriate conversion factor (Gurr, 1984). A relatively recent transesterification method for all classes of lipids using methanol-benzene (4:1) with acetyl chloride circumvents all the above procedures providing samples ready for injection into GC-columns (Lepage and Roy, 1986).

Cholesterol and Fatty Acid Analysis of Beef

Cholesterol Composition

Data on cholesterol vary depending on muscle, sex, grade, cooking, etc. However, values also differ based on the analytical method used to quantify cholesterol content.

Sweeney and Weihrauch, (1976) provide a useful summary on different methods of cholesterol measurement and the appropriate values. Reports for cholesterol in lean beef range from 41.8mg/100g (Stromer et al., 1966) using the Liebermann-Burchard reagent to that of 114-mg/100g (Kritchevsky and Tepper, 1961) using colorimetry. Tu et al., (1967) found cholesterol values of 56mg/100g,

62mg/100g, 60mg/100g and 54mg/100g for *longissimus dorsi*, *semimembranosis*, *serratus ventralis*, *psoas major* and *semitendinosus* muscles respectively. Work by Del Vecchio et al., (1955) showed cholesterol values in muscle to be lower than cholesterol in adipose tissue. Hood and Allen (1971) found muscles containing higher fat contents to have higher cholesterol values, they attributed these differences to differences in the amount of fat.

Sex differences have also been implicated when Terrell et al. (1969) claimed higher cholesterol values for steers than heifers. Conversely, Hood and Allen (1971) found bulls to be higher than heifers in cholesterol and heifers to be higher than steers. Eichorn et al. (1986) furnished data comparing bulls versus steers and concluded that steers carcasses contained significantly more cholesterol due to the higher percentage of fat. Information with regard to the relationship between the age of the animal and muscle cholesterol is lacking. Stromer et al. (1966) and Del Vecchio et al. (1955) reported that younger animals were higher in cholesterol. However, Hood and Allen (1971) showed that contrary to the above, cholesterol content does not decrease with aging.

Hoelscher et al. (1988) and Rhee et al. (1982a) reported the presence of 114mg cholesterol/100g and 137mg cholesterol/100g raw subcutaneous adipose tissue, respectively. Intermuscular fat was said to contain 105 mg cholesterol/100g raw tissue. Rhee et al. (1982a) stated that marbled fat (intramuscular) contained cholesterol content values more similar to those of the intermuscular fat fraction. Rhee et al. (1982b) demonstrated that only the "Practically Devoid" marbling category for steaks contained significantly less cholesterol (wet basis) than did raw steaks from any of the other seven marbling groups studied. The influence of fat level on the cholesterol content of ground

beef was examined by Kregal et al. (1986). Their investigations indicate that as fat levels increased in raw patties, cholesterol values also increased.

GLC determination of total cholesterol was reported by Punwar (1975). Using 5 α-cholestane as an internal standard, Punwar (1975) found corned beef hash to contain 30.5 mg cholesterol/100g sample. While in another study Punwar and Derse (1978) reported beef to contain 55.3mg cholesterol/100g sample. Quantitative analysis of cholesterol in food by GLC has also been determined by Kaneda et al. (1980) although their data did not include beef.

During this year, two publications using gas chromatography-mass spectrometry (GC-MS) have been brought to light. Ulberth and Reich (1992) described a precise and accurate GC-MS method for the determination of cholesterol in processed foods and Stewart et al. (1992) examined cholesterol content of eggs, milk and dairy products. Both teams of investigators report lower values for cholesterol content in foods compared with USDA and AOAC approved methodology. Due to the specificity and sensitivity of the mass spectrometer it has been recommended that a revision of the cholesterol values in food be conducted based on this method (Stewart et al., 1992).

Fatty Acid Composition

Rumen micro-organisms influence the lipid metabolism of the host in two ways: by the changes wrought in dietary lipids before these come to be digested in the intestine, and by the synthesis of microbial lipids. Ruminant depot fats are harder than those of non-ruminants and contain much more stearic acid with lower concentrations of C18:2 and their composition is relatively independent of the unsaturated acid content of the diet (Munton and Fison, 1964). Strong reducing

conditions in the rumen are conducive for hydrogenation of C18 unsaturated acids ingested by ruminants, either from pasture or oil-seed meals (Dryden et al., 1973). Experiments have verified that this hydrogenation takes place to a large extent (McDonald et al., 1977). Both mixed bacteria and mixed protozoa hydrogenate chloroplast lipid and the protozoa can hydrogenate oleic acid, linoleic acid or linseed oil (Munton and Fison, 1964). The source of the hydrogen used by the microbes to carry out this reduction is hydrogen gas formed by microbial fermentation.

The dietary lipids as they leave the rumen, are thus mainly in the form of free fatty acids (more or less hydrogenated) together with some unchanged triglycerides. To this is added a quantity of lipid synthesized by micro-organisms containing large proportions of odd numbered fatty acids and branched chain acids (Gurr, 1984). Strains of *Ruminococcus* and *Bacteroides* incorporate iso-butyric acid and iso-valeric acid into branched chain acids containing ten or twelve more carbon atoms and also into aldehydes (Gurr, 1984).

Despite the generally accepted view that, because of hydrogenation in the rumen, the fatty acid composition of the depot fat of adult ruminants is refractory to change, published values for cattle show wide variations (Hilditch and Williams, 1964; Skelley et al., 1978; Westerling and Hedrick, 1979, and Yoskimura and Namikawa, 1983). Gurr (1984) reported the fatty acid composition of beef suet (perinephric fat) to contain 3% C14:0, 26%, C16:0, 9% C16:1, 8% C18:0, 45% C18:1, 2% C18:2 and 7% remaining fatty acids. National Livestock and Meat Board (1990) report composite data for beef cuts and ground beef containing 38.24% saturated fatty acids (SFA), 42.08% monounsaturated fatty acids (MUFA) and 3.43% polyunsaturated fatty acids (PUFA).

In the study for bulls and steers by Elchorn et al. (1985), bull tissue (*semitendinosus*, *longissimus* and *triceps brachii* muscle; subcutaneous and perinephric adipose tissue) contained higher ($P<0.01$) percentage of PUFA than that of steers at all sampling sites. Thus higher percentages of C18:2, C18:3 and C20:4 were observed in bull tissue. *Semitendinosus* and *triceps brachii* muscles of both bulls and steers contained 6-10% more unsaturated fatty acids compared with *M. longissimus*. All steer samples also contained slightly higher percentages of C16:0 at all sampling sites with significant differences being detected for *M. longissimus*. Investigations by Westerling and Hedrick (1979) showed significant differences for C18:2 and C20:4 between steers and heifers with heifers showing reduced amounts of PUFA. These same writers reported significant differences between subcutaneous and intramuscular fat for the following fatty acids: C16:0, C18:1, C18:2, C18:3, C20:1 and C20:4 with approximately 3% higher values for C18:2 intramuscular fat. Gillis and Eskin (1973) and Hood and Allen (1971) noted significant differences between bulls and steers for C14:0, C16:1, C18:1 and C18:2. They attributed those compositional differences to the possible effect of sex hormones (androgens) on enzyme systems such as desaturase.

Breed type showed very little difference in fatty acid profile for beef breeds fed similar diets and to the same degree of fatness (Larick and Turner, 1989; Sumida et al., 1972). Gillis and Eskin (1973) observed significant differences for breed types between Limousin (n=2) and Simmental sires (n=2) for C14:0 and C16:1. Age also plays a significant role in fatty acid composition as determined by Leat (1975). He found that the amount of stearic acid in subcutaneous fat declined from 20% to 5% during the period 1-2 years post partum. Perinephric fat on the other hand exhibited an increase in stearic acid from 10% to 15% at birth to

40% at 1 year of age and then declined up to 2 years of age. When stearic acid levels were above 10%, inverse changes were noted for C18:1 and below 10% in C16:1.

Larick and Turner (1989) examined the influence of grain versus grain-on-grass diets in cattle. These scientists found that muscles from grain-on-grass cattle had greater increases in PUFA than those fed primarily grain diets. They also found that neutral lipids were higher in C18:2 and C18:3, C20:3, C20:4 and C22:5. Beef from steers raised on pasture had more branched C15:0, C18:0, C18:3, C20:3, C20:4 and C22:5 and less C16:0 and C17:0 than beef produced by steers fed grain (Westerling and Hedrick, 1979; Melton et al., 1982; Brown et al., 1979).

A more recent trend in order to achieve modest changes in depot fat composition, is to feed so called 'protected' fat to ruminants. In this process, fat particles are coated with a protein which is crosslinked by treatment with formaldehyde (Gurr, 1985). Fatty acids are therefore protected against saturation by micro-organisms in the rumen and pass into the intestine for release and absorption. Romans, et al. (1989) showed increases in linolenic acid in non-ruminants when protected fats were used although this was not as pronounced in ruminants. Minor increases in linoleic acid were produced by Huerta-Leidenz et al. (1991), when they fed 30% whole cotton seed to cattle, as determined within perenephric fat. High oleate sunflower oil protected by means of calcium alginate (Ekeren et al., 1992) revealed no modification of perianal adipose tissue composition amongst heifers. Ekeren et al. (1992) concluded that metabolic changes occurred before deposition into adipose tissue and that the stearoyl-

coenzyme A desaturase may resist any net modification of bovine adipose tissue composition.

Sturdivant et al. (1992) examined black Wagyu cattle from Japan and found that among fat quality grades C14:0 tended ($P<0.07$) to decrease with increasing quality grade. Commensurate with the decrease in palmitic acid seen with fat quality grade was a general (though not significant) increase in C18:1. Japanese black cattle (9 steers) were compared with Holsteins (8 steers and 2 heifers) by Yoshimura and Namikawa (1983). Japanese Black were higher in C18:C16 ratio than Holstein cattle.

Only subtle shifts in the percentage of some fatty acids were seen by Vanderwert et al. (1988) when they compared "Select" and "Choice" Limousin steers. Choice cattle had a greater percentage of palmitic and oleic acid and less linoleic acid than "Select" cattle. Smith et al. (1989) reported similar values as mentioned above. Values given by these authors also substantiate published data from the National Livestock and Meat Board (1990). Wood (1984) concluded that low levels of fat contain relatively high levels of saturated fatty acids and low concentrations of unsaturated fatty acids. Consequently, undeveloped fat tissue (i.e. measured by no nett change in weight of animals) in lean ruminant carcasses exhibit higher melting points of extracted lipid (Bensadoun and Reid, 1965).

Time on feed studies in relation to fatty acid composition have been conducted by Hidorouglou et al. (1987); Westerling and Hedrick (1978) and Sumida et al. (1972). Sumida et al. (1972) carried out a four year study to determine the effects of feeding regime on fatty acid composition. Pasture steers were finished on pangola grass without supplemental feed while feedlot steers were finished on rolled barley, pineapple bran, soybean meal and blackstrup

molasses. They found that in year one and year four the level of myristic acid was higher in feedlot steers and that of stearic acid was higher in pasture steers. Palmitioleic and oleic acids were not affected by treatment in either year. Palmitic acid content was significantly higher in year one feedlot steers but the smaller difference in year four was not significant.

Feedlot periods of 76, 104 and 146 days were utilized in the study by Hidorouglou et al. (1987). Their work revealed that grain feeding increased the proportion of oleic acid as steers remained longer in the feedlot. A higher percentage of total saturated fatty acids at 76 days was also noticed (primarily due to stearic acid). Research work by Westerling and Hedrick (1978) involved feeding steers and heifers on fescue grass for 180 days. Following the slaughter of one group, remaining groups were full-fed under drylot conditions for 56 and 112 days. Total saturated fatty acids decreased ($P<0.05$) with days on feed for both subcutaneous and intramuscular fat (i.e. stearic acid being the major contributor to this decline and oleic acid increasing concommitantly). No significant differences were observed between feed lot animals though pasture fed animals were significantly different for all fatty acids measured except C20:1. These results were in agreement with those of Rumsey et al. (1972).

PAPER I. SENSORY, CHEMICAL AND ULTRASONIC MEASUREMENTS OF THE PALATABILITY ATTRIBUTES OF BOVINE SKELETAL MUSCLE

Sensory, chemical and ultrasonic measurements of the palatablIity attributes of bovine skeletal muscle

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ABSTRACT

Sensory, chemical and ultrasonic evaluations of bovine *longissimus thoracis* muscle were compared between entire and castrated male animals. Two hundred and ninety nine bulls and steers of three frame sizes (small, medium and large) were serially slaughtered following varying lengths of time on feed—180, 210 and 240 days. Bulls exhibited significantly lower ($P<0.05$) fat thickness and marbling scores and greater ($P<0.05$) hot carcass weight and rib eye areas than those of steers. All palatability attributes were ranked significantly lower ($P<0.05$) for bulls while Warner-Bratzler myofibrillar force and residual force values were higher than those of steers. Frame size had a significant impact ($P<0.05$) on lipid content, marbling score and moisture content as well as fat thickness, lipid content, marbling score, moisture, tenderness, connective tissue, myofibrillar force and residual force for bulls and steers respectively. Time on feed showed no significant change in marbling deposition amongst bulls while steers demonstrated significant increases in marbling between 180 and 240 days. Palatability remained unchanged for both bulls and castrated counterparts based on feed time. Steaks from small framed steers were the most tender as exhibited by high sensory panel scores and low myofibrillar force measurements. Sensory panel ratings based on marbling scores revealed no significant difference ($P<0.05$) except for modest and moderate degrees of marbling for juiciness and flavor amongst bulls. On the other hand, steers containing slightly abundant degrees of marbling were significantly more tender, lower in connective tissue score and flavorful than categories with less marbling. Poor correlations were achieved between marbling and tenderness ($r = 0.138$ and 0.111 for bulls and steers, respectively) while sensory tenderness and myofibrillar shear force measures were highly correlated ($r=0.609$ and 0.710

for bulls and steers respectively). R^2-values for ultrasonic and palatability parameters were extremely low. Lipid and marbling R^2-values in relation to ultrasound appeared promising.

INTRODUCTION

Despite the production economies associated with raising young bulls for beef as well as the generally accepted custom of rearing bulls for meat in Germany, Belgium, Italy and Sweden (Dransfield, 1984; Fabiansson et al., 1984), male calves in the USA and in other major beef producing countries are castrated and raised as steers. Castration is considered to ease the management of live animals, reduce the prevalence of dark cutting in beef and enhance eating quality (Rhodes, 1969).

Data on the palatability of young bulls versus steers has been variable. Reagan et al. (1971) concluded that steers were more tender, more flavorful and rated higher for overall satisfaction while Arthaud et al. (1977) reported more desirable sensory evaluations for steer beef in terms of tenderness, juiciness and flavor. On the contrary, Klastrup et al. (1983) and Vanderwert et al. (1985) found no significant difference in sensory evaluation. The reduced tenderness of bulls has been attributed to an increased amount of cross-linked collagen (Boccard et al., 1979; Gerrard et al., 1987) and lower amounts of fat cover (Crouse et al., 1983; Riley et al., 1983) the latter leading to a higher incidence of cold induced toughness. Aberle et al. (1981) suggested that a short feeding period of a high energy diet may improve tenderness by increasing the proportion of heat soluble collagen and elevate protein synthesis and turnover rates. This in turn would provide a layer of fat to serve as insulation against cold shortening.

Numerous time on feed studies for young animals have reported increases in tenderness and flavor (Miller et al., 1987; Kropf et al., 1975; Leander et al., 1978). Improved tenderness is said to be short lived, and in fact, extended feeding times

have shown a decline in palatability (Crouse and co-workers, 1978). Breed type variances for palatability have been investigated by a number of writers. Koch et al. (1976) reported Jersey carcasses to be the most tender of all the British breeds and to have higher palatability than Angus carcasses (Ramsey et al., 1963). Charolais, Simmental and Limousin crossbreds when compared to Hereford and Angus crossbreds were less juicy and less tender (Adams et al., 1977; Mains et al., 1981).

