INFLUENCE OF SEX ON THE HISTOLOGICAL AND TENDERNESS CHARACTERISTICS OF BEEF

By 4589

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INTRODUCTION

Meat, be it red meat, poultry or fish, has been an important part of man's diet since his primitive beginning. Since that time meat consumption has moved steadily upwards with beef consumption increasing even more dramatically. The high rate of beef consumption has been associated with greater production, higher consumer earning power and consumer's demand for beef.

Palatability of beef has economic importance in today's cattle industry. Tenderness makes an important contribution to the total variability in beef palatability; i.e., relatively tender meat always has been considered highly desirable. For years fundamental research has been undertaken to determine what biological and physiochemical interrelationship may be required to produce uniformly tender beef. Specific traits contributing to the variance in beef tenderness need further clarification.

Beef is becoming a luxury product, although palatability is extremely variable. Beef quality as a measure of palatability, is evaluated largely on maturity and marbling. Numerous studies in recent years have indicated that consumers are critical of the meat they buy. When the consumer buys meat, she wants to be sure that it is uniform and does not contain excess fat. For this reason research on meat tenderness has been actively pursued to provide consumer and merchandiser with more precise and objective methods for quality, hence palatability control.

As a result of improvement in management practices and balanced

feeding, younger cattle are being used for slaughter purposes. This emphasizes the need for new research to re-evaluate the maturity — marbling relationship. These qualities must be appraised more carefully in relationship to tenderness.

Improved histological tools, including the electron microscope, development of cryostat and the emerging discipline of histochemistry have re-awakened interest in histological methods for studying tenderness.

The present study was designed to provide further information on

1. the muscle fiber diameter and tenderness relationship
2. the importance of sarcomere length as an estimator of the state of muscle contraction, and a predictor of tenderness in muscle sample heated to a particular temperature
3. influence of sex and age on the histological and tenderness characteristics of beef.

REVIEW OF LITERATURE

Maturity

In general, carcass maturity and/or chronological age of the animal at the time of slaughter influences tenderness. It is the common belief that veal is more tender than calf, calf is more tender than beef and young beef more tender than old beef such as that obtained from cows, bulls and stags (Palmer, 1963).

Chronological age and physiological maturity are the terms used in the industry and by the research workers as synonyms, although there is a real difference between these terms. Chronological age is the calendar age while the physiological age is determined by the visual characteristics of bone, cartilage and muscle and is commonly referred as carcass maturity. Although this subjective evaluation of physiological age is not very accurate, it recognizes that carcasses differ with respect to the influence of sex, nutrition and other physiological factors (Berg and Butterfield, 1968).

Hiner and Hankins (1950); Hiner et al. (1955) and Tuma et al. (1963) working on beef from a wide chronological age range reported an inverse relationship between animal age and tenderness. Alsmeyer et al. (1959) obtained quite different results while conducting a study on cattle 5-30 months of age. Their study indicated an increase in tenderness with advancing maturity within this age range. Palmer et al. (1958) found no significant differences in tenderness among cattle of a narrow age range. In another study, his data, involving 538 carcasses ranging

in age from 5 to 99 months showed that age accounted for only 6% of the variation in tenderness (Palmer, 1963).

Goll et al. (1962) studied the rate of connective tissue solubilization brought about by collagenase treatment, relative to chronological age, in veal, cow, aged cow and steer groups. A decreasing rate of solubilization was noted as age increased with the exception of the steer group. The study indicated the existence of more frequent or stronger cross linkages within and among the tropocollagen molecules of more mature collagen which affected the meat tenderness independent of the amount of connective tissue.

Tuma et al. (1962a) compared the tenderness of twenty-four Hereford females, 18, 42 and 90 months of age with a "slight amount" or a "slightly abundant" degree of marbling. Tenderness decreased significantly as animal age increased with the greatest difference occuring between the 18 and 42 months-old animals. The 42 and 90 months-old animals were similar in tenderness. Therefore they suggested that animal age may be more critical with regard to tenderness at a point between 18-42 months of age.

Webb et al. (1964) conducted a study on cattle 12, 24 and 60 months of age to determine the effect of chronological age, physiological stress and post-mortem environment on muscle composition and tenderness. They reported that the meat from younger cattle was significantly more tender than from older cattle.

Romans et al. (1965) found that neither maturity, marbling, nor core location within the muscle had a significant effect on tenderness as determined by the Warner Bratzler shear, even though a trend for the more mature carcasses to be less tender was present.

Walter et al. (1965) in a study found that wide extremes in maturity influenced the eating quality of beef more than the wide extremeties in marbling.

Field et al. (1966) studied the influence of age and marbling on palatability of bovine carcasses slaughtered at 300 to 699 days of age. They found that the age of the steers and heifers did not affect any palatability characteristics when marbling was held constant. When the age was held constant, higher marbling scores in bulls were more closely associated with higher sensory ratings than were higher marbling scores in steers and heifers.

Dunsing (1959) also reported a decrease in tenderness with advancing animal age.

Because of low and conflicting relationships, the value of age and marbling in determining the eating quality of beef is being questioned. More information is needed, particularly from animals 300 to 699 days of age, since a large percent of beef produced is from cattle within this age range.

Marbling

The accumulation of intramuscular fat, referred to as marbling as an indication of quality has been studied by many researchers. Variations in the quantity of intramuscular fat at different anatomical locations within the longissimus muscle have been reported by Doty and Pierce (1961), Lawrie (1961) and Blumer et al. (1962). Cook et al. (1964) noticed that the distribution of intramuscular fat was uniform between the 10th and 13th vertebrae of bovine longissimus muscle and decreased towards both extremeties.

Conflicting research findings exist concerning the relationship between marbling and palatability. Evidences exist to suggest that the presence of marbling influences in some way and to some extent the palatability of meat. Kropf and Graf (1959); Palmer et al. (1958); Simone et al. (1958); Kauffman et al. (1964) and Field et al. (1966) have conducted studies showing that intramuscular fat was related positively to the meat palatability factors. Blumer et al. (1963) reviewed studies involving 2600 cattle carcasses and found that from 1 to 36% of variation in tenderness can be attributed to marbling. Pearson (1966) also stressed the importance of intramuscular fat in regard to meat quality. On the contrary Ramsbottom (1945); Cover et al. (1958); Cover and Hostetler (1960); Walter et al. (1965); Wellington and Stouffer (1959); Suess et al. (1966) and Judge et al. (1959) have shown that marbling may not be directly related to tenderness.

Tuma et al. (1962 and 1963) studied the influence of marbling and animal age on palatability, color and chemical properties of beef muscle. They found that the association between marbling and tenderness varied with animal age. The longissimus muscle was a darker red with advancing animal age and also increased upon aging 14 days. Within age group correlations between the value notations and panel tenderness, shear, pH, marbling and ether extract were generally low and variable in sign. Walter et al. (1965) and Romans et al. (1965) found that percent water decreased as the percent ether extract and marbling score increased.

Blumer et al. (1962) reported that the highest amount of ether extract was associated with coarse marbling, whereas Carpenter et al. (1961) found in pork studies that a higher percentage of extractable fat was associated with finely dispersed small sized fat cells in the muscle.

Tuma et al. (1961) experimenting on muscles from 6 months-old calves and 90 months-old cows found that the calf muscle had more moisture content than the cows. Similar findings were reported by Goll et al. (1963).