Thus with variations in palatability based on breed, time on feed and sex, the objective of this study was to further investigate the influence of these parameters on the palatability attributes of bulls and steers. In addition, ultrasound data were used in order to find any significant influence of these parameters on palatability measures.

MATERIALS AND METHODS

Source of Materials

Cattle used in this study were of three frame sizes; raised as small, medium and large framed crossbred calves from either Rhodes or McNay research farms at Iowa State University. Cattle for each frame size were of similar genetic make up and environmental influence as described by Reiling et al. (1992). Three hundred animals, one half of the calves being randomly castrated, were allotted at random to one of three slaughter dates with equivalent frame size representation, maintaining an approximately equivalent age of 18 months for each group of cattle. Cattle were fed an 80% concentrate ration (on a dry-matter basis) and serially slaughtered between mid-June and mid-August with a 30 day interval between each slaughter date. Feed times corresponded to 180, 210 and 240 days on feed.

Ultrasonic Scanning

Twenty four hours prior to slaughter, cattle were scanned in order to produce real-time ultrasound images by means of an Aloka 500V (Corometrics Medical Systems, Inc.) with a 3.5 MHz 17cm linear array transducer. Two scan images were collected on all animals: one with the dorsal point of the transducer located at the 12-13th vertabrae and the ventral point being between the 12-13th ribs; the other with the transducer aligned parallel to the length of the *longissimus thoracis* muscle across the 11-13th ribs. (Rouse et al., 1992 and Duello et al., 1992). The images were stored on VHS tape and later interpreted for backfat thickness, rib eye area and grey scale analysis as an indicator for marbling.

Carcass Treatments

Cattle were slaughtered at a commercial packing plant and then following approximately a 24 hr chill, carcasses were "quartered" between the twelfth and thirteenth thoracic vertebrae. An experienced USDA meat grader was used to evaluate each carcass for quality and yield grade in accordance with USDA standards (USDA, 1987).

Samples of *longissimus thoracis* muscle, 2.5 cm, were excised from the right side of each carcass between the 12th and 13th ribs. Samples were shipped to Iowa State University, deboned and a thin sliver at the surface of the 12th and 13th rib was removed for proximate analysis. The remainder of each muscle sample was vacuum packed (Dixiepac, 100) and aged for 12 days prior to freezing at -20°C.

Muscle samples for proximate analysis were denuded by dissecting the intermuscular and subcutaneous fat. Prepared strips were frozen and pulverized for 30 seconds in liquid nitrogen by means of a Waring-blender. Samples were packaged in polyethylene bags and stored at -20°C until required.

Proximate Analysis

The percentages of moisture and fat were determined on powdered muscle samples, in duplicate, by oven drying and hexane, respectively (AOAC, 1980).

Sensory Evaluation

Steaks were thawed for 48 hours at 2°C and then broiled to an internal temperature of 64.4°C. The internal temperature of steaks were monitored at the

geometric center using copper-constantan thermocouples attached to an Omega Digital Trendicator. Steaks were broiled to an internal temperature of 40°C, turned, and then broiled further and taken off to reach an internal temperature of 64.4°C. Once removed from the broiler, steaks were individually wrapped in aluminum foil, placed in a 60°C warming oven, removed and cut into approximately 1.27 x 1.27 x 3.18 cm sample cubes. Two samples, randomly chosen were served to a 8-10 member trained sensory panel. Panelists were individually seated in booths in a room separated from the preparation area and provided with water to rinse their palates between samples. Red fluorescent lights were used to eliminate any biases due to the color of samples. Panelists were trained by means of a variety of samples using the following sensory definitions: Myofibrillar tenderness - measure of how easily the sample breaks down into smaller pieces after three chews; Connective tissue - amount of insoluble connective tissue that remains in the mouth after thorough chewing; Juiciness - the sensation of free fluids released from the meat upon chewing; Flavor intensity - measure of the perceived intensity of beef flavor and Flavor - measure of the sensation perceived as beef flavor. Scores for tenderness (T), connective tissue (CT), juiciness (J), flavor intensity (FI) and flavor (F) were based on an eight point descriptive scale (tenderness 1= extremely tough, 8 = extremely tender; connective tissue, 1 = abundant, 8 = none; juiciness, 1 = extremely dry, 8 = extremely juicy; flavor intensity, 1 = extremely bland, 8 = extremely intense; flavor, 1 = extremely off flavor, 8 = extremely flavorful). Values for each sensory attribute of a steak was reported as an average.

Warner-Bratzler Shear Force Measurements

Cores were removed from each of three sections (central, medial, lateral) of cooked steaks previously cooled to room temperature (25°C). Three 1.3 cm diameter cores were removed parallel with the axis of the muscle fiber and used for Warner-Bratzler Shear (WBS) assessment. Shear force measurements were carried out using the WBS-force attachment of the Instron Universal Testing Machine (Instron Corp. Canton, MA). A 50kg load cell and a cross-head speed of 100 mm/minute was used. Shear force deformation curves were recorded by means of a chart recorder using a chart speed of 100 mm/minute. Each core was sheared twice along the long axis and the values averaged. Moller (1981), reported higher correlations with sensory panel data by dividing curves into two parts. The first yield point was identified as the compression/myofibrillar force corresponding to the myofibrillar component of tenderness while the second yield point relates to the residual force of the connective tissue component of tenderness. Values for each steak are reported in kg/cm^2 using a conversion factor of 0.36.

Statistical Analysis

In order to account for missing data, least squares means and associated standard errors of the mean were computed using the Statistical Analysis System (SAS, 1985). Initially, the procedure GLM of SAS (1985) was used to conduct an analysis of variance to determine significant differences due to sex, frame size and time on feed (model: Y = sex, frame, feed time, sex x frame, sex x feed time, frame x feed time). Analysis of individual sexes (model: Y = frame, feed time, frame x feed time) were also conducted. All remaining sources of variation were placed in

the error term. Least significant difference tests were carried out to determine differences for all significant effects. Simple correlations were established for all palatability attributes while multiple regression analysis was conducted for ultrasonic and sensory parameters. Factor analysis of gray scale histogram data [mean, standard deviation (stdDev), minimum (min), maximun (max), sum, Kurtosis (Kur), skewness (skew) and mode], performance data [fat thickness (fat), sex, age, and weight (wt)], Fourier spectrum texture analysis [Fourier distance mean (fdm) and standard deviation (fdsd), Fourier power intensity mean (fim) and standard deviation (fisd) and Fourier intensity count (fic)] and moment descriptors (m11) were used to generate prediction models as reported by Wilson et al., (1992). Unless stated otherwise, tests of hypothesis are reported as significant using α = 0.05.

RESULTS AND DISCUSSION

Overall data on the bulls and steers slaughtered during Summer 1991 are presented in Table 1 and 2. Bull carcasses had significantly lower ($P<0.05$) marbling scores and fat thickness and greater ($P<0.05$) hot carcass weights and rib eye areas when compared to steer carcasses. It should be pointed out that the populations size sampled in this study consisted of 299 animals and therefore significant differences were easily detected. Palatability attributes for bulls and steers given in Table 2 shows values that are extremely close. Bulls once again ranked significantly lower than steers for all palatability attributes measured while Warner-Bratzler myofibrillar force and Warner-Bratzler connective tissue (residual) force were significantly higher ($P<0.05$). Research by Jacobs et al. (1977) and Field (1971) report no significant difference between bulls and steers for tenderness, juiciness, flavor and overall satisfaction. However, they used fewer animals in their studies. Despite statistically significant values reported herein for palatability, these differences may not necessarily be detectable by consumers.

Analysis of variance for bulls and steers conducted for carcass and palatability traits, listing individual sources of variation are given in Table 3. Sex, frame size and time on feed were highly significant ($P<0.01$) for fat thickness, lipid content, and moisture. Sex and frame size were significant for marbling score, tenderness, connective tissue and residual force, while sex x frame size was highly significant ($P<0.01$) for fat thickness, time on feed being significant ($P<0.05$) for moisture content and sex being significant ($P<0.05$) for juiciness, flavor and flavor intensity. All remaining two way interactions were not significant.

Table 1. Selected overall means and standard deviations of parameters for bulls and steers from Rhodes and McNay research farms, 1991

Parameter	Bulls	Steers
Number	148	151
Hot carcass wt (kg)	$362.2^{x} \pm 32$	$322.2^{y} \pm 37$
Marbling score[a]	$945^{x} \pm 62$	$1017^{y} \pm 87$
Fat thickness (mm)	$7.5^{x} \pm 3.2$	$11.3^{y} \pm 2.4$
Rib eye area (cm^2)	$32.0^{x} \pm 3.6$	$29.7^{y} \pm 4.1$

[a] 900 = Slight^{0}, 1000 = Small^{0}.

$^{x\text{-}y}$ Means within the same row bearing different superscripts are significantly different ($P<0.05$).

Table 2. Least squares means and standard errors for palatability attributes of *longissimus thoracis* muscle from bulls and steers originating from Rhodes and McNay farms, 1991

Parameter	Bulls	Steers	SEM[a]
Tenderness	6.8[x]	7.1[y]	0.031
Connective Tissue	6.9[x]	7.0[y]	0.027
Juiciness	6.5[x]	6.7[y]	0.031
Flavor	6.6[x]	6.7[y]	0.026
Flavor Intensity	6.6[x]	6.7[y]	0.029
Myofibrillar force (kg/cm^2)	2.61[x]	2.23[y]	0.042
Residual force (kg/cm^2)	2.51[x]	2.18[y]	0.039

[a] SEM is the standard error of the mean.

[x-y] Means within the same row bearing different superscripts are significantly different ($P<0.05$).

Table 3. Analysis of variance for *longissimus thoracis* muscle traits of bulls and steers slaughtered from Rhodes and McNay research farms, summer, 1991

Variable	Source	df	Sum of Squares	F	P>F
Fat Thickness	Sex	1	1.2180	65.85	0.0001
	Frame	2	0.4882	13.20	0.0001
	Time	2	0.8714	23.55	0.0092
	Sex x Frame	2	0.1765	4.77	0.0001
	Sex x Time	2	0.3097	8.37	0.0847
	Frame x Time	4	0.0214	0.29	0.8395
Lipid (%)	Sex	1	93.5932	42.32	0.0001
	Frame	2	43.8465	9.91	0.0001
	Time	2	61.0807	13.81	0.0001
	Sex x Frame	2	5.1813	1.17	0.3114
	Sex x Time	2	11.2690	2.55	0.0802
	Frame x Time	4	7.3811	0.83	0.5042
Marbling Score	Sex	1	3.959×10^5	72.37	0.0001
	Frame	2	1.280×10^5	11.70	0.0001
	Time	2	2.098×10^4	1.92	0.1489
	Sex x Frame	2	1.289×10^5	1.18	0.3094
	Sex x Time	2	2.649×10^5	2.42	0.0907
	Frame x Time	4	7.181×10^4	0.33	0.8599

Table 3 cont.

Variable	Source	df	Sum of Squares	F	P>F
Moisture	Sex	1	238.4509	48.74	0.0001
	Frame	2	103.7523	10.60	0.0010
	Time	2	45.4178	4.64	0.0104
	Sex x Frame	2	10.3209	1.05	0.3497
	Sex x Time	2	33.1609	3.39	0.0552
	Frame x Time	4	12.0591	0.62	0.6513
Tenderness	Sex	1	3.4612	23.67	0.0001
	Frame	2	2.0255	6.92	0.0012
	Time	2	0.0490	0.17	0.8457
	Sex x Frame	2	0.5069	1.73	0.1786
	Sex x Time	2	0.1675	0.57	0.5646
	Frame x Time	4	1.0683	1./83	0.1239
Connective Tissue	Sex	1	1.0601	8.97	0.0030
	Frame	2	0.9301	3.94	0.0206
	Time	2	0.2439	1.03	0.3575
	Sex x Frame	2	0.6713	2.84	0.0600
	Sex x Time	2	0.0585	0.25	0.7809
	Frame x Time	4	1.4427	3.05	0.0174

Table 3 cont.

Variable	Source	df	Sum of Squares	F	P>F
Juiciness	Sex	1	1.9690	13.84	0.0002
	Frame	2	0.2345	0.82	0.4397
	Time	2	1.0581	3.72	0.0255
	Sex x Frame	2	0.6019	2.12	0.1255
	Sex x Time	2	0.2349	0.83	0.4391
	Frame x Time	4	0.3185	0.56	0.6921
Flavor Intensity	Sex	1	1.6030	9.82	0.019
	Frame	2	0.1608	0.49	0.611
	Time	2	0.1917	0.59	0.556
	Sex x Frame	2	0.3238	0.99	0.372
	Sex x Time	2	0.2167	0.66	0.516
	Frame x Time	4	1.3062	0.47	0.758
Flavor	Sex	1	0.5087	5.11	0.0246
	Frame	2	0.2671	1.34	0.2634
	Time	2	0.6368	3.20	0.0424
	Sex x Frame	2	0.0189	0.10	0.9093
	Sex x Time	2	0.0031	0.02	0.9847
	Frame x Time	4	0.4549	1.14	0.3372

Table 3 cont.

Variable	Source	df	Sum of Squares	F	P>F
Myofibrillar Force	Sex	1	10.8025	40.41	0.0001
	Frame	2	1.3532	2.53	0.0814
	Time	2	0.5920	1.11	0.3319
	Sex x Frame	2	1.3166	2.46	0.0871
	Sex x Time	2	1.1086	2.07	0.1277
	Frame x Time	4	0.0893	0.08	0.9874
Residual Force	Sex	1	7.7666	33.22	0.0001
	Frame	2	2.0845	4.46	0.0124
	Time	2	0.4469	0.96	0.3856
	Sex x Frame	2	1.1218	2.40	0.0926
	Sex x Time	2	0.6005	1.28	0.2787
	Frame x Time	4	0.3439	0.37	0.8315

Due to the highly significant influence of sex in the model, individual sexes were analyzed using frame size, time on feed and frame size x time on feed interaction as sources of variation. Table 4 represents the analysis of variance for *longissimus thoracis* muscle traits of bulls slaughtered from Rhodes and McNay farms, Summer, 1991. Frame size was significant for lipid content, marbling score and moisture content. Time on feed was found to be significant ($P<0.05$) for lipid content. Frame size x time on feed interaction was highly significant ($P<0.01$) for connective tissue scores only. All remaining effects were non-significant ($P<0.05$).

As with bulls, a similar breakdown based on frame size, time on feed and frame size x time on feed effect was made in Table 5. Steers showed highly significant effects ($P<0.01$) based on frame size for fat thickness, lipid content, marbling score, moisture, tenderness, connective tissue ($P<0.01$) myofibrillar force and residual force. Longer time on feed was highly significant ($P<0.01$) for fat thickness, lipid content, marbling scores and moisture content. All remaining effects were non-significant. ($P<0.05$).