Webb et al. (1964) working on cattle 12, 24 and 60 months of age found no significant differences between total ether extract and moisture content of the muscle. A significant correlation of -.45 for 50 samples was obtained between ether extract and nitrogen.

Fiber Diameter

Studies by meat researchers were being conducted to compare the differences in fiber diameter and sarcomere length with tenderness variation.

Brady (1937) conducted studies on four beef muscles and obtained a low correlation between fiber diameter and shear force. He reported that the muscle texture is related to tenderness, i.e., muscles of coarse grain being less tender than those of the fine grain.

Hiner et al. (1953) working on cattle 10 weeks-old to 9 years old obtained a significant fiber diameter-tenderness relationship, that is, as fiber diameter increased in size, tenderness decreased correspondingly. They further reported that as the chronological age of the animal increases, fiber diameter also increases.

Joubert (1956) demonstrated that a close relationship existed between diameter of the muscle fiber and total musculature in lamb. He also stated that as the animal increased in weight from birth to maturity, the muscle fibers increased in size until their maximum growth capacity was reached.

Tuma et al. (1962a) observed a gradual increase in fiber diameter

with increasing animal age for the longissimus muscle. Their study indicated that the larger the fiber diameter, the higher the shear force when the data is uncorrected for animal age. Waviness of muscle fibers in about 40% of the samples was also observed especially in animal 18 months and older. A statistically significant "position effect" for the longissimus muscle was also noticed, the dorsal (medial) position being more tender. Alsmeyer et al. (1965) also reported similar results for the "position effect."

Swanson et al. (1965) found a great deal of muscle variation in fiber size both among positions along the longissimus muscle and among different locations within each position for animals of the same weight and grade. They found that fiber size is not a good indicator of longissimus muscle area.

Romans et al. (1965) conducted a study to provide information regarding the role played by carcass maturity and marbling in determining beef quality. They found that muscle fiber diameters were significantly larger in the moderate marbling group than in the slight marbling group. A trend towards larger fiber diameter was noted for the more mature carcasses. Fiber diameter was not significantly correlated with tenderness.

Eisenhut et al. (1965) reported that the amount of physical stress controlled muscle fiber diameter. In their study, carcass sides which were subjected to great stretching (stress) were significantly more tender than comparable sides in which the stress was medium.

Herring et al. (1965 and 1966) observed that muscles which had smaller fiber diameter had long sarcomeres and vice versa. They obtained a highly significant simple correlation of -.67 for sarcomere length and fiber diameter which tends to substantiate the fact that there is

an association between fiber diameter and sarcomere length when muscles are either stretched or contracted. A highly significant effect of age and sarcomere length was observed on fiber diameter.

Wang et al. (1956) showed that muscle fiber extensibility had a negative relationship to tenderness with correlations from -.43 to -.65.

In the light of the new tools available for histological research, the relationship between microscopic structure and tenderness needs further study.

Sarcomere Length

During recent years considerable interest has been focused on the relationship between post-mortem muscle contraction and tenderness. The extent of stretch or contraction induced by pre-rigor treatment is reflected by sarcomere length.

Locker (1960) postulated that some of the variations in sarcomere length among muscles may be due to stretch induced in the muscles during vertical suspension.

Welbourne et al. (1968) conducted a study to find the relationship among shear values, sarcomere lengths and cooling procedures in turkeys. They stated that measuring the sarcomere length determines the effect of the chilling procedures on length of muscle fibrils and their correlation with shear values. Chilling treatments resulted in a progressive shortening of sarcomere lengths in breast and thigh muscle with decreasing temperatures.

Herring et al. (1965a) observed that when muscles shortened, corresponding decreases in sarcomere length, increases in fiber diameter and decreases in tenderness were found and vice versa. Muscles which

increased in sarcomere length and had a corresponding decrease in fiber diameter also tended to show a decrease in shear force. They further reported that in comparison with horizontally placed sides, the vertically placed sides had greater sarcomere length in psoas major, latissimus dorsi and rectus femoris muscles.

Howard and Judge (1968) compared the sarcomere length in bovine longissimus muscle to other variables for predicting tenderness in medial and lateral muscle positions. They observed that the medial muscle position was less tender, had shorter sarcomeres and had higher correlations between sarcomere length and tenderness than the lateral muscle position. When comparing sarcomere length to shear resistance for all locations of the longissimus muscle, very small differences in contractile state were associated with marked differences in tenderness.

Gothard et al. (1966) conducted histological studies of post-mortem changes in sarcomere length as related to bovine muscle tenderness. They observed that as the rapid phase of contraction began, muscle fibers contracted at various rates. "Rapidly" contracting fibers were normally straight in appearance, while adjacent "slower" contracting fibers were usually wavy or "kinked" in appearance. They found that the kinking was due to a pulling influence of more highly contracted fibers on those more relaxed. Considerable lengthening of sarcomeres normally occured during aging period.

Sex and Sex Hormone

It has been assumed that certain sex or sex conditions as such have little effect on tenderness. This assumption has been held with barrow-gilt and steer-heifer differences in particular (Palmer, 1963).

Cahill et al. (1956) made a study on 40 cattle of similar age and breeding in four lots of ten head each. The four groups consisted of steers, stilbestrol implanted steers, bulls and stilbestrol implanted bulls were slaughtered at about 16 months of age. Their results indicated that all carcasses were acceptable in tenderness. Differences in tenderness of broiled steaks between groups were not pronounced at either 3 or 13 days post-mortem.

Pilkington et al. (1959) conducted carcass studies with steers, bulls and stilbestrol-implanted bulls sold as slaughter calves to find out the effect of sex on palatability and tenderness. They found no significant difference in tenderness of broiled steaks as determined by Warner-Bratzler shear between steers, stilbestrol implanted bulls and bulls slaughtered as calves.

Adams and Arthaud (1963) studied the influence of sex and age on tenderness of beef. A significant difference among sexes was found. Steers were significantly more tender than bulls, whereas no significant difference was observed between the tenderness of bulls and heifers or between heifers and steers.

Cahill (1964) studied the effect of sex differences in beef carcasses relative to cutability and palatability and found that steers and heifers of similar age, breed and marbling are equal in tenderness.

Field et al. (1966) found that bulls 500 to 599 and 600 to 699 days old were significantly less tender than steers and heifers of comparable age. Sensory scores for flavor and juiciness of steers and heifers were significantly higher than for bulls in the older age group. Marbling scores for these two older groups were significantly higher in steers and heifers than in bulls. Significantly higher palatability rating

favoring the steers and heifers were obtained when all steer and heifer carcasses were compared with bull carcasses.

Woods (1962) found little difference in carcass grade score, dressing percent, area of longissimus muscle or fat covering over the 12th rib between implanted and non-implanted steers or between control and steers fed stilbestrol. The combination of an implant and oral feeding of stilbestrol did not appear to influence carcass characteristics.

Bailey et al. (1966) investigated the quality factors of the longissimus muscle from young bulls and steers and showed that the stilbestrol implant caused a decrease in percent ether extract of steers, but tended to increase slightly the intramuscular fat in bulls. Differences in tenderness and flavor between bulls and stilbestrol treated steers were non significant.

In a study on lamb carcass characteristics, Knight and Foote (1965) observed that differences between wethers and ewes were minimal. Results substantiated slight differences in conformation, but otherwise close similarity was the rule. Longissimus muscle measurement, cut-out proportions and carcass component percents indicated little discrepancy between the wethers and ewes.

Microwave Cookery

Electronic cooking is done by microwaves, which are transmitted by a magnetron tube located at the back of the compartment of the microwave oven. The waves are absorbed by the food; they create molecular activity within the food and as a result, heat is produced and the food is cooked. Since electronic ovens are now being used in homes, it is of interest to know how cooking in such ovens compares with conventional cooking.

Fielder et al. (1962) reported that electronic and deep-fat cooking resulted in greater cooking losses than did broiling or oven roasting. Carpenter and King (1965) and Braniff et al. (1961) also reported similar findings.

Monk et al. (1964) studied the cooking losses for poultry meat and found less weight losses occurred during electronic cooking than during broiling or oven roasting.

Headley and Jacobson (1960) compared the effect of electronic and conventional cookery on lamb from 8 animals and found that electronic cookery was four times as fast as conventional roasting. Shrinkage in size was greater in the electronic range than in the electrically heated oven. Their study indicated that conventionally roasted meat was juicier with better lamb flavour than the electronically cooked meat.

In a study conducted by Apgar et al. (1959) to compare the quality of pork patties, roasts and chops cooked electronically (with and without browning unit), conventionally and in an institutional electronic oven, it was found that conventional cooking required about five times the cooking time of electronic cooking. No significant differences in total-cooking loss occurred due to cooking method of either the patties or roasts. Cooking electronically resulted in significantly less weight loss in chops. Tenderness scores for the conventionally-cooked chops, though not significant, tended to be slightly more desirable than for those electronically cooked.

In view of the variable methods used and reported in the literature, Hedrick et al. (1968) conducted a study to determine the effects of cooking methods, core diameter, temperature of sample core when sheared and core position on Warner-Bratzler shear value of beef short loin steaks.

Their studies indicated that shear force values were significantly affected by method of cookery, temperature of sample cores when sheared, sample core size and sample core locations. They found that shear values were significantly affected by all treatments. Sample cores from steaks cooked in deep fat had higher shear values than sample cores sheared hot. Sample cores from the central and lateral locations had higher shear values than sample cores from the medial position.

Marshall et al. (1960) compared the preparation losses, cooking time and yield on the basis of internal temperature and oven temperature. It was observed that total cooking loss was higher and drip loss was double that from conventionally roasted meat. Portions of the electronically cooked roasts were hard, dry and were rated lower in appearance, tenderness, juiciness and flavor than that cooked by the conventional method.

Pollack et al. (1960) and Law et al. (1968) also compared the effects of electronic cooking vs. conventional cooking and observed similar results.

MATERIALS AND METHODS

Materials

This project was designed to obtain production and carcass data on bulls and steers. The cattle used in this experiment were from the University of Nebraska breeding project herd. At weaning, the calves were allotted at random to the slaughter and treatment groups.

The calves were halter broke and started on feed the first thirty days after weaning. For the remainder of the experiment, the calves were given free access to a pelleted ration while tied to individual feeders overnight. Total consumption was recorded for each animal. The pelleted ration consisted of 58% shelled corn, 35% alfalfa meal, 5% molasses, 1% soybean meal and 1% mineral. The ration was calculated to contain 64.4% total digestible nutrients. Approximately two pounds of grass hay per head was also fed during the day. Stilbestrol implants were used on part of the bulls and steers. The treatments were as follows: Treatment 1 - steers without implants (1SU), Treatment 2 - steers with 48mg implant (2ST), Treatment 3 - bulls without implant (3BU), Treatment 4 - bulls with 96mg implant (4BT), and Treatment 5 - bulls with 192mg implant (5BTH).

The bulls and steers were slaughtered on two dates. The early slaughter date was 212 days after the adjustment period. The late slaughter date was 240 days after the adjustment period. The animals were 13 to 15 months old when slaughtered. The experiment was a completely randomized design (table 1). These treatments were randomly distributed in both sexes and ages. Slaughter dates were also randomized. Only one

treatment was used for one animal. The cattle were slaughtered in a commercial packing plant. The carcasses were graded to the nearest one-third of a grade by a representative of Meat Grading Branch, Livestock Division, C&M.S., U.S. Department of Agriculture. The grades ranged from average standard to low choice and marbling ranged from practically devoid to small+.

TABLE 1. EXPERIMENTAL DESIGN

mg. of stilbestrol implant	0mg	48mg	96mg	192mg	Total
*SEX					
Steers	11	13	-	-	24
Grade	Good+	Good+			
Marbling level	Small-	Small-			
Bulls	17	-	10	12	39
Grade	Standard+		Good	Good	
Marbling level	Trace+		Slight	Slight	
Total	28	13	10	12	63

*Sexes were not represented equally in each treatment group.

Sample Selection

The right side of each carcass was returned to the meat laboratory and was cut into closely trimmed boneless retail cuts according to Murphey et al. (1960). The longissimus muscle from the 9-10-11 rib cut of the

right side was used for the Warner-Bratzler Shear Test and histological studies. The medial position was selected for taking the cores from these cuts. The samples were supplied by the Nebraska Project and stored in a freezer at -23.5C until used.

Methods

Histological

One-half inch cores for sarcomere length determination and fiber diameter measurement were taken from the medial position of each sample after it was thawed for 12 hours in a refrigerator. Sarcomeres were extracted from the muscle using the method of Locker (1960) and described in appendix I. The sarcomeres were measured with a Wild Heerburg phase contrast microscope using oil immersion lens, 1500 magnification and an eyepiece micrometer.

The procedure for the measurement of fiber diameter was that of Tuma et al. (1962) and is listed in appendix II. A total of 50 fibers were measured for each sample using a Bausch and Lomb compound microscope fitted with an eyepiece micrometer at 150 magnification. An average fiber diameter was then determined for each sample and was converted to microns.

Wavy fibers were evaluated by counting the number found in 15 fields for every sample.

Techniques used for the measurement of fat distribution, staining of sections and the photographic techniques were those used by Reddy (1969) and described in appendix III, IV, V, and VI.

Deep Fat Cookery and Shear Evaluation

Each sample was divided in the frozen condition into two halves. One half was used for deep fat frying and the other half was saved for electronic cooking. The group of samples which was to be used for deep fat cookery was removed from the freezer and thawed in a 3.3C cooler until pliable (about 10-12 hours). A core, one inch in diameter was removed from each sample just before cooking was started. Care was taken in removing the core parallel with muscle fibers orientation. Each core was cooked to an internal temperature of 79.5C in vegetable oil pre-heated to 90-95C on an electric hot plate. The internal temperature was determined by a recording potentiometer (Honeywell) equipped with a thermocouple placed exactly in the center of the core. To assure uniform cooking on all sides and to prevent moisture loss each core was enclosed in two or three polyethylene bags one over the other in the form of layers. These bags were "Whirl Pack"and were almost of the size of the meat core. Although the cores were cooked in vegetable oil, they were untouched with oil due to these polyethylene bags. In this way, each core was cooked in steam and natural juices by wrapping it closely in the polyethylene bags. Therefore this deep fat cookery was actually a modified moist-heat cookery. Not more than three cores were cooked in the containers at a time and each with its covering of the polyethylene bags was allowed to hang with a string from a stand in the middle of the oil container. This assured uniform cooking of the cores on all sides. Average cooking time recorded was 7 minutes. After cooking, each core was cooled to an internal temperature of 37.5C. The average of three shears on each core was determined by Warner-Bratzler shear.