When viewing frame size (Table 6) bulls showed a significant difference in weight from small to large frame. Steers also showed a similar trend. Marbling scores for bulls varied significantly ($P<0.05$) between small and large framed carcasses and declined with an increase in size. Steers also significantly decreased ($P<0.05$) in marbling score with an increase in frame size but the differing effect was only between the small framed and the rest of the carcasses. This greater propensity to fatten for small framed animals has been documented by Neumann and Lusby (1986) and Minish and Fox (1979). Fat thickness did not significantly differ ($P<0.05$) between frame sizes for bulls while rib eye areas increased significantly based on increase in frame size. In contrast, fat thickness

Table 4. Analysis of variance for *longissimus thoracis* muscle traits of bulls slaughtered from Rhodes and McNay research farms, summer, 1991

Variable	Source	df	Sum of Squares	F	P>F
Fat Thickness	Frame	2	0.0437	1.49	0.2291
	Feed Time	2	0.0759	2.59	0.0786
	Frame x Time	4	0.078	1.33	0.2609
Lipid (%)	Frame	2	29.6364	7.60	0.0007
	Time	2	23.1388	5.93	0.0034
	Frame x Time	4	10.4699	1.34	0.2576
Marbling Score	Frame	2	4.40172×10^4	5.22	0.0065
	Time	2	1.9052×10^3	0.23	0.7980
	Frame x Time	4	1.0841×10^4	0.64	0.6326
Moisture	Frame	2	25.0492	5.77	0.0039
	Time	2	9.7275	2.24	0.1104
	Frame x Time	4	7.5052	0.86	0.4873
Tenderness	Frame	2	0.2639	0.87	0.4208
	Time	2	0.0929	0.31	0.7364
	Frame x Time	4	0.7906	1.30	0.2714

Table 4 cont.

Variable	Source	df	Sum of Squares	F	P>F
Connective Tissue	Frame	2	0.2836	1.16	0.3151
	Time	2	0.1817	0.75	0.4761
	Frame x Time	4	2.2041	4.53	0.0018
Juiciness	Frame	2	0.0882	0.30	0.7428
	Time	2	1.0253	3.46	0.0641
	Frame x Time	4	0.2321	0.39	0.8142
Flavor Intensity	Frame	2	0.0239	0.11	0.8969
	Time	2	0.2310	1.05	0.3518
	Frame x Time	4	1.7733	1.76	0.1400
Flavor	Frame	2	0.3117	0.39	0.6806
	Time	2	0.3552	1.68	0.1906
	Frame x Time	4	0.9725	0.62	0.6619
Myofibrillar Force	Frame	2	0.2926	0.48	0.6182
	Time	2	1.6278	2.68	0.0718
	Frame x Time	4	0.2260	0.19	0.9452
Residual Force	Frame	2	0.1417	0.30	0.7420
	Time	2	0.9762	2.06	0.1314
	Frame x Time	4	0.3061	0.32	0.8623

Table 5. Analysis of variance for *longissimus thoracis* muscle traits of steers slaughtered from Rhodes and McNay research farms, summer, 1991

Variable	Source	df	Sum of Squares	F	P>F
Fat Thickness	Frame	2	0.6246	14.07	0.0001
	Feed Time	2	1.1381	25.64	0.00001
	Frame x Time	4	0.0120	0.14	0.9689
Lipid (%)	Frame	2	54.3027	9.41	0.0001
	Time	2	74.6503	12.94	0.0001
	Frame x Time	4	2.3781	0.21	0.9347
Marbling Score	Frame	2	9.6510×10^4	7.21	0.0010
	Time	2	4.6815×10^4	3.5	0.0329
	Frame x Time	4	1.1085×10^3	0.04	0.9967
Moisture	Frame	2	40.1784	10.36	0.0001
	Time	2	27.4693	7.09	0.0012
	Frame x Time	4	3.7035	0.48	0.7521
Tenderness	Frame	2	2.2590	8.01	0.0005
	Time	2	0.1227	0.44	0.6480
	Frame x Time	4	0.5443	0.96	0.4291

Table 5 cont.

Variable	Source	df	Sum of Squares	F	P>F
Connective Tissue	Frame	2	1.3049	5.69	0.0042
	Time	2	0.1189	0.52	0.5961
	Frame x Time	4	0.1477	0.32	0.8626
Juiciness	Frame	2	0.7439	2.72	0.0691
	Time	2	0.2558	0.94	0.3944
	Frame x Time	4	0.6398	1.17	0.3262
Flavor Intensity	Frame	2	0.2109	1.10	0.3355
	Time	2	0.2832	1.48	0.2316
	Frame x Time	4	0.1018	0.27	0.8997
Flavor	Frame	2	0.2841	0.85	0.4297
	Time	2	0.0908	0.27	0.7625
	Frame x Time	4	0.4845	0.72	0.5765
Myofibrillar Force	Frame	2	2.3594	5.08	0.0074
	Time	2	0.0543	0.12	0.8897
	Frame x Time	4	0.0989	0.11	0.9801
Residual Force	Frame	2	3.0493	6.61	0.0018
	Time	2	0.0554	0.12	0.8688
	Frame x Time	4	0.4527	0.49	0.7424

Table 6. Means and standard deviations of selected carcass parameters for small, medium and large framed bulls and steers from Rhodes and McNay research farms, 1991

	Bulls			Steers		
Parameter	Small	Medium	Large	Small	Medium	Large
Number	51	48	47	50	52	49
Hot carcass wt (kg)	$295.7^{x} \pm 27.2$	$337.7^{y} \pm 3.3$	$375.8^{z} \pm 35.1$	$289.2^{x} \pm 25.2$	$319.8^{y} \pm 7.6$	$358.3^{z} \pm 26.0$
Marbling Score[a]	$967.0^{x} \pm 74$	$942.0^{xy} \pm 55$	$924.0^{y} \pm 57$	$1054.0^{x} \pm 93$	$1002.0^{y} \pm 66$	$997.0^{y} \pm 77$
Fat Thickness (mm)	$8.5^{x} \pm 3.2$	$8.0^{x} \pm 2.8$	$7.5^{x} \pm 2.5$	$13.3^{x} \pm 4.5$	$12.0^{y} \pm 3.0$	$9.25^{z} \pm 2.8$
Rib eye area (cm^2)	$30.3^{x} \pm 2.3$	$32.3^{y} \pm 3.6$	$33.8^{z} \pm 3.3$	$28.9^{x} \pm 2.1$	$29.7^{x} \pm 2.8$	$29.7^{x} \pm 3.0$

[a] 900 = $Slight^{0}$, 1000 = $Small^{0}$.

$^{x-z}$ Means within the same row, for each sex group, bearing different superscripts are significantly different ($P<0.05$).

for steers decreased significantly with increase in frame size while differences in rib eye area remained non-significant.

Time on feed for both bulls and steers (Table 7) showed a significant increase in weight. This weight increase seems to be associated with a significant increase in fat thickness for steers while increased rib eye area among bulls may account for weight gains. Marbling deposition did not show any significant changes for bulls with increased feed time. On the other hand, steers demonstrated a significant increase in marbling with time on feed between days 180 and 240. Miller et al. (1987); Dolezal and Tatum (1983) and Belk et al (1991) also found significant increases in marbling deposition for steers with increases in time on feed. May et al. (1992) reported that marbling increased curvilinearly with days on feed being between 0–196 days.

Data of certain chemical, sensory and Warner-Bratzler shear force measurements of longissimus thoracis muscle are provided in Table 8. There was a significant difference ($P<0.05$) between small framed and medium and large framed animals when considering lipid values. Small framed animals had significantly lower moisture levels than their counterparts. Sensory panel and Warner-Bratzler data for bulls stratified on the basis of frame size revealed no significant difference for every parameter tested. Steers exhibited a different pattern and this may be related to lipid content. Tenderness and connective tissue scores were significantly higher ($P<0.05$) for the small framed animals than their larger framed contemporaries. This difference was also verified by the myofibrillar and residual forces from Warner-Bratzler peak force measurements. Juiciness scores also differed significantly in favor of small framed animals probably due to the higher fat content. Studies by Campion et al. (1975), and Seideman et al.

Table 7. Means and standard deviations of selected carcass parameters for bulls and steers from Rhodes and McNay research farms, stratified on the basis of days on feed

	Bulls			Steers		
Parameter	180	210	240	180	210	240
Number	48	50	50	50	49	52
Hot carcass wt (kg)	$305.0^{x} \pm 20$	$339.2^{y} \pm 26.2$	$359.5^{z} \pm 28.3$	$301.7^{x} \pm 18.4$	$324.1^{y} \pm 21.6$	$358.3^{z} \pm 26.0$
Marbling Score[a]	$942.0^{x} \pm 57$	$951.0^{x} \pm 59$	$944.0^{x} \pm 53$	$999.0^{x} \pm 93^{x}$	$1011.0^{xy} \pm 62$	$1043^{y} \pm 55$
Fat Thickness (mm)	$7.0^{x} \pm 2.2$	$8.2^{xy} \pm 1.9$	$8.5^{y} \pm 2.3$	$8.4^{x} \pm 3.2$	$12.0^{y} \pm 3.0$	$13.8^{z} \pm 2.8$
Rib eye area (cm^2)	$30.2^{x} \pm 2.1$	$32.3^{x} \pm 3.4$	$33.4^{y} \pm 3.1$	$29.1^{x} \pm 2.0$	$29.7^{x} \pm 2.8$	$29.9^{x} \pm 2.7$

[a] $900 = Slight^{0}$, $1000 = Small^{0}$,

$^{x-z}$ Means within the same row, for each sex group, bearing different superscripts are significantly different ($P<0.05$).

Table 8. Least squares means of certain chemical, sensory and Warner-Bratzler shear force measurements of *longissimus thoracis* muscle of bulls and steers stratified on the basis of frame size

	Bulls				Steers			
Parameter	Small	Medium	Large	SEM[a]	Small	Medium	Large	SEM[a]
Lipid (%)	4.38^{x}	3.29^{y}	3.53^{y}	0.202	5.91^{x}	4.79^{y}	4.45^{y}	0.238
Moisture (%)	72.92^{x}	73.84^{y}	73.79^{y}	0.213	71.15^{x}	72.13^{y}	72.39^{y}	0.029
Tenderness	6.9^{x}	6.8^{x}	6.8^{x}	0.055	7.2^{x}	6.9^{y}	6.9^{y}	0.055
Connective Tissue	6.9^{x}	6.9^{x}	6.8^{x}	0.049	7.1^{x}	6.7^{y}	6.9^{y}	0.022
Juiciness	6.5^{x}	6.6^{x}	6.5^{x}	0.055	6.8^{x}	6.7^{xy}	6.6^{y}	0.055
Flavor Intensity	6.6^{x}	6.6^{x}	6.6^{x}	0.049	6.8^{x}	6.7^{x}	6.7^{x}	0.032
Flavor	6.6^{x}	6.5^{x}	6.5^{x}	0.047	6.8^{x}	6.7^{x}	6.8^{x}	0.055
Myofibrillar Force (kg/cm^2)	2.62^{x}	2.56^{x}	2.55^{x}	0.078	2.05^{x}	2.31^{y}	2.32^{y}	0.071
Residual Force (kg/cm^2)	2.45^{x}	2.43^{x}	2.51^{x}	0.069	1.98^{x}	2.26^{y}	2.30^{y}	0.068

[a] SEM is the standard error of the mean.

$^{x-y}$ Means within the same row, for each sex group, bearing different superscripts are significantly different ($P<0.05$).

(1975), using hamburgers ranging from 3-7% fat, have shown positive effects of lipid on tenderness and juiciness. A relatively recent study by Campion et al. (1975) reached consistent conclusions to those of Blumer (1963) when average choice steaks (5.4±2% ether-extractable lipid) were positively correlated (although low) between muscle lipid and eating quality measurements.

Scientists in the USA (Tatum, 1981 and Dikeman, 1987) have maintained that a value of approximately 3% ether-extractable lipid is necessary to provide juicy, tender beef. However, in a Danish study, high levels of tenderness, juiciness, and flavor were found in grilled steaks averaging only 1.6% extractable lipid (Liboriussen et al., 1977). The internal temperature of doneness was 50°C. Young bulls of various breeds used in their study averaged between 7 and 8 (on a 10 point scale) for tenderness, juiciness and flavor. Liboriussen and coworkers (1977) results are similar to those reported in this study. It has also been shown by Norris et al. (1971) and Parrish et al. (1973) that sensory scores for tenderness, juiciness, flavor and Warner-Bratzler sheer force measurements were not significantly affected by the degree of marbling. Parrish et al. (1973) showed that higher temperatures of doneness, however, adversely affected tenderness.

Values for certain chemical, sensory and Warner-Bratzler shear force measurements of *longissimus thoracis* muscle stratified on the basis of days on feed are laid out in Table 9. Lipid content increased with time on feed but only significantly between 180 days and 210 days, and 210 days and 240 days for bulls and steers respectively. Moisture values decreased significantly ($P<0.05$) coinciding with increases in lipid level. Lawrie et al. (1963) reported that the amount of water in a muscle is inversely proportional to the amount of lipid present.

Table 9. Least squares means of certain chemical, sensory and Warner-Bratzler shear force measurements of *longissimus thoracis* muscle of bulls and steers stratified on the basis of days on feed

	Bulls				Steers			
Parameter	180	210	240	SEM[a]	180	210	240	SEM[a]
Lipid (%)	3.12^{x}	4.02^{y}	4.03^{y}	0.202	4.27^{x}	4.85^{x}	5.99^{y}	0.238
Moisture (%)	73.87^{x}	73.18^{y}	73.48^{y}	0.213	72.35^{x}	72.04^{x}	71.30^{y}	0.038
Tenderness	6.9^{x}	6.9^{x}	6.8^{x}	0.055	7.1^{x}	7.1^{x}	7.0^{x}	0.055
Connective Tissue	6.8^{x}	6.9^{x}	6.9^{x}	0.049	7.0^{x}	7.0^{x}	7.1^{x}	0.002
Juiciness	6.6^{x}	6.6^{x}	6.5^{x}	0.055	6.8^{x}	6.7^{x}	6.7^{x}	0.055
Flavor Intensity	6.7^{x}	6.6^{x}	6.6^{x}	0.049	6.7^{x}	6.7^{x}	6.7^{x}	0.032
Flavor	6.5^{x}	6.6^{x}	6.5^{x}	0.047	6.7^{x}	6.7^{x}	6.7^{x}	0.055
Myofibillar Force (kg/cm^2)	2.56^{xy}	2.2^{y}	2.7^{x}	0.078	2.25^{x}	2.25^{x}	2.07^{x}	0.071
Residual Force (kg/cm^2)	2.51^{x}	2.52^{x}	2.64^{x}	0.069	2.17^{x}	2.22^{x}	2.17^{x}	0.068

[a] SEM is the standard error of the mean.

$^{x-y}$ Means within the same row, for each sex group, bearing different superscripts are significantly different ($P<0.05$).

No significant palatability difference ($P<0.05$) could be reported between feed times amongst bulls and steers. Warner-Bratzler myofibrillar force and residual force values were also non-significant for steers. On the other hand, bulls showed significantly higher ($P<0.05$) values for myofibrillar force between 210 and 240 days on feed. Residual force was not significantly affected.