Microwave Cookery

The other half of the samples were used for this cookery method. The same method was used for thawing and for taking the core as in the case of deep fat cookery. A "Radarange, MARK IV Microwave Oven" (Raytheon) was used for this method of cooking. All the cores were cooked to an internal temperature of 79.5±2C which is almost the same temperature used for deep cooking. Average cooking time recorded was 25 seconds. After cooking, each core was allowed to cool to 37.5C. The average of three shears on each core was determined by the Warner-Bratzler shear.

Statistical Analysis

Data obtained on the histological and tenderness characteristics were analyzed by analysis of variance, correlation coefficients and multiple regression analysis. A stepwise regression analysis was done to compare the contributions made by the individual variables when combined with other variables, to the prediction of tenderness.

RESULTS AND DISCUSSION

Means and standard deviations for the histological and palatability characteristics of the bovine *longissimus* muscle are given in table 2.

Fiber Diameter

There was a significant ($P<.01$) increase in fiber diameter mean from 65.05 to 69.07 microns with an increase in animal age (table 3). An increase of 0.108 microns was observed for every one day increase in the age of the animal (table 4). This is in agreement with the results of Tuma *et al.* (1962) and Hiner *et al.* (1953). Fiber diameter was not found to be significantly associated with tenderness which does not agree with the findings of the aforementioned workers but agrees with the results of Romans *et al.* (1965). Covington (1970) also found that fiber diameter increases with advancing maturity even though he used physiological maturity rather than chronological age.

Increase in the fiber diameter with the increase in the age of the animal can be explained according to Brody (1945) that during the auto-accelerating phase of growth the fiber continues to increase in thickness and this, together with increase in its length, accounts almost entirely for the development of any particular muscle. During the subsequent auto-inhibitory phase, when the growth in general slows down, the increment in fiber size becomes progressively smaller until mature size is obtained.

Fiber diameter was found to be negatively correlated ($r=-.33$) with sarcomere length (table 5) which indicates that as fiber diameter increases

TABLE 2. MEANS AND STANDARD DEVIATIONS OF HISTOLOGICAL AND TENDERNESS CHARACTERISTICS OF BOVINE *LONGISSIMUS* MUSCLE

Variable	Means	Standard deviations
Age (days)	467.46	18.88
Fiber diameter (Microns)	67.58	5.90
Percent wavy fibers	52.12	10.09
Sarcomere length (Microns)	2.06	0.14
Total muscle area (sq. cm.)	56.58	2.02
Number of fat deposits I	19.87	6.44
Number of squares with fat deposits I	15.70	4.68
Area of fat deposits I (sq. mm.)	0.50	0.23
Number of fat deposits II	1.84	1.85
Number of squares with fat deposits II	5.68	11.52
Area of fat deposits II (sq. mm.)	0.49	0.47
Number of fat deposits III	2.37	1.13
Number of squares with fat deposits III	26.49	17.00
Area of fat deposits III (sq. mm.)	4.70	4.26
Total fat area (sq. mm.)	5.66	4.24
Total number of squares with fat	45.54	17.48
Total number of fat deposits	24.14	7.42
Average size of fat deposits (sq. mm.)	0.30	0.31
Percent fat (Histological)	6.73	6.09
Number of squares of fat/unit muscle area	0.20	0.15
Shear value, fat cooking (kg)	18.73	6.04
Shear value, microwave cooking (kg)	17.98	5.40

TABLE 3. ANALYSIS OF VARIANCE FOR HISTOLOGICAL AND TENDERNESS CHARACTERISTICS OF BOVINE LONGISSIMUS MUSCLE

Variables	TREATMENT		AGE (REGRESSION)		ERROR
	M.S.	Calculated F value	M.S.	Calculated F value	M.S.
Fiber diameter (Microns)	28.34	0.88	252.01	7.82**	32.20
Percent wavy fibers	291.55	3.23*	12.65	0.14	90.20
Sarcomere length (Microns)	0.03	1.50	0.17	8.50**	0.02
Total muscle area (sq. cm.)	6.85	1.73	0.07	0.01	3.94
No. of fat depo. I	17.96	0.48	361.17	9.76**	36.98
No. of sqrs. with fat depo. I	32.23	1.58	67.84	3.32	20.40
Area of fat depo. I (sq. mm.)	0.06	1.20	0.02	0.40	0.05
No. of fat depo. II	5.23	1.56	0.01	0.002	3.35
No. of sqrs. with fat depo. II	91.33	0.67	148.53	1.09	135.52
Area of fat depo. II (sq. mm.)	0.37	1.72	0.01	0.04	0.22
No. of fat depo. III	1.27	1.06	4.38	3.68	1.19
No. of sqrs. with fat depo. III	354.74	1.23	160.28	0.55	287.77
Area of fat depo. III (sq. mm.)	14.80	0.79	4.89	0.26	18.69
Total area of fat (sq. mm.)	14.21	0.76	5.49	0.29	18.52
Total no. of sqrs. with fat	301.10	0.98	321.92	1.04	306.73
Total no. of fat depo.	36.25	0.74	431.45	8.86**	48.68
Average size of fat depo. (sq. mm.)	0.07	0.70	0.18	1.80	1.80
Percent fat (Histological)	49.53	1.37	29.47	0.81	36.08
No. of sqrs. of fat/unit muscle area	0.02	1.00	0.02	1.00	0.02
Shear value, fat cooking (kg)	112.80	3.62*	84.58	2.72	31.09
Shear value, microwave cooking (kg)	35.91	1.28	99.65	3.55*	28.02

* $P < 0.05$ sig. level.
** $P < 0.01$ sig. level.

there is a decrease in the sarcomere length. Herring et al. (1965) observed similar correlation between fiber diameter and sarcomere length. No significant correlation was observed between shear (fat cooking or microwave cooking) and fiber diameter. This shows that fiber diameter is not a good indicator of tenderness. Tuma et al. (1962) also found that if the effect of animal age was removed, fiber diameter was a poor indicator of tenderness. Fiber diameter was also found to be negatively correlated ($r=-.24$) with number of fat deposits of category III which points out that as fiber diameter increases in size there is a recuction in the number of larger fat deposits.

Sarcomere Length

The decrease in sarcomere length means from 1.98 to 1.90 microns with advancing age of the animal was of significant magnitude ($P<.01$), (table 3). A decrease of .003 microns was observed with every one day increase in the animal age (table 4). Howard (1966) found the same trend between sarcomere length and age. Covington (1968) also reported a trend for sarcomere length to decrease as maturity increased but this relation was not significant.

Waviness of the Muscle Fibers

Waviness of muscle fibers was observed in all the samples. The importance of taking into consideration the percentage of wavy fibers is that there apparently is a relationship between post-mortem muscle contraction and tenderness. Contraction of the muscle is brought about by the sliding of thin actin filaments into the space between the thick filaments.

There was no significant effect found on the percent wavy fibers by

TABLE 4. LEAST SQUARES MEANS AND ADJUSTED TREATMENT MEANS OF THE HISTOLOGICAL AND TENDERNESS CHARACTERISTICS OF BOVINE LONGISSIMUS MUSCLE

Variables	Mean	ADJUSTED TREATMENT MEANS					F Value	Age (regression)
		1SU	2ST	3BU	4BT	5BTH		
Fiber diameter (Microns)	67.45	65.05	66.81	68.53	69.07	67.79	0.88	.108**
Percent wavy fibers	51.49	50.66	46.32	58.44	50.24	51.79	3.23*	.024
Sarcomere length (Microns)	1.96	1.96	1.98	1.93	1.96	1.90	1.50	-.003**
Total muscle area (sq. cm.)	56.51	57.07	56.97	56.98	56.20	55.33	1.73	.002
No. of fat depo. I	19.73	20.02	18.73	17.97	18.36	19.05	0.48	-.130**
No. of sqrs. with fat depo. I	15.74	17.54	15.03	17.27	15.02	13.85	1.58	-.056*
Area of fat depo. I (sq. mm.)	0.48	0.42	0.46	0.44	0.47	0.60	1.20	.001
No. of fat depo. II	1.81	1.25	0.98	2.28	2.48	2.06	1.56	.001
No. of sqrs. with fat depo. II	5.55	2.77	9.98	5.72	5.17	4.11	0.67	-.083
Area of fat depo. II (sq. mm.)	0.47	0.33	0.28	0.66	0.63	0.45	1.72	-.001
No. of fat depo. III	2.35	2.31	1.87	2.42	2.52	2.72	1.06	-.014*
No. of sqrs. with fat depo. III	26.67	23.98	35.39	22.14	26.07	25.78	1.23	-.086
Area of fat depo. III (sq. mm.)	4.64	3.74	6.52	4.34	4.13	4.47	0.79	-.015
Total area of fat (sq. mm.)	5.59	4.36	7.32	5.46	5.25	5.55	0.76	-.016
Total no. of sqrs. with fat	45.46	43.73	52.73	45.17	46.51	39.16	0.98	-.122
Total no. of fat depo.	24.00	23.61	21.86	26.23	24.24	23.88	0.74	-.142**
Average size of fat depo.	0.31	0.31	0.44	0.27	0.26	0.28	0.70	.003
Percent fat	6.89	6.40	9.26	4.33	6.55	7.91	1.37	.037
No. of sqrs. of fat/unit muscle area	0.21	0.17	0.26	0.22	0.19	0.21	1.00	-.001
Shear value, fat cooking (kg)	18.53	14.90	15.66	21.38	20.52	20.19	3.62*	.063*
Shear value, microwave cooking (kg)	17.90	15.91	16.22	19.12	19.61	19.64	1.28	.068*

* $P < .05$ sig. level.
** $P < .01$ sig. level.

the age of the animal but the mean for the older animals was greater. Tuma et al. (1962a) also observed waviness of the muscle fibers in 40% of their samples (range of waviness within samples 0-85%) with a predominance for animals 18 months and older. Similar findings were reported by Covington (1970) also.

Treatment had a significant ($P < .05$) effect on the percent wavy fibers with the means ranging from 46.32 to 58.44 percent (table 3).

The 't' values for treatment differences are shown in table 7A and treatment means are shown in table 7B. Treatment 1SU was significantly different from treatment 3BU as it decreased the percent wavy fibers by 15.1% from the mean. Similarly treatment 2ST was found to be significantly different from treatments 3BU and 5BTH. The effect of treatment 2ST significantly decreased the percent wavy fibers by 23.5% and 10.6% from the mean when compared with the treatments 3BU and 5BTH respectively. Among the bulls treatment 3BU significantly increased the percent wavy fibers when compared with treatments 4BT and 5BTH.

A significant ($P < .05$) negative correlation ($r = -.28$) was found between sarcomere length and percent wavy fibers which shows that as muscle becomes more wavy its average sarcomere length becomes shorter.

Marbling

Fat is normally deposited at widely varying rates in different parts of the body which results in marked variation in the proportion of fat found in different parts. Although intramuscular fat makes its appearance from the very early life of the animal but the rate of deposition increases with the advancing age of the animal.

The effect of age on the distribution and area of fat deposits is

presented in tables 3 and 4. A highly significant ($P < .01$) effect of age was observed on the number of fat deposits of the category I (i.e., smaller fat deposits), and also on the total number of fat deposits. The general trend was a reduction in the number of small fat deposits and total number of fat deposits with the increase in the age of the animal. This means that as the animal advances in age smaller fat deposits disappear or they unite to form medium sized fat deposits. They may be the reason that there is a reduction in the total number of fat deposits. Table 4 shows a decreasing but non-significant trend in the total area of fat deposits with advancing animal age. Reddy (1969) found that maturity had little influence on the number and area of fat deposits, although the most mature group had the highest fat content.

The number and area of small, moderate and large fat deposits had no significant effect on tenderness.

The differences in the estimation of fat by histological method (used in the present study) and chemical methods (used by some other workers) might be due to the basic differences in the two procedures. In addition, differences in estimation may also be due to the differences in sample size, sample location and to great variation in the quantity and distribution of fat in the longissimus muscle. Wang et al. (1954), Doty and Pierce (1962), Blumer et al. (1962), Cook et al. (1964) and Weir (1953) found extreme variability in fat content within the longissimus muscle. There is also evidence that species and breed differences exist (McMeekan, 1940 and Carpenter et al., 1961).