Experiments have shown that feeding on high energy diet prior to slaughter improves palatability, but the duration is short lived and little benefit is derived from extended feeding (Zinn et al., 1970; Smith et al., 1977; Harrison et al., 1978). Moody and coworkers (1970) cited evidence on the influence of length of feeding high roughage ration on qualitative and quantitative characteristics of beef. They concluded that meat from cattle fed 28 days was less flavorful than from cattle fed longer periods, marbling increased with time on fed and cattle fed for 56 days produced rib steaks which were more flavorful and juicier than those at 28 or 112 days. Increases in tenderness have also been reported by Miller et al. (1975) Leander et al. (1978) and Shinn et al. (1976), although no change in taste panel tenderness scores or Warner-Bratzler shear force were determined between 28 and 77 days by Matthews and Bennett (1962). Crouse and coworkers (1978) used varying lengths of time on feed (105-365 days) and found that longer feeding periods for cattle to reach slaughter weight tended to decrease taste panel acceptability. Dolezai et al. (1982) fed steers for 100-230 days and found that extending time on feed beyond 100 days provided little additional palatability assurance. This conclusion is shared by numerous writers (Bowling et al., 1978); Epley et al., 1968; Dikeman et al., 1981)

Table 10 provides a breakdown of certain chemical and carcass measurements for bulls based on time on feed and frame size. Marbling scores

Table 10. Least squares means for selected carcass measurements of *longissimus thoracis* muscle from bulls based on frame size and time on feed

	Group[a]									
	11	12	13	21	22	23	31	32	33	SEM[b]
Marbling[c]	961^{vw}	947^{vw}	917^{w}	985^{v}	941^{vw}	922^{w}	953^{vw}	936^{w}	935^{w}	16.10
Fat Thickness (mm)	7.0^{wx}	8.3^{vwx}	6.6^{x}	9.6^{v}	7.5^{vwx}	8.1^{vwx}	9.0^{vw}	9.2^{v}	7.8^{vwx}	0.86
Lipid Content (%)	3.74^{wxy}	3.01^{yz}	2.69^{z}	4.96^{v}	3.12^{xyz}	3.92^{wxy}	4.33^{vw}	3.71^{wxy}	4.04^{vwx}	0.350
Moisture (%)	73.28^{wx}	73.89^{vw}	74.38^{v}	72.35^{x}	73.89^{vw}	73.38^{vwx}	73.16^{wx}	73.74^{vw}	73.57^{vw}	0.369

[a] Group designations = 10x Time on feed (1 = 180 days, 2 = 210 days, 3 = 240 days) + frame size (1 = small, 2 = medium, 3 = large).

[b] SEM is the standard error of the mean.

[c] 900 = $Slight^{0}$, 1000 = $Small^{0}$.

[v-z] Means within the same row having different superscripts are significantly different ($P<0.05$).

indicate no significant ($P<0.05$) difference between large framed animals irrespective of slaughter period. Medium framed animals during the third slaughter phase also fell into this category. Small framed bulls fed for 210 days showed significantly greater ($P<0.05$) amounts of marbling than large framed animals. There was no significant difference between small and medium framed bulls from the first slaughter period and medium and small bulls from phase two and three, respectively. Fat thickness measurements were significantly different between large framed animals during the first slaughter period and small and medium framed animals from slaughter periods two and three. There was also a significant difference between large framed bulls fed 180 days and small framed bulls fed for 180 and 210 days, respectively. Large variations in intramuscular lipid content were seen between the bulls. Large framed animals fed for the shortest time period did not differ significantly in intramuscular lipid, from medium framed bulls in both slaughter periods one and two. Significantly lower ($P<0.05$) values were also observed for intramuscular lipid contents between bulls of medium frame size fed 180 days and those of small and large frame fed 240 days. Small framed bulls also had the highest amount of intramuscular lipid. Moisture values were not significantly different between small framed bulls from the first slaughter date and those of medium and large framed bulls during the first and third slaughter phases. Included in those group were also medium and large framed bulls of the second slaughter date and small framed bulls of the third slaughter phase. A significant moisture difference is also noted for large framed bulls from the first slaughter phase and small framed bulls from the first and third slaughter periods respectively.

Small framed steers fed for the longest duration (Table 11) had the highest marbling scores and were significantly different from medium and large framed

Table 11. Least squares means for selected carcass measurements of *longissimus thoracis* muscle from steers based on frame size and time on feed

	Group[a]									
	11	12	13	21	22	23	31	32	33	SEM[b]
Marbling[c]	1040^{wxy}	978^{z}	981^{z}	1046^{wx}	990^{yz}	996^{xyz}	1072^{w}	1022^{wxyz}	1029^{wxyz}	20.0
Fat Thickness (mm)	10.8^{wxy}	8.4^{yz}	6.3^{z}	12.8^{vw}	11.6^{vwx}	8.1^{xy}	15.9^{u}	13.6^{uv}	11.9^{vw}	0.90
Lipid Content (%)	4.89^{vwxy}	4.03^{y}	3.92^{y}	5.84^{uv}	4.59^{wxy}	4.16^{xy}	6.88^{u}	5.73^{uvw}	5.29^{vwx}	0.274
Moisture (%)	71.85^{uv}	72.61^{u}	72.56^{u}	71.32^{vw}	72.23^{uv}	72.56^{u}	70.38^{w}	71.57^{v}	72.04^{uv}	0.301

[a] Group designations = 10x Time on feed (1 = 180 days, 2 = 210 days, 3 = 240 days) + frame size (1 = small, 2= medium, 3 = large).

[b] SEM is the standard error of the mean.

[c] 900 = Slight^{0}, 1000 = Small^{0}.

[u-z] Means within the same row having different superscripts are significantly different ($P<0.05$).

steers fed for shorter periods. Small framed steers from the first slaughter period had marbling scores that were not significantly different ($P<0.05$) from the third slaughter period irrespective of frame size or that of small framed steers fed for 210 days. Values for fat thickness among steer carcasses were significantly different ($P<0.05$) between small framed steers fed for the full period and all others except medium framed animals fed full term. Medium and large framed steers from the first slaughter phase had significantly lower ($P<0.05$) fat covers than all other animals except its medium framed counterpart from the same slaughter period. Medium framed steers fed the full period were not significantly different from those carcasses within the same feed group as well as small and medium framed animals in slaughter group two.

Lipid values for small framed steers from the third slaughter period were not significantly different from steers of the same frame size from slaughter period two and medium framed steers fed for 240 days. Large framed steers fed for 240 days were not significantly different from all steers in slaughter period two and small and medium framed steers fed for 180 and 240 days respectively. No significantly difference could be reported for steers fed 180 days and those fed 210 days of medium and large frame. Moisture values for small framed steers were lower than medium and large framed counterparts although a significant difference was detected between the steers from the first and third slaughter periods. No significant difference was detected among all steers in the first slaughter period, medium and large framed steers in the second and large framed steers in the third slaughter period. There was no significant difference for moisture between steers of small frame size fed 180 days, medium framed steers fed 210 days and medium and large framed steers fed 210 and 240 days on feed respectively.

It is evident from the palatability data summarized in Table 12 that no significant differences ($P<0.05$) existed for tenderness among bulls. Connective tissue scores varied little among bulls except between large framed bulls of the first slaughter period and remaining carcasses. Medium and large framed carcasses from trial one and two also showed no significant difference in connective tissue. The remaining carcasses did not differ significantly on the basis of connective tissue.

Juiciness scores varied significantly between medium frame bulls fed 180 days and all those bulls fed full term. Flavor intensity scores were higher ($P<0.05$) for medium bulls of the first slaughter period than large framed bulls of the same period. Flavor differences were perceived only between large framed bulls of the first feed time and small framed bulls of the second feed time. Myofibrillar shear force values differed amongst bulls for those of medium frames fed 210 days and that of large framed fed 240 days. Residual force measurements varied significantly between bulls of small frame fed 180 days and that of large frame fed 240 days.

Palatability and Warner-Bratzler shear force measurements for steers based on frame size and time on feed are reported in Table 13. A significant difference in tenderness is reported between small framed steers fed 180 and 240 days with that of large framed steers fed 240 days. In addition the aforementioned small framed steers were rated significantly higher than all remaining counterparts. Furthermore, small framed steers from slaughter periods one and three differed significantly for connective tissue from the remainder except for small and medium framed steers fed 210 and 240 days respectively. Higher juiciness scores ($P<0.05$) were noted for small steers from the first time frame when contrasted with remaining steers.

Table 12. Least squares means for palatability and Warner-Bratzler shear force measurements of *longissimus thoracis* muscle of bulls based on frame sizes and time on feed

	Group[a]									
	11	12	13	21	22	23	31	32	33	SEM[b]
Tenderness	6.9^{x}	6.9^{x}	6.7^{x}	6.9^{x}	6.7^{x}	6.9^{x}	6.9^{x}	6.8^{x}	6.7^{x}	0.096
Connective Tissue	6.9^{xy}	7.1^{x}	6.6^{z}	6.9^{xy}	6.8^{xy}	7.1^{x}	7.0^{xy}	7.0^{xy}	6.9^{xy}	0.086
Juiciness	6.6^{xy}	6.8^{x}	6.6^{xy}	6.3^{y}	6.6^{xy}	6.6^{xy}	6.4^{y}	6.4^{y}	6.4^{y}	0.095
Flavor Intensity	6.6^{xy}	6.8^{x}	6.3^{y}	6.6^{xy}	6.5^{y}	6.7^{xy}	6.6^{xy}	6.5^{xy}	6.5^{xy}	0.082
Flavor	6.5^{xy}	6.5^{xy}	6.2^{y}	6.7^{x}	6.6^{xy}	6.6^{xy}	6.5^{xy}	6.4^{xy}	6.6^{xy}	0.157
Myofibrillar Force (kg/cm^2)	2.5^{xy}	2.5^{xy}	2.6^{xy}	2.5^{xy}	2.4^{y}	2.5^{xy}	2.7^{xy}	2.6^{xy}	2.8^{x}	0.137
Residual Force (kg/cm^2)	2.3^{y}	2.4^{xy}	2.5^{xy}	2.4^{xy}	2.4^{xy}	2.4^{xy}	2.6^{xy}	2.5^{xy}	2.7^{x}	0.120

[a] Group designations = 10 x Time on feed (1 = 180 days, 2 = 210 days, 3 = 240 days) + frame size (1 = small, 2 = medium, 3 = large).

[b] SEM is the standard error of the mean.

[x-z] Means within the same row of havingf different superscripts are significantly different ($P<0.05$).

Table 13. Least squares means for palatability and Warner-Bratzler shear force measurements of *longissimus thoracis* muscle from steers of three frame sizes slaughtered at three different time intervals

	Group[a]									
	11	12	13	21	22	23	31	32	33	SEM[b]
Tenderness	7.3^{x}	7.0^{yz}	7.0^{yz}	7.1^{yz}	7.0^{yz}	7.0^{yz}	7.3^{x}	7.0^{yz}	6.9^{z}	0.09
Connective Tissue	7.2^{y}	6.9^{x}	6.9^{x}	7.1^{xy}	6.9^{x}	6.9^{x}	7.2^{y}	7.0^{xy}	6.9^{x}	0.08
Juiciness	6.9^{y}	6.7^{x}	6.7^{x}	6.7^{x}	6.7^{x}	6.8^{x}	6.8^{x}	6.7^{x}	6.6^{x}	0.09
Flavor Intensity	6.9^{x}	6.6^{x}	6.9^{x}	6.7^{x}	6.8^{x}	6.8^{x}	6.8^{x}	6.7^{x}	6.7^{x}	0.10
Flavor	6.8^{x}	6.8^{x}	6.8^{x}	6.7^{x}	6.7^{x}	6.7^{x}	6.8^{x}	6.7^{x}	6.6^{x}	0.08
Myofibrillar Force (kg/cm^2)	2.08^{xy}	2.28^{xy}	2.31^{xy}	2.07^{xy}	2.38^{y}	2.32^{xy}	2.01^{x}	2.29^{xy}	2.33^{xy}	0.01
Residual Force (kg/cm^2)	2.06^{y}	2.22^{xy}	2.23^{xy}	2.00^{y}	2.32^{xy}	2.31^{xy}	1.90^{y}	2.2^{xy}	2.39^{x}	0.11

[a] Group designations = 10 x Time on feed (1 = 180 days, 2 = 210 days, 3 = 240 days) + frame size (1 = small, 2 = medium, 3 = large).

[b] SEM is the standard error of the mean.

[x-z] Means within the same row of having different superscripts are sigificantly different ($P<0.05$).

The sensory panel was unable to detect any differences in flavor and flavor intensity from steaks from these steers. Warner-Bratzler myofibrillar force determinations were not significantly different except for medium framed steers from the second feed period and small framed steers of the third feed phase. Except for small framed steers that did not differ significantly in terms of residual force measurement and the significant difference between small framed steers and large framed steers fed 240 days, no differences were apparent. Johnson et al. (1988) reported that bulls aged 6 days postmortem had higher shear force values than steers but at 13 day postmortem equivalent shear force values were obtained.

Evidence provided by Field (1971) and Arthaud et al. (1977) clearly displays the greater toughness experienced by sensory panels or shear force values from bull meat. However, work conducted by Dransfield (1984) showed only one score to be lower ($P<0.05$) between bulls and castrates—that of juiciness of roasted *M. longissimus* from bulls. Rate of carcass chilling is considered to be the principal reason for tenderness differences between bulls and steers. However, following electrical stimulation (Crouse et al., 1983; Riley et al., 1983) and aging, bull beef was still found to be relatively tough, thus, cold shortening could not be attributed to the cause. Work by Boccard et al. (1979) claimed that the concentration of collagen is higher in bulls while Hinch and Thwaites (1982) and Gerrard and coworkers mention that a difference in the type of collagen has been found; bulls having a lower concentration of heat soluble collagen (i.e. an increase in cross-linked collagen). The concensus view from many papers (Cross et al., 1973; 1974; Liboriussen et al., 1977; Hedrick et al., 1969; Jacobs et al., 1977) is that young entire males are of comparable eating quality to that of their castrated counterparts and that toughening due to collagen is only significant when extremes of age are

compared. Results reported in this work substantiate previous work in terms of high scores for bulls and their general acceptability in terms of eating quality.

A breakdown of palatability and shear force data based on marbling is provided in Tables 14 and 15 for bulls and steers, respectively. No significant palatability difference between bulls could be reported except for juiciness, flavor intensity and myofibrillar force for Modest and Moderate degrees of marbling. Steers also exhibited a similar pattern whereby a significant difference could be detected for those muscles between the Slightly Abundant degree of marbling and the remainder of marbling categories for tenderness, connective tissue, myofibrillar and residual force. The high degree of marbling had a significant impact in terms of flavor between Traces and Slightly abundant groupings. Residual force values for steers varied but declined with a concomitant increase in marbling. Significant differences ($P<0.05$) were obtained between the chategories of Traces and Slight and that of Modest, Moderate and Slightly abundant. Evidence here substantiates previous findings attesting to the fact that marbling and tenderness are poorly correlated, (Parrish, 1974; Crouse et al., 1978, and Goll et al., 1965).

As indicated above, no significant differences could be detected for the marbling categories in relation to tenderness (except for prime grades) and this is evidenced by the poor correlations between marbling and tenderness ($r = 0.138$ and 0.111 for bulls and steers, respectively). Table 16 and 17 show strong correlations between tenderness and myofibrillar force ($r= -0.609$ and -0.719 for bulls and steers respectively) and between residual force and connective tissue ($r = -0.441$ and -0.539 for bulls and steers respectively). Significantly high correlations were also noted for moisture and lipid content ($r = -0.871$ for bulls and r

Table 14. Least squares means for palatability and certain carcass attributes of *longissimus thoracis* muscle from bulls stratified on the basis of marbling category

	Marbling Category				
	Traces	Slight	Small	Modest	Moderate
Number	28	93	23	3	1
Tenderness	6.7^{x}	6.9^{x}	6.9^{x}	7.1^{x}	6.6^{x}
Connective Tissue	6.8^{x}	6.9^{x}	6.9^{x}	7.1^{x}	6.7^{x}
Juiciness	6.4^{xy}	6.6^{xy}	6.6^{xy}	6.8^{x}	6.1^{y}
Flavor Intensity	6.6^{x}	6.6^{x}	6.6^{x}	6.8^{x}	6.1^{y}
Flavor	6.6^{x}	6.5^{x}	6.6^{x}	7.0^{x}	6.5^{x}
Myofibrillar Force(kg/cm^2)	2.68^{xy}	2.66^{xy}	2.43^{xy}	1.95^{x}	2.85^{y}
Residual Force(kg/cm^2)	2.60^{x}	2.55^{x}	2.44^{x}	2.29^{x}	2.03^{x}

$^{x-y}$ Means within the same row bearing different superscripts are significantly different ($P<0.05$).