THE FOLLOWING DOCUMENT(S) IS OVERSIZED AND IS BEING FILMED IN SECTIONS TO INSURE COMPLETENESS AND CONTINUITY

TABLE 5. CORRELATION COEFFICIENTS COMPARING THE HISTOLOGICAL AND TENDERNESS CHARACTERISTICS OF BOVINE LONGISSIMUS MUSCLE

	Fiber diameter (Microns)	Percent wavy fibers	Sarcomere length (Microns)	Total muscle area (sq. cm.)	No. of fat deposits I	No. sqrs. with fat deposits I	Area of fat depo. I	No. of fat depo. II	No. sqrs. with fat depo. II	Area of fat depo. II (sq. mm.)	No. of fat depo. III	No. sqrs. with fat depo. III	Area of fat depo III sq. mm.)
Fiber diameter (Microns)	1.00												
Per cent wavy fibers	0.23	1.00											
Sarcomere length (Microns)	-0.33**	-0.28*	1.00										
Total muscle area (sq. cm.)	0.04	0.03	-0.04	1.00									
No. of fat deposits I	0.16	-0.21	0.19	0.01	1.00								
No. of squares with fat deposits I	-0.05	-0.11	0.17	-0.04	0.56**	1.00							
Area of fat deposits I (sq. mm.)	0.17	-0.12	-0.12	-0.18	0.33*	0.05	1.00						
No. of fat deposits II	0.00	0.05	0.04	0.11	0.23	0.06	0.17	1.00					
No. of sqrs. with fat deposits II	-0.09	-0.04	0.06	0.14	0.08	0.02	-0.05	0.17	1.00				
Area of fat deposits II (sq. mm.)	-0.09	0.04	0.14	0.08	0.21	0.11	0.07	0.87**	0.17	1.00			
No. of fat deposits III	-0.24*	-0.04	-0.01	-0.01	0.26*	0.11	-0.05	-0.03	0.04	0.01	1.00		
No. of sqrs. with fat deposits III	-0.06	-0.14	0.08	0.04	-0.08	0.13	-0.27*	-0.30	0.06	-0.18	0.26*	1.00	
Area of fat deposit III (sq. mm.)	-0.06	-0.12	0.08	0.08	-0.02	-0.15	-0.22	-0.12	-0.03	0.01	0.19	0.91**	1.00
Total area of fat (sq. mm.)	-0.06	-0.11	0.08	0.07	0.03	-0.14	-0.16	-0.01	-0.02	0.13	0.19	0.87**	0.99**
Total no. of squares with fat	-0.01	-0.01	0.14	0.04	0.16	0.20	-0.18	-0.04	0.13	0.10	0.20	0.85**	0.81**
Total no. of fat deposits	0.11	-0.21	0.17	0.03	0.96**	0.52**	0.32**	0.44**	-0.02	0.40**	0.36**	-0.11	-0.02
Average size of fat deposits (sq. mm.)	0.02	-0.08	-0.07	0.09	-0.38**	-0.42**	-0.24*	-0.19	-0.09	-0.14	-0.06	0.61**	0.67**
Percent fat (Histological)	-0.01	-0.10	-0.01	-0.13	-0.32**	-0.24*	-0.16	-0.13	-0.10	-0.19	-0.06	0.49**	0.48**
No. of sqrs. of fat/unit muscle area	-0.08	-0.08	0.11	0.10	0.09	-0.18	-0.14	0.11	0.01	0.17	0.08	0.77**	0.91**
Shear value, fat cooking (kg)	0.13	0.02	-0.15	-0.25*	0.17	0.14	0.23	0.03	-0.12	0.01	-0.07	-0.21	0.19
Shear value, microwave cooking (kg)	0.13	-0.03	-0.04	-0.29*	0.01	-0.03	0.07	0.00	-0.16	0.04	-0.17	-0.00	0.04

* P .05 sig. level.
** P .01 sig. level.

TABLE 5. CORRELATION COEFFICIENTS COMPARING THE HISTOLOGICAL AND TENDERNESS CHARACTERISTICS OF BOVINE LONGISSIMUS MUSCLE

1.00															
0.56**	1.00														
0.33*	0.05	1.00													
0.23	0.06	0.17	1.00												
0.08	0.02	-0.05	0.17	1.00											
0.21	0.11	0.07	0.87**	0.17	1.00										
0.26*	0.11	-0.05	-0.03	0.04	0.01	1.00									
-0.08	0.13	-0.27*	-0.30	0.06	-0.18	0.26*	1.00								
-0.02	-0.15	-0.22	-0.12	-0.03	0.01	0.19	0.91**	1.00							
0.03	-0.14	-0.16	-0.01	-0.02	0.13	0.19	0.87**	0.99**	1.00						
0.16	0.20	-0.18	-0.04	0.13	0.10	0.20	0.85**	0.81**	0.81**	1.00					
0.96**	0.52**	0.32**	0.44**	-0.02	0.40**	0.36**	-0.11	-0.02	0.05	0.16	1.00				
-0.38**	-0.42**	-0.24*	-0.19	-0.09	-0.14	-0.06	0.61**	0.67**	0.64**	0.40**	-0.39**	1.00			
-0.32**	-0.24*	-0.16	-0.13	-0.10	-0.19	-0.06	0.49**	0.48**	0.45**	0.29*	-0.32**	0.62**	1.00		
0.09	-0.18	-0.14	0.11	0.01	0.17	0.08	0.77**	0.91**	0.92**	0.75**	0.11	0.58**	0.41**	1.00	
0.17	0.14	0.23	0.03	-0.12	0.01	-0.07	-0.21	0.19	-0.19	-0.11	0.14	0.21	-0.04	-0.13	1.00
0.01	-0.03	0.07	0.00	-0.16	0.04	-0.17	-0.00	0.04	0.04	0.06	-0.02	0.06	0.14	0.04	0.72**
No. of fat deposits I	No. sqrs. with fat deposits I	Area of fat depo. I	No. of fat depo. II	No. sqrs. with fat depo. II	Area of fat depo. II (sq. mm.)	No. of fat depo. III	No. sqrs. with depo. III	Area of fat depo. III sq. mm.)	Total area of fat (sq. mm.)	Total no. of sqrs. with fat	Total no. of fat deposits	Average size of fat deposits (sq. mm.)	Percent fat (Histological)	No. sqrs. of fat/ unit muscle area	Shear value, fat cooking (kg)

	Shear value, microwave cooking (kg)
1.00	

* P .05 sig. level.
** P .01 sig. level.

END

OF

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Shear

A significant ($P < .05$) effect of treatment on shear value, deep fat cooking was noted in table 3. Means for the shear value due to five different treatments ranged between 14.90 to 21.38 (table 4). Treatments 1SU and 2ST had the lowest shear values comparing all treatments. Whereas stilbestrol treated bulls (4BT and 5BTH) recorded a significantly lower shear value than untreated bulls. Treatment with stilbestrol significantly ($P < .05$) decreased the proportion of percent wavy fibers in both bulls and steers. These results generally suggest that the greater the proportion of wavy fibers, the higher the shear value and the less tender will be the meat and vice versa.

The 't' values for treatment differences are shown in table 7A and treatment means are shown in table 7B. Results indicate that treatment 3BU was significantly different from treatments 1SU, 2ST, 4BT and 5BTH. Treatment 3BU significantly increased the shear value (mean = 18.53) by 34.9%, 30.8%, 4.6% and 6.4% when compared with treatments 1SU, 2ST, 4BT and 5BTH respectively. These tables also indicate that treatment 5BTH was significantly different from treatment 2ST as it increased the mean shear value by 24.4% when compared to treatment 2ST. These results generally indicate that bull meat is slightly less tender than that of the steers which agrees with the findings of Adams and Arthaud (1963). Further, it appears from the means of the shear values that hormone treatment produced significantly ($P < .05$) more tender beef than beef from untreated bulls.

The results in the present study do not agree with Cahill et al. (1956), Pilkington et al. (1959) and Suess (1966) who found no significant differences in tenderness between treated and untreated animals.

This lack of agreement may be due to differences in cooking methods, final cooking temperatures and the temperatures at which the shear value was recorded. These results agree with the findings of Field et al. (1966) who reported that bulls 500 to 599 and 600 to 699 days old were significantly less tender than steers and heifers of comparable age. These workers obtained significantly higher palatability ratings favoring the steers and heifers when these were compared with bull carcasses. They did not use any stilbestrol implants.

The results of the present study (table 4) indicated that age had a positive significant effect on the tenderness (shear deep fat cooking and microwave cooking). Shear value increased with the advancing animal age. These results agree with the studies of Hiner and Hankins (1950), Tuma et al. (1962a) and Dunsing (1959). Palmer (1963) reviewing the relation of age with tenderness stated that beef carcass maturity or age of the animal at the time of slaughter influences tenderness.

The step-wise regression analysis used to rank the variables in the order of their values as predictor of tenderness are shown in tables 6A and 6B. When the shear value, fat cooking was a dependent variable (table 6A) sex ranked first on the influence of tenderness. The rest of the independent variables namely area of small fat deposit, total muscle area, age and number of smaller fat deposits did not apparently influence tenderness.

When shear value, microwave cooking was a dependent variable (table 6B), sex ranked third while total muscle area was more closely related to tenderness.