Table 15. Mean values for certain carcass and palatability attributes of *longissimus thoracis* muscle from steers stratified on the basis of marbling category

	Marbling Category					
	Traces	Slight	Small	Modest	Moderate	Slightly Abundant
Number	5	63	55	2	4	1
Tenderness	6.9^{x}	7.0^{x}	7.1^{x}	7.1^{x}	7.1^{x}	7.5^{y}
Connective Tissue	6.9^{x}	7.0^{x}	7.1^{xy}	7.1^{xy}	7.1^{xy}	7.6^{z}
Juiciness	7.0^{x}	6.7^{x}	6.7^{x}	6.8^{x}	6.8^{x}	6.7^{x}
Flavor Intensity	6.9^{x}	6.8^{x}	6.7^{x}	6.7^{x}	6.8^{x}	6.7^{x}
Flavor	6.5^{x}	6.7^{xy}	6.8^{xy}	6.6^{xy}	6.9^{xy}	7.1^{y}
Myofibrillar Force(kg/cm^2)	2.45^{x}	2.36^{x}	2.13^{x}	2.10^{x}	2.32^{x}	1.67^{y}
Residual Force(kg/cm^2)	2.34^{x}	2.35^{x}	2.14^{xy}	1.93^{y}	1.89^{y}	1.51^{z}

$^{x-z}$ Means within the same row bearing different superscripts are significantly different ($P<0.05$).

Table 16. Correlation coefficients between certain physical, chemical and sensory characteristics of *longissimus thoracis* muscle from bulls

	Marbling	Moisture	Tenderness	Connective Tissue	Juiciness	Flavor	Flavor Intensity	Myofibrillar Force	Residual Force
Moisture	-0.547**								
Tenderness	0.138	-0.155							
Connective Tissue	0.151	-0.166	0.695**						
Juiciness	0.066	-0.065	0.539**	0.380**					
Flavor	0.068	-0.128	0.325**	0.062	0.423**				
Flavor Intensity	0.003	-0.092	-0.047	0.323**	0.159	0.255*			
Myofibrillar Force	-0.169	0.218*	-0.609**	-0.426**	-0.415	-0.204*	0.085		
Residual Force	-0.219*	0.277**	-0.581**	-0.441**	-0.444	-0.345**	0.008	0.779**	
Lipid Content	0.678**	-0.871**	0.203*	0.142	0.095	0.034	0.062	-0.254**	-0.302**

* P<0.05.

** P<0.01.

Table 17. Correlation coefficients between certain physical, chemical and sensory characteristics of *longissimus thoracis* muscle from steers

	Marbling	Moisture	Tenderness	Connective Tissue	Juiciness	Flavor	Flavor Intensity	Myofibrillar Force	Residual Force
Moisture	-0.685**								
Tenderness	0.111	-0.130							
Connective Tissue	0.158	-0.182	0.750**						
Juiciness	0.026	0.054	0.519**	0.448**					
Flavor	0.074	-0.131	0.185*	0.129	0.344**				
Flavor Intensity	0.001	-0.064	0.246*	0.278*	0.341**	0.49**			
Myofibrillar Force	-0.146	0.197*	-0.719**	-0.571**	-0.298**	-0.117	-0.200*		
Residual Force	-0.293*	0.302*	-0.639**	-0.539**	-0.218**	-0.142	-0.206*	0.753**	
Lipid Content	0.737**	-0.933**	0.119	0.159	-0.029	0.074	0.065	-0.196*	-0.317*

* $P<0.05$.

** $P<0.01$.

= -0.933 for steers). Residual and myofibrillar forces also showed strong correlations while the remainder of the correlations were poor.

A recent proposal to split the small category for marbling was considered (Table 18) at Small ^{50} and a comparison made between samples lying below Slight ^{50}, between Slight ^{51} to Small^{50} and up to Moderate ^{0}. It was not possible to provide any evidence to justify this proposal though differences did exist between Slight^{50} and remaining chategories for tenderness. Using marbling scores below Slight^{50} as a demarkation point may be a more appropriate cut off point.

Table 19 shows the R^2-values for palatability attributes from all possible regression analyses for bulls and steers using ultrasound data. Ultrasound measures accounted for 10.1% and 23.6% of the variations in tenderness of the model prediction equation for bulls and steers, respectively. In addition, connective tissue, moisture, myofibrillar force and residual force accounted for 9.7%, 46.8%, 10.3%, 12.1% and 10.1%, 56.9%, 20.9% and 25.1% of the variation in the model prediction equation for bulls and steers respectively. Extractable lipid accounted for 51.9% and 63.3% of the variation in the prediction equation for bulls and steers. Considering the low correlation between extracted lipid and marbling with tenderness as well as the fairly high accountability for variation in the prediction equation for the above parameters, signal measures were by no means able to predict tenderness in this study. Rouse et al. (1992) reported R^2-values being 0.44 and 0.41 for marbling and ether extract respectively for year 1990. Wilson et al., (1992) reported correlations to the order of 0.74. Thus the prediction equation is more suitable in accounting for lipid/intramuscular fat measurement. Use of ultrasound speckle by Brethour (1990) was found to be highly correlated (r = 0.67)

Table 18. Least squares means for palatability and shear force attributes of *longissimus thoracis* muscle of steers stratified on the basis of marbling

Parameter	Slight^{50}	Small^{50}	Moderate^{0}
Number	26	84	36
Tenderness	6.9^{x}	7.1^{y}	7.1^{y}
Connective Tissue	6.9^{x}	7.0^{x}	7.0^{x}
Juiciness	6.7^{x}	6.7^{x}	6.8^{x}
Flavor	6.7^{x}	6.7^{x}	6.7^{x}
Flavor Intensity	6.7^{x}	6.7^{x}	6.7^{x}
Myofibrillar Force (Kg/cm^2)	2.45^{x}	2.21^{y}	2.13^{y}
Residual Force (Kg/cm^2)	2.34^{x}	2.23^{xy}	2.02^{y}

$^{x-y}$ Means within the same row bearing different superscripts are significantly different ($P<0.05$).

Table 19. R^2-values for chemical, physical and sensory measures of bulls and steers using ultrasound data

Dependent Variable[a]	Bulls	Steers
Ether extractable lipid	0.519	0.633
Marbling	0.468	0.502
Tenderness	0.101	0.236
Connective Tissue	0.097	0.101
Moisture	0.468	0.569
Myofibrillar force	0.103	0.209
Residual Force	0.121	0.251

[a] Dependent variable = mean + std. dev. + max + min + skew + Kurt + mode + fat + hide + area + wt and age + fdm + fdsd + fim + fisd + fic + M11.

to marbling, but generally accounted for 25 to 30% of the marbling score variance. An attempt by Brethour (1990) to classify carcasses by grade led to a classification accuracy ranging from 53 to 94%. By means of "heuristically" rectifying the scale, an overall classification accuracy of 77.5% was achieved. Park and Whittaker (1990) reported an R^2 of 0.76 for ether extractable fat with ultrasound frequency analysis of Fourier spectra. In the publication of Chen et al. (1989), percent intramuscular fat was determined by image processing. Their results indicate that image processing did not give direct measurements of percent fat in the rib eye. One apparent reason is that these methods measured the amount of fat on a two-dimensional surface, while the chemical method is a three-dimensional measurement. Further refinment of the tissue characterization by ultrasound is needed to more accurately measure intramuscular fat (Duello et al., 1990).

CONCLUSION

For all palatability attributes and Warner-Bratzler shear force measurements conducted in this study, bulls ranked significantly lower or tougher than their castrate counterparts. Time on feed did not alter sensory panel scores amongst bulls or steers. Small framed steers were significantly more tender than all other frame size animals and this may have been related in their ability to fatten earlier as well as their greater marbling scores. Large framed bulls fed 180 and 240 days as well as medium framed bulls fed 210 days ranked the lowest in terms of tenderness, connective tissue, and juiciness. Large framed steers fed 240 days were equivalent in tenderness to medium and large framed bulls irrespective of slaughter period.

Based on the new proposal for quality grades of beef, no strong evidence to support palatability differences between slight, small or modest degrees of marbling could be found.

Correlation coefficients between marbling and tenderness were extremely poor while those of myofibrillar force measurements in relation to tenderness were 0.719 for steers.

Ultrasound data did not reflect meaningful palatability information. However, R^2-values between ultrasound and percent lipid and marbling were 0.519, 0.468 and 0.633 and 0.502 for bulls and steers, respectively.

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PPAPER II. EFFECT OF SEX, FEED TIME AND LIPID CONTENT ON THE CHOLESTEROL AND FATTY ACID COMPOSITION OF BOVINE *LONGISSIMUS THORACIS* MUSCLE

Effect of sex, feed time and lipid content on the cholesterol and fatty acid composition of bovine *longissimus thoracis* muscle

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ABSTRACT

Cholesterol content (mg % equivalent) and fatty acid composition of *longissimus thoracis* (LT) muscle, from bulls (N=50) and steers (N=59) fed for three different time periods, was determined by gas chromatography-mass spectrometry (GC-MS) and gas chromatography, respectively, following stratification by lipid content. Time on feed did not alter ($P<0.05$) cholesterol content, but significantly increased ($P<0.01$) the amount of lipid. A significant difference ($P<0.05$) was observed for cholesterol content between bulls and steers as well as within different lipid content groups of the LT muscle. Regression equations for cholesterol content of bulls and steers were generated between 0-8% intramuscular fat. Values for cholesterol reported herein are lower (≈ 15%) than previously reported results due to the superior analytical tool, GC-MS, permitting direct measurement of cholesterol based on molecular weight. Fatty acid compositional changes of muscle varied slightly among bulls and steers. Steers displayed a significant increase in C14:0 when fed for the full period. Stearic acid content declined significantly ($P<0.05$) as bulls remained longer on feed while their castrate counterparts exhibited this same trend, but was significant only up to 210 days on feed. Prolonging the feed time revealed higher proportions of unsaturated fatty acids. Lipid contents higher than 5% showed significant increases for C18:2 and significant decreases for C18:0 among bulls. Muscle of steers on the other hand showed significant increases in C18:2 when lipid levels were greater than 3%.

INTRODUCTION

There is general agreement among many health practitioners and professionals that persons susceptible to chronic atherosclerosis should monitor their consumption of saturated fat, cholesterol and total calories (Olson, 1987; Ross and Glomset, 1976). The fact that all animal products contain cholesterol (Merck, 1983) and that saturated fats are contained in meat, has led numerous people world-wide to change their eating habits. Reiser et al. (1985) and Kestin et al. (1989) suggested that the effect of dietary cholesterol in normolipidemic subjects is not modified by the nature or amount of dietary fat.

A number of publications have shown that fatty acid composition of bovine fat is influenced by dietary regimen (Sumida et al., 1972; Rumsey et al., 1972), sex (Walden et al., 1968; Eichorn et al., 1985) breed (Rumsey et al., 1972), season (Link et al., 1970) and length of feed (Hidiroglou et al., 1985). Cholesterol content has been reported on the basis of anatomical location (Tu et al., 1967), sex (Terrel et al., 1969), analytical method (Kaneda et al., 1980; Punwar, 1975), marbling content (Stromer et al., 1966) and even cooking methodology (Rhee et al., 1982b).

Intact males are highly efficient producers of lean when compared to castrates (Bailey et al., 1966) and are acceptable in palatability (Field et al., 1977; Jacobs et al., 1977). Research has shown significant differences in cholesterol content (Eichorn et al., 1986) and fatty acid composition (Hood and Ellis, 1976) between intact males and castrates. The present study warrants further examination of cholesterol and fatty acid contents by using gas chromatography, based on prolonged feed times for bulls and steers and lipid content . GC-MS methodology was used in the case of cholesterol quantification.

MATERIALS AND METHODS

Muscle samples from 50 bulls and 59 steers fed for three different feed times (180, 210, 240 days) were handled in the following manner:

Sample Preparation

Longissimus thoracis muscle samples excised between the 12th and 13th rib were denuded by dissecting the intermuscular and subcutaneous fat. Prepared strips were frozen and pulverized for 30 sec. in liquid nitrogen by means of a Waring-blender. Samples were packed in polyethylene bags, stored at -20°C and used as and when required.

Lipid Analysis

Proximate analysis (fat and moisture) of muscle samples were determined by standard methods (AOAC, 1980). Cholesterol extraction was carried by the method described by Lepage and Roy (1986). All tubes were silanized (AOAC, 1990) prior to use. Accurate weights of 0.1g sample were measured into each tube followed by the addition of internal standard, 50µl, of deuterium labelled cholesterol (Merck, Montreal, Canada). Direct transesterification was achieved following the addition of acetyl chloride (200µl) and methanolysis [2ml methanol-benzene; 4:1 (v/v)] at 100°C for 1 hr. A solution of 6% potassium carbonate (5ml) and benzene (2ml) were added following transesterification in order to neutralize the pH. Tubes were vortexed and an aliquot of the benzene fraction transferred to dram vials and dried at 65°C under nitrogen gas. To form derivatives, 100 µl of acetonitrile (Regis Chemical, Morton Grove, IL) and 100 µl of MTBSTFA [N-methyl-

N-(t-butyl-dimethylsilyl) trifluroacetamide, Regis chemical] were added to the dried sample and allowed to incubate at 65°C for 1 hr. Derivatized samples were transferred into injection vials (Sun Brokers, Wilmington, NC), capped, and analyzed by GC-MS (Hewlett-Packard gas chromatogram/mass selective detector, Model 5890/5970B, Avondale, PA). The column was a Hewlett Packard-1 (cross linked methyl silicone gum) column 50m x 0.2mm x 0.11 µm film thickness. The carrier gas was helium with an inlet pressure of 83 kPa (12 psi). An initial oven temperature of 60°C was used and increased with a ramp rate of 35°C/min to 350°C. Injections were made in the splitless mode for 0.2 min, while the injector and detector temperatures were maintained at 300°C. The major ion fragments for cholesterol and 7-D labelled cholesterol were monitored by using selective ion monitoring. Cholesterol was monitored at 368.3 atomic mass units (amu) and its 7-D-isotope at 375.3 amu. A standard curve for the cholesterol standard for concentrations of 37.5mg/25µl to 150mg/100µl provided an correlation coefficient of 0.997. The ratio of unknown sample to internal standard was calculated back relative to the ratio of known purified cholesterol and internal standard. This in turn was converted to mg % equivalents.

Fatty acid methyl esters were also prepared and sampled from the above procedure (Lepage and Roy, 1986). A Hewlett-Packard 5890 gas chromatograph (Avondale, PA) equipped with a flame ionization detector was used with a 15 m capillary column (0.25 mm internal diameter, stationary phase DB-23 0.5 µm thick J. W. Scientific, Folsom, CA). Operating conditions were as follows: helium carrier gas 77 ml/min, inlet and detector temperature 250°C, initial oven temperature 170°C for 0.5 min, increased by 20°C/min to 220°C for 2.5 min. Fatty acid methyl esters were identified by comparison with methyl ester standards (Sigma Chemical

Co., St. Louis, MO). Duplicate samples of the fatty acid peaks were quantified by electronic integration.

Statistical Analysis

Cholesterol content was analyzed by the PROC GLM procedure and least squares means compared by least significant difference (LSD). An α of 0.05 was chosen as the level of significance. The statistical analysis system (SAS, 1985) was used to compute all data analyses.

RESULTS AND DISCUSSION

Values obtained from lipid analyses at different times on feed were used to divide sex categories into three groups: 0-3.0%, 3.1-5.0%, and 5.1-8.0% lipid.

Cholesterol Content

Sex and intramuscular fat content were highly significant (Table 1). *Longissimus thoracis* samples from bulls and steers contained 51.3mg cholesterol/100g tissue and 53.7mg cholesterol/100g tissue, respectively. There was a significant ($P<0.05$) interaction between sex and lipid content while time on feed was non-significant. Means for cholesterol content of bulls and steers based on lipid content are compared in Table 2. A significant ($P<0.05$) increase in cholesterol content was noted with a corresponding increase in lipid amount for both sexes. Table 3 reveals the mean lipid content and marbling scores stratified on the basis of feed time. In all cases, bulls possessed less fat and lower marbling scores than their castrated counterparts. This is probably one reason for the difference in cholesterol content when sex effects are taken into account. These results substantiate those of Eichorn et al. (1986) who reported lower cholesterol levels in intact males rather than castrates, since the latter are fatter. Furthermore, Kregal (1986) showed that as fat level increased in raw patties, cholesterol content also increased.

Hoelscher et al. (1988) and Rhee et al. (1982a) demonstrated that cholesterol content of raw beef intermuscular fat and subcutaneous fat was 108 mg/100g raw tissue and 114 mg/100g raw tissue, respectively and to be much

Table 1. Mean squares for sex, time on feed, lipid content and sex x lipid content interaction

Sources	Degrees of Freedom	Mean Square
Sex	1	114.51***
Time on Feed	2	11.38
Lipid Content	2	206.57***
Sex x Lipid Content	2	31.23*
Error	101	8.75

* P<0.05.

*** P<0.001.

Table 2. Mean cholesterol content of *longissimus thoracis* muscles from bulls and steers based on lipid content

Lipid Content (%)	Bulls	Steers
0 – 3.0	47.74[a]	49.08[a]
3.1 – 5.0	52.23[b]	53.63[b]
5.1 – 8.0	54.95[c]	56.06[c]
SEM[d]	0.396	0.473

[a-c] Means in the same column with different superscripts are significantly different (P<0.05).

[d] SEM is the standard error of the mean.

Table 3. Mean fat content and marbling scores[f] for *longissimus thoracis* muscle of bulls and steers based on time on feed

	Bulls		Steers	
Feed Time (Days)	Lipid (%)	Marbling Score	Lipid (%)	Marbling Score
180	2.30[a]	903[a]	2.54[a]	929[a]
210	3.89[b]	954[b]	4.08[b]	986[b]
240	5.74[c]	1004[c]	5.94[c]	1045[c]
SEM[d]	0.008	8.22	0.108	10.42

[a-c] Means in the same column with different superscripts are significantly different ($P<0.05$).

[d] SEM is the standard error of the mean.

[f] 900 = Sight^0, 1000 = Small^0, 1100 = Modest^0.

higher than raw beef longissimus muscle (62 mg/100g raw tissue). Intramuscular and intermuscular fat are said to contain similar cholesterol values (Rhee et al., 1982a). Thus, cholesterol accumulation within the adipocyte has a significant influence on the total cholesterol content measured in this study.

Regression equations for mg cholesterol/100g wet tissue (Y) on % lipid (X) reported by Tu et al. (1967) and Rhee et al. (1982b) were Y= 48.9 + 1.7X and Y = 54.73 + 0.87X, respectively. Regression equations computed here for intact males and castrates were as follows: Y= 44.4 + 1.83X and Y = 48.9 + 0.72X. Correlation analysis (Table 4) showed marbling score and lipid content were positively correlated with cholesterol content although cholesterol and marbling produced a low correlation coefficient (0.438). Highly significant ($P<0.001$), but negative correlations were obtained for all parameters when related with water.

Values for cholesterol (mg/100g sample) reported by Rhee et al. (1982b) for raw beef *longissimus* steaks with "Traces," "Slight" and "Small" degrees of marbling were 60.66, 59.95 and 64.00, respectively. Fat content (%) for those same steaks were 3.63 6.06 and 7.95, respectively. These values and others (Tu et al., 1967; Eichorn et al., 1986 and Bohac et al., 1988) are considerably higher than reported here. Spectrophotometric methods that have been employed are known to produce erroneous results due to interfering sterols (Sweeney and Weiraugh, 1976), fatty acids, tryptophan and protein (Naber, 1989) and this may account for the elevated values (approx. 15%) in cholesterol content. GC-MS is a powerful separation tool enabling precise detection of cholesterol on a molecular weight basis. Furthermore, recent GC-MS analyses by Ulberth and Reich (1992) and Stewart et al. (1992) on a variety of food items lends itself to a revision of the current tables on composition of cholesterol in food.

Table 4. Correlation coefficients among selected variables for *longissimus thoracis* muscle from bulls and steers

	Lipid Content	Marbling Score	Moisture
Cholesterol	0.438***	0.438***	-0.687***
Lipid Content		0.679***	-0.74***
Marbling Score			-0.581***

*** P<0.001.

Fatty Acid Composition

No significant ($P < 0.05$) sex effects were observed for the nine fatty acids analyzed in this study. Gillis and Eskin (1973) reported significantly higher C14:0, C16:1 C18:2 and lower C18:1 in bulls than steers while Eichorn et al. (1985) presented data reflecting significantly higher ($P<0.01$) percentages of C18:2, C18:3 and C20:4 in bulls. Time on feed was found to be significant ($P<0.05$) for C18:1 and C18:3 while lipid content was significant ($P<0.05$) for C18:1 and C18:2.

Table 5 shows the change in fatty acid composition for bulls and steers based on time on feed. No significant difference was observed for C14:0, C16:0, 16:1 C17:0 and C20:4 for the bulls based on feed time. Steers displayed a similar pattern except that a significant increase was noted for C14:0 at 240 days on feed with no significant difference for C18:2 and C18:3. There was a significant decrease ($P<0.05$) in stearic acid content for bulls as they remained longer on feed while this was only apparent between 180 and 210 days for steers. An increase in C18:1 was observed for both sexes although a significant effect ($P<0.05$) was displayed only at 240 days for the bulls. In addition, a significant decrease ($P<0.05$) was noted for C18:2 amongst bulls between 180 to 210 days on feed.

It should be pointed out that a significant increase in unsaturated fatty acids was obtained with prolonged feeding, while the proportion of saturated fatty acids drops. However, justifying 240 days on feed in the case of bulls, and 210 days for steers remains questionable due to the economies of production. The biological signifance of such increases has yet to be determined.

Table 5. Fatty acid composition of *longissimus thoracis* muscle from bulls and steers stratified on the basis of time on feed

	Bulls			Steers		
Fatty Acid[a]	180	210	240	180	210	240
14:0	2.30^{x}	2.32^{x}	2.26^{x}	2.45^{x}	2.65^{x}	2.92^{y}
16:0	24.85^{x}	25.04^{x}	24.60^{x}	25.41^{x}	25.72^{x}	25.52^{x}
16:1	4.39^{x}	4.47^{x}	4.62^{x}	4.25^{x}	4.35^{x}	4.36^{x}
17:0	1.00^{x}	1.01^{x}	1.01^{x}	1.02^{x}	1.04^{x}	1.08^{x}
18:0	15.53^{x}	15.12^{y}	14.22^{z}	15.82^{x}	14.66^{y}	14.36^{y}
18:1	45.03^{x}	45.35^{x}	46.66^{y}	44.47^{x}	45.06^{y}	45.40^{y}
18:2	5.54^{x}	5.20^{y}	4.95^{y}	5.23^{x}	5.19^{x}	4.92^{x}
18:3	0.26^{x}	0.28^{x}	0.30^{y}	0.32^{x}	0.31^{x}	0.42^{x}
20:4	1.10^{x}	1.21^{x}	1.38^{x}	1.03^{x}	1.02^{x}	1.02^{x}
Total Saturated	43.71^{x}	43.52^{x}	42.09^{y}	44.54^{x}	43.78^{y}	43.88^{y}
Total Unsaturated	56.29^{x}	56.48^{x}	57.91^{y}	55.46^{x}	56.22^{y}	56.12^{y}

[a] Fatty acid composition expressed as a percentage of 9 fatty acid methyl esters.

$^{x-z}$ Means within the same row, for each sex group, bearing different superscripts are significantly different ($P<0.05$).

Table 6. Fatty acid composition of *longissimus thoracis* muscle from bulls and steers stratified on the basis of lipid content

	Bulls			Steers		
Fatty Acid[a]	0–3.0	3.1–5.0	5.1–8.0	0–3.0	3.1-5.0	5.1–8.0
14:0	2.70^{x}	2.70^{x}	2.76^{x}	2.60^{x}	2.72^{xy}	2.83^{y}
16:0	26.65^{x}	26.19^{y}	25.93^{y}	26.19^{x}	25.96^{x}	26.28^{x}
16:1	3.31^{x}	3.35^{x}	3.39^{x}	3.29^{x}	3.3^{x}	3.33^{x}
17:0	0.99^{x}	1.03^{x}	0.99^{x}	1.05^{x}	1.07^{x}	1.09^{x}
18:0	15.42^{x}	15.31^{x}	14.88^{y}	15.06^{x}	15.00^{x}	15.08^{x}
18:1	45.50^{x}	45.74^{x}	45.09^{y}	45.79^{x}	45.34^{x}	44.75^{y}
18:2	4.35^{x}	4.51^{x}	5.67^{y}	4.84^{x}	5.37^{y}	5.35^{y}
18:3	0.33^{x}	0.34^{x}	0.42^{x}	0.29^{x}	0.36^{x}	0.38^{x}
20:4	0.75^{x}	0.83^{x}	0.86^{x}	0.89^{x}	0.90^{x}	0.91^{x}
Total Saturated	45.76^{x}	45.23^{x}	44.56^{y}	44.90^{x}	44.75^{x}	45.28^{y}
Total Unsaturated	54.24^{x}	54.77^{xy}	55.44^{y}	55.10^{x}	55.25^{x}	54.72^{y}

[a] Fatty acid composition expressed as a percentage of 9 fatty acid methyl esters.

$^{x-z}$ Means within the same row, for each sex group, bearing different superscripts are significantly different ($P<0.05$).

Data presented in Table 6 shows fatty acid composition for bulls and steers stratified on the basis of lipid content. No significant ($P<0.05$) differences were noted for C14:0, C16:1, C17:0, C18:3, C20:4 and C16:0, C16:1, C17:0, C18:0, C18:3 and C20:4 between bulls and steers, respectively. An increase in lipid content (>3.0%) significantly decreased C16:0 among bulls while C18:0 also decreased significantly as lipid content exceeded 5%. There was also a significant increase in C18:2 with increase in lipid levels greater than 5%. Also noted was a tendency for C18:1 to increase, though not significantly, and then show a significant decrease in amount.

Steers showed similar trends with regard to C18:1 and C18:2 although the latter showed a significant ($P<0.05$) increase with lipid levels in excess of 3%. A significant decrease ($P<0.05$) was also noted for C14:0 for lipid levels of 0–3% and 5.1–8.0%. Sturdivant et al. (1992) examined black Wagyu cattle from Japan and found that among fat quality grades, C14:0 tended ($P<0.07$) to decrease with increasing quality grade. Commensurate with the decrease in palmitic acid (C16:0) as seen with fat quality grade was a general (though not significant) increase in C18:1 (Sturdivant et al. 1992).

Gillis and Eskin (1973) ascribed compositional differences between bulls and steers to the effect of sex hormones on enzyme systems such as desaturase (Marsh and James, 1962). The influence of changes beyond the rumen affecting fatty acid composition warrants further investigation (Ekeren et al.1992). Work by Hidiroglou et al. (1987) Skelley et al. (1973) and Westerling and Hedrick (1979) showed an increase in unsaturated fatty acid composition with diet and time on feed. Ekeren et al. (1992) concluded that metabolic changes occurred before fatty acid deposition and that stearoyi -CoA desaturase enzyme may resist any net

modification of bovine adipose tissue. Fatty acid values reported for different quality grades for Choice, Select and Standard are similar to those reported here (Smith et al. 1989; Vanderwert et al. 1988). Bensadoun and Reid (1965) and Wood (1984) claimed that undeveloped fat tissue (i.e. carcasses with low levels of fat) contain relatively high concentrations of saturated fatty acids.

CONCLUSION

The effect of sex, lipid content and sex x lipid content was found to be significantly ($P<0.05$) different for cholesterol content. Time on feed was non-significant for cholesterol content. Cholesterol content of *longissimus thoracis* muscle was found to be significantly higher for steers than bulls because steers were fatter than bulls (lipid content and marbling scores). When cholesterol content was stratified on the basis of lipid content, it was also shown that a significant increase in cholesterol was evident with increase in fat regardless of sex. Correlation coefficients between cholesterol, lipid content, marbling score and moisture were highly significant ($P<0.001$). Using the GC-MS technique cholesterol content of *longissimus thoracis* muscle was approximately 15% lower than previously reported data.

Increased time on feed produced a greater proportion of unsaturated fatty acids for both bulls and steers - primarily due to a reduction in stearic acid and an increase in oleic acid content. Increases in lipid content also revealed increases in unsaturated fatty acids. However, this was not consistent; stearic and oleic acid values were significantly reduced, while linoleic acid increased among bulls possessing lipid values in excess of 5%. Steers demonstrated an increase in linoieic acid greater than 3% lipid while oleic acid declined slightly above 5%.

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PAPER III. EFFECT OF POSTMORTEM pH ON THE PHYSICAL, CHEMICAL AND SENSORY CHARACTERISTICS OF BOVINE *LONGISSIMUS THORACIS* MUSCLE

Effect of postmortem pH on the physical, chemical and sensory characteristics of bovine *longissimus thoracis* muscle

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ABSTRACT

Samples of *longissimus thoracis* muscle with postmortem pH in the range of 5.5–6.9 were subjected to a 12 day aging period at 2°C. Sensory panel tenderness, connective tissue, juiciness, flavor intensity, Warner-Bratzler(WB) myofibrillar and residual force and myofibril fragmentation index (MFI) of high pH (6.7-6.9) meat were significantly different ($P<0.05$) from samples of intermediate pH 6.0-6.1. Muscles at pH 6.0-6.1 were the toughest samples. This was confirmed by WB myofibrillar force and MFI. Palatability attributes of normal pH (5.5) samples were not significantly different from dark cutting beef except for flavor and at the high pH extreme, tenderness. The increase in WB myofibrillar force at pH 6.0-6.1, lack of extensive degradation of muscle proteins at this pH and the decreased sarcomere length resulted in tougher meat than low or high pH muscles. Sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS–PAGE) on the dark cutters revealed that the breakdown of troponin-T to 30 Kd was not complete. In addition, the appearance of a 'doublet' on high molecular weight resolution gels may account for the unusal tenderness experienced by sensory panelists.

INTRODUCTION

Pre- and post-slaughter care are important factors in determining the eating quality of meat. Meat tenderness has been shown to be related to the state of muscle contraction (Locker, 1960; Marsh and Leet, 1966), enzymatic action on myofibrillar/cytoskeletal proteins (Parrish et al., 1981; Penny, 1974; Robson et al., 1991), postmortem temperature (Marsh et al., 1981; Parrish et al., 1973) and ultimate muscle pH (Bouton et al., 1957). Muscle fiber size (Crouse et al., 1991) and muscle fiber type (Totland et al., 1988) have also been implicated in this complex tenderness web.

The exact mechanism of meat tenderness has yet to be elucidated. When considering proteolysis, neutral/alkaline protease activity is said to occur while carcass temperatures and pH are high (Dayton et al., 1975; Marsh, 1983), or acidic cathepsins when pH is low (Dutson et al., 1980). Retention of calpain activity under acidic conditions has also been reported (Koohmaraie et al., 1986). However, postmortem pH and temperature are generally agreed to have a significant effect on final muscle tenderness (Locker and Hagyard, 1963; Marsh et al., 1981; Bouton et al., 1957).