The independent variables could explain only 22 to 25% of the variation in tenderness leaving 75 to 78% unexplained.

TABLE 6A. ANALYSIS OF VARIANCE FOR REGRESSION COMPONENTS OF THE HISTOLOGICAL AND TENDERNESS CHARACTERISTICS OF BOVINE LONGISSIMUS MUSCLE

5 Variables Added Stepwise by Contribution

Variables	DF	Contribution To R-SQR	F Value	Regression coefficient	T Value
Sex	61	0.14	10.06**	4.26	2.98*
Area of fat deposits I (sq. mm.)	60	0.04	2.798	3.83	1.27
Total muscle area (sq. cm.)	59	0.02	1.82	-0.49	-1.38
Age (days)	58	0.02	1.64	0.06	1.59
No. of fat deposits I	57	0.03	2.15	0.22	1.47
		0.25			

5 Independent variables with 63 observations.

Variable no. 23 (shear value, fat cooking) is dependent variable.

* P < .05 sig. level.

** P < .01 sig. level.

TABLE 6B. ANALYSIS OF VARIANCE F O R REGRESSION COMPONENTS OF THE HISTOLOGICAL AND TENDERNESS CHARACTERISTICS OF BOVINE LONGISSIMUS MUSCLE

5 Variables Added Stepwise by Contribution

Variables	DF	Contribution To R-SQR	F Value	Regression coefficient	T Value
Total muscle area (sq. cm.)	61	0.08	5.59*	-0.75	-2.37*
Age (days)	60	0.04	3.05	0.05	1.56
Sex	59	0.04	3.05	2.92	2.20*
No. of fat deposits III	58	0.03	2.22	-1.07	-1.79
Total no. of squares with fat	57	0.03	2.22	0.06	1.49
		0.22			

5 independent variables with 63 observations.

Variable 24 (shear value, microwave cooking) is dependable variable.

* P <.05 sig. level.

** P <.01 sig. level.

TABLE 7A. 't' VALUES FOR THE TREATMENT DIFFERENCES ON THE BOVINE LONGISSIMUS MUSCLE*

Treatments	Shear value, fat cooking (kg)	Percent wavy fibers
Treatment 1SU vs treatment 2ST	-0.33	1.12
Treatment 1SU vs treatment 3BU	-3.00**	2.11*
Treatment 1SU vs treatment 4BT	-2.29**	0.10
Treatment 1SU vs treatment 5BTH	-3.15**	-0.39
Treatment 2ST vs treatment 3BU	-2.77**	-3.45**
Treatment 2ST vs treatment 4BT	-2.06*	-0.98
Treatment 2ST vs treatment 5BTH	-2.60*	-2.16*
Treatment 3BU vs treatment 4BT	0.39	2.16*
Treatment 3BU vs treatment 5BTH	0.70	2.28*
Treatment 4BT vs treatment 5BTH	0.18	-0.51

*Based on 't' test. * P <.05 sig. level. ** P <.01 sig. level.

TABLE 7B. TREATMENT MEANS FOR SHEAR VALUE, FAT COOKING AND PERCENT WAVY FIBERS OF BOVINE LONGISSIMUS MUSCLE

Treatments	Shear value, fat cooking (kg)	Percent wavy fibers
Treatment 1 (1SU) (steers - no implant)	14.90	50.66
Treatment 2 (2ST) (steers - 48 mg implant)	15.66	46.32
Treatment 3 (3BU) (bulls - no implant)	21.38	58.44
Treatment 4 (4BT) (bulls - 96 mg implant)	20.52	50.24
Treatment 5 (5BTH) (bulls - 192 mg implant)	20.19	51.79

Mean for the shear value, fat cooking = 18.53.

Mean for percent wavy fibers = 51.49.

The results indicated that the muscle fiber histological characteristics used in this study have little influence on tenderness.

Stilbestrol treatment significantly increased the tenderness in both bulls and steers. It was observed that stilbestrol treated bulls and steers had relatively less proportion of wavy fibers. No other histological characteristics were influenced by stilbestrol treatment.

Means for the Warner-Bratzler shear value indicated that bull muscle was comparatively less tender than that from the steers. Sex had little influence on the histological traits studied in this experiment.

SUMMARY

A study was conducted on the histological and palatability characteristics of beef using the longissimus muscle of the right side of the 9-10-11 rib cut from 24 steers and 39 bulls in the age range of 430-496 days. The grades ranged from average standard to low choice and the marbling level from practically devoid to small+. Implants of stilbestrol were used on part of the bulls and steers in different doses which were as follows: Treatment 1 - steers without implant (1SU), Treatment 2 - steers with 48mg implant (2ST), Treatment 3 - bulls without implant (3BU), Treatment 4 - bulls with 96mg implant (4BT) and Treatment 5 - bulls with 192mg implant (5BTH). The histological and tenderness characteristics studied were fiber diameter, sarcomere length, percent wavy fibers, fat distribution and Warner-Bratzler shear of samples prepared by fat cooking and microwave cooking.

Fiber diameter significantly ($P<.01$) increases from 65.05 to 69.07 microns as the chronological age of the animal increased. A significant ($P<.01$) negative correlation ($r=-.33$) was observed between sarcomere length and fiber diameter. No relationship was found between fiber diameter and shear value. Fat deposits of the category III (large) showed a significant ($P<.05$) negative correlation ($r=-.24$) with the fiber diameter. There was no influence of treatment on fiber diameter.

Sarcomere length means varied from 1.90 to 1.98 microns and were significantly ($P<.01$) influenced by the age of the animal. A decreasing trend was recorded with the increasing chronological age, even though

it was not found to be associated with tenderness.

The wavy fiber means for treatment groups ranged from 46.32 to 58.44 percent and were not significantly influenced by age. Treatment with stilbestrol significantly ($P<.05$) decreased the proportion of wavy fibers in both steers and bulls. Sarcomere length showed a significant ($P<.05$) negative correlation ($r=-.28$) with the wavy fibers. No significant correlation of shear value was observed with the percent wavy fibers.

Age had a highly significant ($P<.01$) effect on the number and area of small fat deposits and also on the total number of fat deposits. The general trend was a reduction in the number of smaller fat deposits and the total number of fat deposits with the increase in the age of the animal. No correlation existed between the fat deposits and tenderness.

A significant ($P<.05$) treatment effect was observed on tenderness. Means for the shear value due to the five different treatments ranged between 14.90 to 21.38. Treatment 1SU and 2ST had the lowest shear values comparing all treatments. Whereas, treatment 4BT and 5BTH recorded the lowest shear values for the bulls.

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APPENDIX

APPENDIX I

SARCOMERE EXTRACTION TECHNIQUE

1. Frozen sample was placed in a cooler at 3.3°C for 12 hours.
2. One-half inch core from the thawed sample was transferred into a Waring blender containing 50 ml. of 0.02M Kcl solution which was previously cooled in a refrigerator.
3. Sample was blended for one minute at a slow speed.
4. One drop of homogenate was placed on a cool clean slide.
5. A coverslip was placed and its edges were sealed with paraffin.
6. Sarcomere length was measured with a phase contrast microscope using a filar micrometer.
7. Twenty-five sarcomeres were measured and an average value was obtained for each sample and was converted to microns.