Yu and Lee (1986), found *longissimus* muscle at intermediate pH (5.8-6.3) to have higher shear force values than at either low (<5.8) or high (>6.3) pH. Data presented by Bouton et al. (1957) and Dransfield (1981) showed a minimum tenderness existed at a pH of approximately 6.1. Purchas (1990) attributed increased shear force values at pH 6.1, at least partly, to sarcomere length,

although Yu and Lee produced evidence to the contrary with no significant differences being found for sarcomere length at all pH groups.

Previous work by Yates et al. (1983) and Samejima and Wolfe (1976) to determine postmortem degradation patterns of myofibrillar proteins showed troponin-T breakdown following incubation of samples at adjusted pH. Yu and Lee (1986) used subcutaneous injections of epinephrine in order to achieve high pH meat samples and found pronounced degradation of proteins associated with the thin filiment (tropomyosin, α-actinin and troponin). The objective of the present study was to investigate naturally occurring dark cutting, and normal pH beef with respect to sensory, physical and chemical parameters.

MATERIALS AND METHODS

From part of a larger study, carcasses from three slaughter periods having a pH ≥ 6.0 (n=14) were classified as dark cutting. Carcasses consisted of 3 bulls and 1 steer, 3 bulls and 2 steers, and 4 bulls and 1 steer from slaughter periods 1, 2 and 3 respectively. From the same study, 15 control samples of similar sex (pH = 5.5) were also obtained.

Sampling

Samples of *longissimus thoracis* (LT) 2.5 cm were excised 24 hr. postmortem from the right side of each carcass between 12th and 13th ribs. A Beckman Chem-Mate pH meter equipped with a surface probe was used to measure pH a 24hr postmortem. Samples were vacuum packed (Dixiepac 100) and aged for 12 days prior to freezing and used as and when required.

Ultimate pH Measurements

Ten grams of frozen sample were allowed to thaw to room temperature for 1.5hr. Samples were homogenized in 90 ml de-ionized water (pH 7.0). The filtrate of each suspension was used to measure the ultimate pH using an Accumet 925 meter and combined glass electrode.

Sensory Evaluation

Steaks were thawed for 48 hours at 2°C and then broiled to an internal temperature of 64.4°C. The internal temperature of steaks were monitored at the geometric center using copper-constantan thermocouples attached to an Omega

Digital Trendicator. Steaks were broiled to an internal temperature of 40°C, turned, and then broiled further and taken off to reach an internal temperature of 64.4°C. Once removed from the broiler, steaks were individually wrapped in aluminum foil, placed in a 60°C warming oven, removed and cut into approximately 1.27 x 1.27 x 3.18 cm sample cubes. Two samples, randomly chosen were served to a 8-10 member trained sensory panel. Panelists were individually seated in booths in a room separated from the preparation area and provided with water to rinse their palates between samples. Red fluorescent lights were used to eliminate any biases due to the color of samples. Panelists were trained by means of a variety of samples using the following sensory definitions: Myofibrillar tenderness - measure of how easily the sample breaks down into smaller pieces after three chews; Connective tissue - amount of insoluble connective tissue that remains in the mouth after thorough chewing; Juiciness - the sensation of free fluids released from the meat upon chewing; Flavor intensity - measure of the perceived intensity of beef flavor and Flavor - measure of the sensation perceived as beef flavor. Scores for tenderness (T), connective tissue (CT), juiciness (J), flavor intensity (FI) and flavor (F) were based on an eight point descriptive scale (tenderness 1 = extremely tough, 8 = extremely tender; connective tissue, 1 = abundant, 8 = none; juiciness, 1 = extremely dry, 8 = extremely juicy; flavor intensity, 1 = extremely bland, 8 = extremely intense; flavor, 1 = extremely off flavor, 8 = extremely flavorful). Values for each sensory attribute of a steak was reported as an average of panelists scores.

Warner-Bratzler Shear Force Measurements

Cores were removed from each of three sections (central, medial, lateral) of cooked steaks previously cooled to room temperature (25°C). Three 1.3 cm diameter cores were removed parallel with the axis of the muscle fiber and used for Warner-Bratzler Shear (WBS) assessment. Shear force measurements were carried out using the WBS-force attachment of the Instron Universal Testing Machine (Instron Corp. Canton, MA). A 50kg load cell and a cross-head speed of 100 mm/minute were used. Shear force deformation curves were recorded by means of a chart recorder using a chart speed of 100 mm/minute. Each core was sheared twice along the long axis and the values averaged. Moller (1981), reported stronger correlations with sensory panel data by dividing curves into two parts. The first yield point was identified as the compression/myofibrillar force corresponding to the myofibrillar component of tenderness while the second yield point relates to he residual force of the connective tissue component of tenderness. Values for each steak are reported in kg/cm^2 using a conversion factor of 0.36.

SDS-PAGE Electrophoresis

Isolation of myofibrils from *longissimus thoracis* muscles was carried out according to the procedure described by Goll et al. (1974). Protein content of the myofibril preparation was determined using the modified method of Robson et al. (1968)

Sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by using a modification of Laemmii (1970) with 5% polyacryiamide (acrylamide/bisacrylamide = 100:1 w/w) and 12% polyacrylamide (acrylamide/bisacrylamide = 37:1, w/w) slab gels (pH 8.9) of 16 cm x 14 cm x 0.15

cm dimension. The 12% gels were layered with 5% stacking gel. Protein samples were incubated at 50°C for 20 minutes in 0.5 ml tracking dye solution [30mM Tris-HCl, pH 8.00, 3mM EDTA, 3% (w/v) SDS, 30% (v/v) gylcerol and 0.3% (w/v) pyronin Y] and 0.1 ml of 2-mercaptoethanol (Wang, 1982).

Gels were stained with comassie brilliant blue [0.2% w/v) commassie brilliant blue R-250, 7% 9 v/v) acetic acid, 40% (v/v) ethanol]. Standards used include myosin (205 K); β-galactosidase (116 K); phosphorylase B (97.4 K), bovine albumin (66 K); egg albumin (45 K) and carbonic anhydrase (29 K) (supplied by Sigma Chemical Company).

Myofribil Fragmentation Index Determination

Myofibril Fragmentation Index (MFI) was determined on each steak by a modified procedure described by Parrish and DePuigar (1990). Three cores from each steak (medial, central and lateral) were finely scissor-minced and readily apparent pieces of fat and connective tissue removed. Four grams of this sample were homogenized for 30 seconds in 10 volumes (v/w) of a 2°C isolating medium of 100mM KCl, 20mM K phosphate, 1mM EGTA, 1mM $MgCl_2$ and 1 mM sodium azide. The homogenate was then passed through a polyethylene strainer to remove connective tissue and debris. A stirred amount (0.25ml) of the homogenate was diluted in 2.4 volumes (v/w) of the same isolating medium. Duplicates from each sample were prepared. The diluted myofibril suspension was stirred for 10 seconds and dispensed into a cuvette. Absorbance of this suspension was measured immediately at 540 nm using a Spectronic 20 Bausch and Lomb Colorimeter. The average absorbance measurements were multiplied by a factor of 200 to give an MFI for that particular steak.

Sarcomere Length

Sarcomere length was determined as described by Culler et al. (1978) on diluted myofibril suspensions from the short MFI method. Protein concentrations (0.5 ± 0.05 mg/ml) were determined by the biuret method of Gornall and coworkers (1949). A drop of the myofibril suspension was placed on a glass slide. The microscope slide was partitioned into imaginary quadrants and 24 myofibrils per quadrant were measured using a Vicker's image splitter attached to a Zeiss photomicroscope. The photomicroscope was adapted with a Neofluar 40X phase objective. Average sarcomere lengths in μm were determined using the following equation:

$$\frac{\text{Sum of lengths measured/sum of numbers of sarcomeres}}{(1.33 = \text{correction factor}).}$$

Statistical Analysis

The Satistical Analysis System (SAS 1985) was used to calculate least square means and standard errors and to detect treatment differences. Least significant difference tests were carried out to detect the effect of pH on palatabiiity, MFI and shear force measures. All tests of hypothesis are reported as signifcant using α=0.05.

RESULTS AND DISCUSSION

Carcasses for this study were classified on the basis of visual appearance (PSE; pale, soft, exudative or dark color) and pH. LT samples were divided into three different groups; low/normal pH (5.5), intermediate pH (6.0-6.1) and high pH (6.7-6.9).

Table 1 shows the distribution of carcasses by weight, maturity, and marbling score. The three groups were of similar maturity but carcasses within each marbling category averaged as 'Slight' in terms of degrees of marbling; higher scores being evident at the higher pH.

Results of physical and palatability attributes for *longissimus thoracis* muscles based on pH are given in Table 2. A significant difference was observed for sensory tenderness between high pH and remaining samples. A curvilinear relationship was obtained for sensory tenderness and Warner Bratzier shear force values by Bouton et al. (1957) and Purchas (1990). Thus a slight increase in toughness, though not significant in this case, was experienced at the intermediate pH range by all panelists.

Connective tissue, juiciness and flavor intensity differed significantly between high pH and intermediate pH ranges. No significant difference existed between normal pH samples and those at other pHs for these same sensory parameters.

Juiciness scores tended to be reduced in the intermediate pH range and improved on either side of this. Purchas (1990) reported that expressed juice decreased linearly with increase in pH but were not different after adjusting for pH. Flavor scores for normal pH and dark cutting beef were found to be significantly different ($P<0.05$), although no significant difference was detected amongst dark

Table 1. Selected carcass traits from carcasses varying in pH[a]

	pH		
Trait	5.5	6.0–6.1	6.7–6.9
No. of Carcasses	15	10	4
Carcass wt (kg)	316.8 ± 11.3	317.1 ± 13.4	346.4 ± 12.3
Maturity	A	A	A
Marbling Score[b]	941 ± 25	957 ± 30	985 ± 15

[a] Means ± standard deviations.

[b] Slight ^{0} = 900, Small ^{0} = 1000.

Table 2. Mean palatability, shear force, MFI and sarcomere length values for *longissimus thoracis* muscle for different pH

	pH			
Trait	5.5	6.0-6.1	6.7–6.9	SEM[a]
Tenderness	6.7^{x}	6.5^{x}	7.4^{y}	0.16
Connective Tissue	6.8^{xy}	6.7^{x}	7.1^{y}	0.10
Juiciness	6.6^{xy}	6.4^{x}	7.0^{y}	0.13
Flavor Intensity	6.7^{xy}	6.5^{x}	7.0^{y}	0.13
Flavor	6.7^{x}	4.2^{y}	3.9^{y}	0.16
Myofibrillar Force (kg/cm^2)	2.6^{x}	2.7^{x}	1.6^{y}	0.21
Residual Force (kg/cm^2)	2.5^{x}	2.8^{x}	1.8^{y}	0.22
Sarcomere Length (μm)	2.10^{y}	2.01^{x}	2.10^{y}	0.01
MFI	51.6^{x}	48.0^{x}	60.0^{y}	3.80

[a] SEM is the standard error of the mean.

$^{x-y}$ Means bearing the same superscripts as not significantly different ($P<0.05$).

cutters themselves. Researchers (Jeremiah et al. 1988) found that steaks from steers under minimum preslaughter stress were rated higher than their counterparts from bulls when under normal preslaughter stress. This would suggest that steer steaks contain appropriate flavor character notes.

Myofibril fragmentation indexes reported in Table 2 also showed a significant and dramatic effect at the high pH range when compared to intermediate pH range Myofibril fracture at high pH is almost instantaneous. It has been said that in the region of pH 6.8, tenderness is excessive (Bouton et al. 1957) and is associated with a jellylike consistency. Samples at this high pH were significantly different from all samples between pH 5.5 and pH 6.1. Measurements of sarcomere length at normal pH and pH 6.7-6.9 were significantly different ($P<0.05$) from those at pH 6.0-6.1.

Data in Table 3 are correlation coefficients between various physical, chemical and sensory characteristics of *longissimus thoracis* muscle from dark cutting beef. The most striking and significant relationships were between MFI, sensory tenderness, myofibrillar force measurement and residual force measurement. Correlations between MFI and the aforementioned parameters were 0.73, -0.77 and -0.76 respectively. Sensory tenderness scores were highly significantly correlated to myofibrillar force (-0.80) and residual force (-0.69). No significant correlation were computed between sarcomere length and other variables. Marbling, MFI and sensory tenderness were significantly correlated, although they were of low magnitude.

Figures 1 and 2 show SDS-PAGE patterns of purified myofibrils from *longissimus thoracis* muscles of various pHs following a twelve day aging period.

Table 3. Correlation coefficients between physical, chemical and sensory characteristics of *longissimus thoracis* muscle of dark cutting beef

	Myofibril Fragmentation Index	Sensory Tenderness Score	Myofibrillar Force	Residual Force	Fat Content
Marbling	0.34*	0.35*			
Sensory Tenderness Score	0.73**				
Myofibrillar Force	-0.77**	0.80**			
Residual Force	-0.76**	-0.69**	0.80**		
Sarcomere Length	0.26	0.34	-0.34	-0.39	0.16

* $p < 0.05$.

** $p < 0.01$.

SDS-5% gels were used to separate the high molecular weight proteins titin and nebulin while SDS-12% were used for greater resolution of the lower molecular weight protein spectrum.

Samejiwa and Wolfe (1976) and Yates et al. (1983) found alterations in myosin and troponin-T to be most noticeable at pH 5.4 at 37°C when compared to samples of pH 7.0 at 4°C. Olson and Parrish (1977) demonstrated marked and specific proteolysis in bovine muscle undergoing postmortem storage. They found the simultaneous disappearance of troponin-T and the concomitant appearance of a 30,000 Dalton component to be indicative of CAF activity. This intriguing finding was also shown to occur only in tender and not tough bovine *longissimus* (MacBride and Parrish, 1977). In all the dark cutters analyzed here (Fig. 1), troponin-T remained visible while traces of the 30Kd component were also seen. This could be due to further degradation of the 30Kd or insufficient troponin-T breakdown. Control samples at pH 5.5 showed marked intensities of 30Kd while the tough steer showed negligible amounts of this product. PSE beef (pH 5.5) had a very similar protein degradation pattern to the tough steer except that troponin-I appears to have disintegrated.

Figure 2 shows SDS-5% polyacrylamide gel electrophoretograms of purified myofibrils from *longissimus* muscles of various pH. The tough steer at three days postmortem shows distinct nebulin and titin bands (T_1 and T_2). A twelve day aging trial showed no nebulin present for all samples. Furthermore, titin is present in the T_2 form when considering the aging conditions used in this work. Certain degradation compounds were also present for aged samples between the migration bands of titin and nebulin. Their origin has yet to be elucidated although Fritz and Greaser (1991) found various degradation products using Western blots.

Figure 1. 12% polyacrylamide gel electrophoretograms, containing a 5% stacking gel, of myofibrillar/cytoskeletal proteins of bovine *longissimus thoracis* muscle. Lane 1 represents molecular weight standards; lane 2,. myosin standard; lane 3, troponin-T standard; lane 4, beef sample at 3 days postmortem; lane 5, pH 5.5 (normal beef); lane 6, pH 5.5 (PSE); lane 7, pH 6.0; lane 8, pH 6.1; lane 9, pH 6.4; lane 10, pH 6.7 and lane 11, pH 6.9 respectively. Protein concentrations in lanes 1-3 were 60µg while lanes 5-11 were 120µg

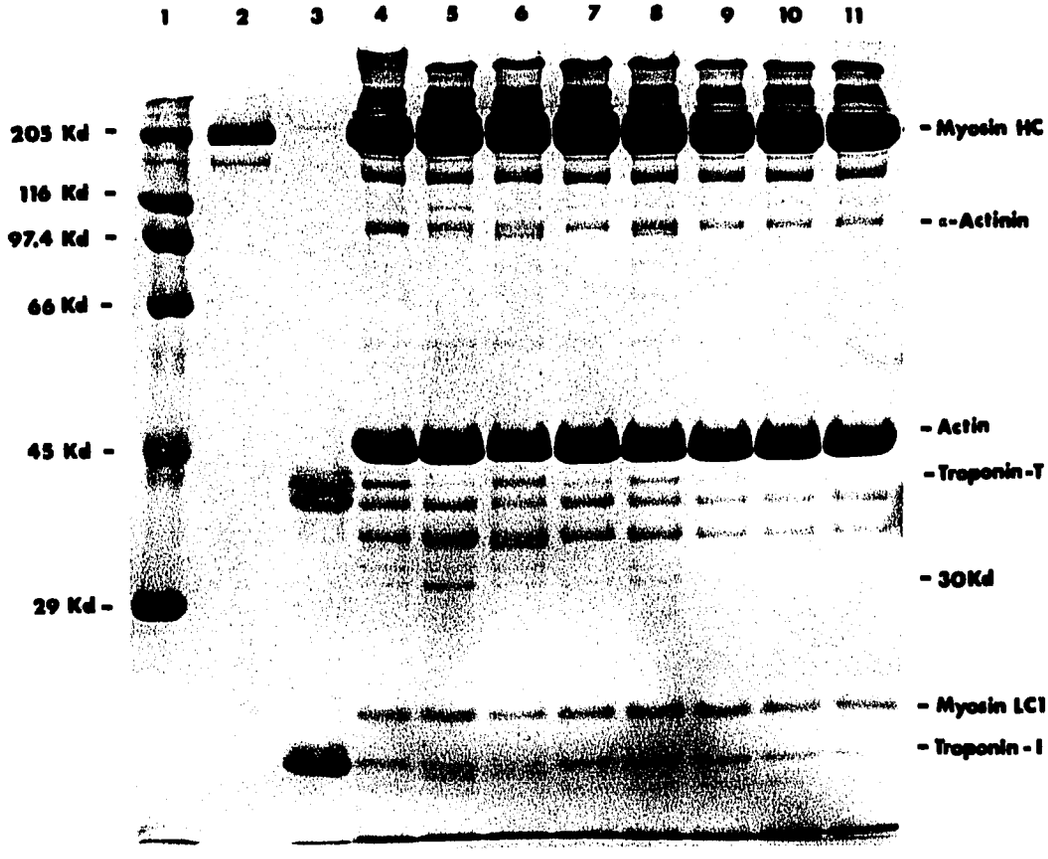
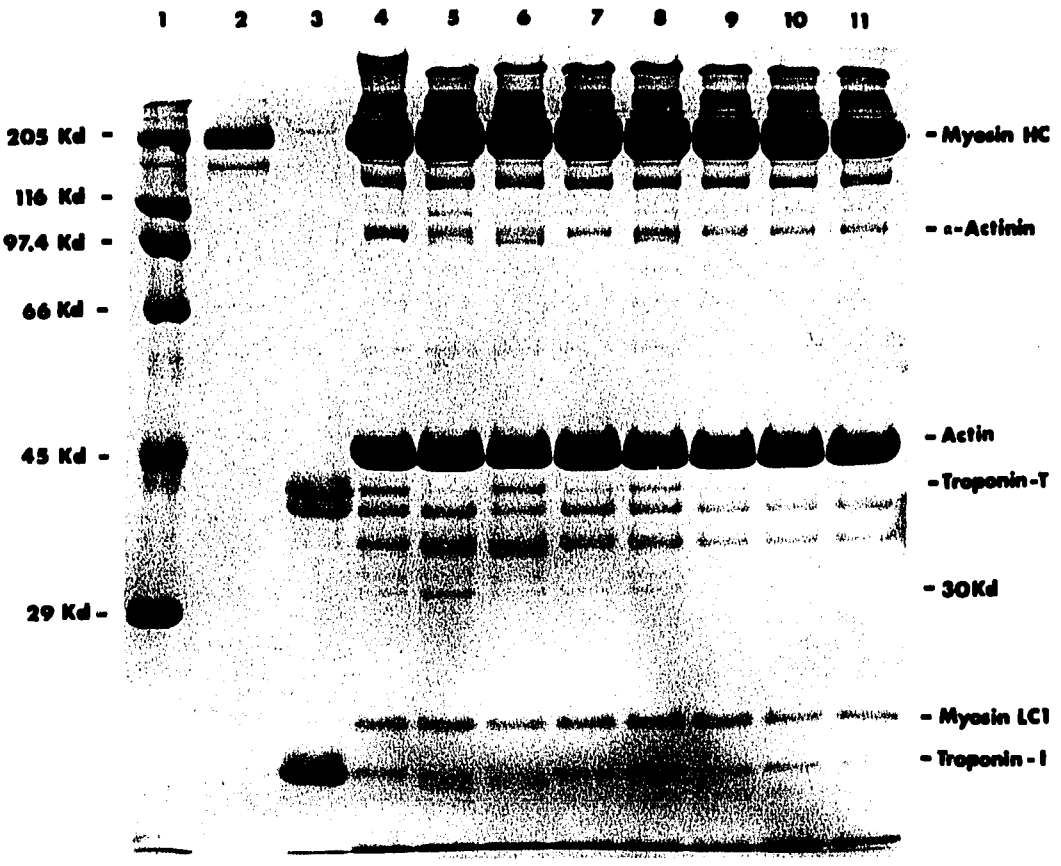

1
2
3
4
5
6
7
8
9
10
11
205 Kd
116 Kd
97.4 Kd
66 Kd
45 Kd
29 Kd
- Myosin HC
- α-Actinin
- Actin
- Troponin-T
- 30Kd
- Myosin LC1
- Troponin-I

Figure 2. 5% polyacrylamide gel electrophoretograms of myofibrillar/cytoskeletal proteins of bovine *longissimus thoracis* muscle. Lane 1 represents beef sample at 3 days postmortem; lane 2, pH 5.5 (normal beef); lane 3, pH 5.5 (PSE); lane 4, pH 6.0; lane 5, pH 6.1; lane 6, pH 6.4; lane 7, pH 6.7 and lane 8, pH 6.9 respectively. Protein concentrations in each lane were 120µg


1
2
3
4
5
6
7
8
9
10
11
205 Kd
116 Kd
97.4 Kd
66 Kd
45 Kd
29 Kd
- Myosin HC
- α-Actinin
- Actin
- Troponin-T
- 30Kd
- Myosin LC1
- Troponin-I

Muscle samples representing pH 6.0 through 6.9 also revealed a "doublet" of slightly lower molecular weight than nebulin even though pH 6.1 showed this band to be of lesser intensity.

Dayton et al. (1976) and Goll et al. (1989) reported that calpains are maximally active between pH 6.5 and 8.5. However, Dransfield (1992) claims from modelling postmortem tenderization, that as pH of muscle falls to about 6.1, calpain I is activated with the increase in calcium ion concentration to about 10^{-4}M. Koohmaraie et al., (1988) has cited evidence of 25% calpain I activity being retained even at pH 5.5. It is evident that due to the lack of lysosomal activity and neutral proteases, samples at the intermediate pH range were susceptible to limited degradation. This conclusion is supported by Yu and Lee (1986) when they examined samples of *longissimus* muscle between pH 5.8-6.3 by electron microscopy, and found limited degradation of Z-lines, thick and thin filaments.

This unusual myofibrillar protein degradation pattern i.e. the incomplete degradation of troponin-T (when compared to normal pH samples) and unknown degradation products of molecular weights of the order of 400Kd appearing as a 'doublet' may account for some of the differences in tenderness between dark cutting beef and beef at normal pH.

Titin and nebulin, the gigantic, longitudinally running proteins of the myofibril are anchored at one end of the Z-line. Titin is a known substrate for calpain (Zeece et al., 1986) and the role of calpain over nebulin degradation has yet to be elucidated (Robson et al., 1991). The intermediate filament, desmin, (encircling the Z-line periphery and connecting myofibrils) is rapidly degraded by calpain (O'Shea et al.,1979). Synemin, a protein colocalized with desmin at the Z-line periphery, (Bilak et al., 1989) may also be degraded by calpain (Robson et al., 1991). It is

possible that titin, nebulin, desmin and synemin play a greater role in tenderness than any of the other myofibrillar proteins during aging at high pH. Thus the preferential degradradation of the titin, nebulin, desmin and synemin should not be ruled out.

CONCLUSION

Dark cutting beef at the high pH extreme was found to be significantly different in terms of tenderness when compared to normal pH and intermediate pH samples. In addition, significant differences were noted for connective tissue, juiciness, flavor intensity, MFI, Warner Bratzler myofibrillar and residual force and sarcomere length when contrasted between high and intermediate pH. Highly significant correlations were also obtained between tenderness, MFI and Warner-Bratzier myofibrillar force. No significant difference was found between samples of normal and intermediate pH except for flavor.

SDS-PAGE revealed limited amounts of troponin-T breakdown for dark cutting beef. Protein degradation patterns of beef at pH 6.1 were unique and differed from samples at either pH extreme. PSE beef had a similar degradation pattern to 3 day aged samples except that troponin-I was not present on 12% gels. Dark cutting beef also revealed an additional 'doublet' on 5% gels.

Further work is required to elucidate the mechanism by which high pH meat imparts a tenderness effect over control samples. Myofibrillar protein degradation patterns based on an early time course postmortem has yet to be determined. The role of calpastatin over calpain in high pH muscle promises to be an area of research that would help elucidate the possible mechanisms involved in tenderization. Samples in this study were aged for a twelve day period but earlier and later postmortem samples would have helped to provide information on enzyme activity and myofibrillar changes.

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GENERAL SUMMARY

Palatability of Bulls and Steers

Conflicting evidence published about the palatability of bull and steer beef has related these differences primarily to the amount of cross-linked collagen and cold shortening exhibited amongst bulls. Electrical stimulation is said to obviate cold shortening while young bulls have limited amounts of cross-linked colagen. Furthermore, aging to thirteen days postmortem has been demonstrated to produce meat of equivalent tenderness between entire males and castrates. Time on feed studies have shown various endpoints necessary to improve palatability attributes in beef. Thus, a study was conducted to determine palatability differences between bulls and steers of three frame sizes slaughtered to varying endpoints, thirty days apart.

Bulls were rated significantly lower than steers for all palatability attributes measured in this study. However, differences were minor and may not necessarily be detectable. Warner-Bratzler myofibrillar force and residual force values were also higher amongst bulls than steers. Due to the highly significant effect of sex in the model, bulls and steers were analyzed separately.

Small framed steers were the most tender and deposited the highest amount of intramuscular and subcutaneous fat. Marbling has been shown to be poorly correlated to tenderness while larger amounts of fat cover have been attributed to the slow cooling of carcasses. The early postmortem period is one of tremendous importance for CAF activity while carcass temperatures and pH are high. It is imperative that CAF and calpastatin activity be evaluated between small, medium and large framed animals during the early period postmortem; in order to elucidate

palatability differences that may be revealed either by frame size or fat thickness. To date, no such evidence has been documented.

Both bulls and steers increased significantly in weight with time on feed. This increase in weight is manifested by an increase in fat thickness for steers while bulls exhibited increases in rib eye area.

Organoleptic properties and Warner-Bratzler measures were not significantly affected by frame size for bulls. However, small framed steers showed significantly higher scores for tenderness, juiciness and connective tissue when compared to large framed steers. This was verified by myofibrillar and residual force measurements. Flavor and flavor intensity was found to be not significantly different. Time on feed did not significantly effect any palatability attributes amongst bulls or steers. Warner-Bratzler measures were not affected significantly except for bulls fed 210 and 240 days. It may be that feeding times used here were too far extended to detect decernible differences.

When viewing palatability traits based on marbling chategory for steers, no significant differences in tenderness between 'Traces' to 'Moderate' chategories were detected. A significant difference was observed between the 'Slightly Abundant' chategory and remainder for tenderness, myofibrillar force, connective tissue and residual force measures. Prime steaks were thus superior in sensory qualities than all other steaks. A proposal to split the marbling chategory of 'Small' at 'Small^{50}' was found to be unwarranted. The use of 'Slight^{50}' as a demarkation point to expand the 'Choice' chategory appears more appropriate.

Results here confirmed previously reported data on the correlation between marbling and tenderness ($r = 0.138$ and 0.111 for bulls and steers respectively). Strong correlations were observed between tenderness and myofibrillar force ($r = -$

0.609 and -0.719 for bulls and steers respectively) and between residual force and connective tissue (r = -0.871 for bulls and -0.933 for steers). Correlations between lipid and marbling score were high (r = 0.678 and 0.737 for bulls and steers respectively).

R^2-values based on ultrasound data in order to ascertain palatability attributes were very poor. R^2-values for ultrasonic measures with lipid and marbling were 0.519, 0.468 and 0.633 and 0.502 for bulls and steers respectively.

Cholesterol and Fatty Acids

Cholesterol and fatty acids were extracted and transesterified by the modified procedure of Lepage and Roy, (1985). Cholesterol measurement utilizing a GC-MS revealed that (1) bulls had significantly lower amounts of cholesterol than steers; (2) time on feed did not significantly alter cholesterol content but increased the amount of lipid; (3) values for cholesterol reported in this study were lower than previously reported data since cholesterol was quantified on the basis of molecular weight.

It should be borne in mind that the method used above is extremely dangerous. Some of the chemicals used are hazardous and carcinogenic. Incubation of sample tubes at 100°C should be carried out in a contained environment while the glass tubes themselves must be replaced frequently.

GC was used to measure fatty acids present in *longissimus thoracis* muscle. Increased time on feed produced a greater proportion of unsaturated fatty acids for both bulls and steers - primarily due to a reduction in stearic acid. Increases in lipid content also revealed increases in unsaturated fatty acids. However, this was not consistent; stearic and oleic acid values were significantly reduced, while linoleic

acid increased amongst bulls possessing lipid values in excess of 5%. Steers demonstrated an increase in linoleic acid beyond 3% lipid while oleic acid declined slightly above 5%.

Dark Cutting Beef

Carcasses in this study were classified on the basis of appearance and pH. Bouton et al., (1957) and Purchas (1990) showed a curvilinear relationship for sensory panel tenderness scores and myofibrillar shear force values at various pH. These results confirmed previous data and showed that (1) dark cutting beef at the high pH extreme were significantly different in terms of tenderness, MFI and Warner-Bratzler myofibrillar force when compared to beef at intermediate pH; (2) sarcomere lengths at high pH were significantly greater than that at intermediate pH; (3) flavor scores for dark cutters were significantly lower than samples at normal pH; (4) highly significant correlations were obtained between sensory tenderness scores, MFI and Warner-Bratzler myofibrillar force.

SDS-PAGE showed limited amounts of troponin-T breakdown and thus limited amounts of 30Kd components in dark cutting beef. Further breakdown of 30Kd component should not be excluded. The presence of degradation products of the order of 400Kd may account for the unusual tenderness differences revealed between dark cutters and normal pH beef. PSE beef was similar to 3 day aged samples except that troponin-I was not present on 12% gels.

Further work is required to elucidate the mechanism by which high pH renders a greater tenderness effect over control samples. The role of calpastatin over calpain at high pH in meat systems warrants future investigation. The myofibrillar protein degradation patterns of dark cutting beef based on an early postmortem

period needs to be determined. This would shed light on the critical role of titin breakdown as it relates to tenderness.

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