Reference: Locker, R. L. (1960)

Degree of muscular contraction as a factor in tenderness of beef. Food Res. 25:304.

APPENDIX II

PROCEDURE FOR MEASURING FIBER DIAMETER

1. Samples were fixed in 4% Neutral Formaline for 48 hrs.
2. One-eighth inch thick sections were sliced from each core.
3. Section was placed in a Waring blender containing enough physiological saline just covering the blades.
4. It was blended at slow speed for 30 seconds.
5. Part of the contents were poured on a petri dish. Fifty straight fibers were measured from each sample using a Bausch and Lomb compound microscope with a 10 X objective and 15 X SK filar micrometer. Wavy fibers were not included due to the inability to accurately measure their diameter.

Reference: Tuma, H. J.; J. H. Venable, R. R. Wuthier and R. L. Henrickson (1962)
Relationship of fiber diameter to tenderness and meatiness as influenced by bovine age.
J. Anim. Sci. 21:33

APPENDIX III

PROCEDURE TO MEASURE THE FAT DEPOSITS

1. A half inch core was cut at the medial position from each of the samples.
2. Each core was cut transversely approximately at the middle, at right angles to the fiber direction in the muscle.
3. A 3-5 mm thick section of the muscle was removed from each core and pressed lightly between folds of paper towel to remove excess moisture.
4. The sample was transferred to a metal specimen block kept on the freeze bars of the cryostat microtome at -20°C, on which a few drops of OCT Compound was placed.
5. Sufficient OCT compound was used so that the sample was completely surrounded by it.
6. Immediately the quick freeze attachment was placed on the top of the muscle and was held for about one minute.
7. After freezing, the specimen block with the frozen sample was mounted in the chuck of the microtome and sections were cut at 12 microns thickness.
8. Sections were transferred to the surface of the pre-cooled slides kept in the kryostat and thawed immediately.
9. The sections were dried at the room temperature for about half an hour and this fixed the sections to the slides uniformly well.
10. Within an hour after sectioning, the sections were stained.

APPENDIX IV

STAINING PROCEDURE FOR FAT

Staining fat is accomplished by using dyes that are soluble in fat or lipid material. For this reason, a fat stain is termed a physical stain. Fat and lipids are chemically related substances. They are soluble in ether, chloroform, alcohol, xylene, acetone and benzene. Since dehydrating and clearing agents for paraffin and celloidin are fat solvents, fat stains are done on frozen sections.

The staining solution used was Oil Red O.

Stock solution consists of the following:

Oil Red O	0.5 gm.
99% isopropanol (isopropyl alcohol)	100 ml

This is stock saturated solution. It is very stable.

Working solution was prepared from the stock solution just before use

Oil Red O Stock Solution	6 ml.
Distilled water	4 ml.

Working solution is unstable and therefore should be prepared fresh for routine use, let stand for 5 to 10 minutes and then filtered.

Staining:

1. Sections were stained in the working solution for 10 to 15 minutes.
2. They were washed in cooled water.
3. Nuclei were stained breifly (10 to 30 seconds) in Harris's Hematoxylin.
4. Phenol-glycerol-jelly was used to mount the sections.

Results: Fat - Red

Nuclei - Blue

Reference: Lillie, R.D. (1965) Histopathologic Technique and Practical Histochemistry, McGraw-Hill Book Co. New York.

APPENDIX V

PHOTOGRAPHIC TECHNIQUE FOR THE STAINED SECTIONS

1. Slides stained with Oil Red O and Harris's Hematoxylin were placed on the negative carriage of a SIMMON OMEGA D-2 photographic enlarger which was fitted with a 50 mm lens (WOLLENSAK, f4.5).
2. A micrometer (graticule) disc rule containing 196 squares with a total area of 57.76 sq. mm was placed faced down on the coverslip of the stained slide, so that both the disc etchings and the specimen were in close proximity at the focal plane.
3. Exposures were made on medium contrast paper (F_3) with a timing of 15 seconds and aperture 3.
4. Photographs were developed in Dektol, washed in water and fixed in a fixative. After final washing they were glazed.

APPENDIX VI

MEASUREMENT OF FAT DEPOSITS

1. From the photographs, total muscle area covered by the grid was determined by counting the number of squares overlapping the section. Portion of the section covering half or more of the little square was counted as one. Less than half a square was not included.
2. The total area covered by the grid was 57.76 sq. mm. and each square was 0.29 sq. mm.
3. To facilitate measurement, the fat deposits were arbitrarily divided into three categories:

   Category I: (Small) comprised of fat deposits covering less than half a square (0-0.15 sq. mm)

   Category II: (Moderate) included fat deposits between half and one square (0.15 to 0.29 sq. mm)

   Category III: (Large) consisted of fat deposits exceeding one square (0.29 sq. mm)
4. Analysis of variance was performed to determine the data groups which could further be subjected to F test for significance.
5. Correlation coefficients were calculated between the physical and histological characteristics of longissimus dorsi muscle.

APPENDIX VII

PROCEDURE FOR DEEP FAT COOKERY OF MEAT CORES

1. Cooking of the samples was conducted on three days. Therefore the samples were divided into three groups.
2. Each sample was divided in the frozen condition into two halves, the other half being saved for electronic cooking.
3. Each group of samples which was to be used for deep fat cooking was allowed to thaw in a cooler at 3.3C for twelve hours.
4. A core, one inch in diameter was removed from each sample just before cooking was started. Care was taken in removing the cores that the muscle fibers in the core were parallel to each other lengthwise.
5. Each core was cooked to an internal temperature of 79.5C in vegetable oil pre-heated to 90-95C in a deep pan on an electric hot plate.
6. The internal temperature was determined by a recording potentiometer equipped with thermocouples, placed in the center of the core.
7. To assure uniform cooking on all sides and to provent moisture loss each core was enclosed in three polyethylene bags, one above the other in the form of layers. These bags were "Whirl Pack" and were almost the size of the meat core. Although the cores were cooked in vegetable oil, but due to these polyethylene bags they were untouched with oil.(modified moist-heat cookery).
8. To effect uniform cooking, at a time not more than three cores were cooked and these were allowed to hang with string from a stand, in the middle of the deep pan, covered with polyethylene bags, while the thermocouple was in the middle of the core.
9. After cooking, each core was cooled to 37.5C internal and was sheared three times using Warner-Bratzler Shear.

APPENDIX VIII

PROCEDURE FOR MICROWAVE COOKING OF MEAT CORES

1. The other half of the samples were used for this method of cooking.
2. Same method was used for thawing and for taking core as in the case of deep fat cooking
3. "Radarange, MARK IV Microwave Oven" (RAYTHEON) was used for electronic cooking.
4. All the cores were cooked to an internal temperature of 90±C. This internal temperature was the same as that for deep fat cooking.
5. After cooking, each core was cooled to 37.5C internal and was sheared on Warner-Bratzler Shear.

APPENDIX IX

CARCASS INFORMATION

Carcass No.	Sex	Age (Days) at Slaughter	Treatment Given	Carcass Grade	Marbling Level
6006	Steer	474	2	Good-	Small
6008	Bull	471	5	Good-	Slight
6009	Steer	471	1	Good	Small
6013	Steer	496	1	Choice-	Small+
6015	Steer	496	2	Choice-	Small+
6016	Steer	494	1	Good+	Slight+
6018	Steer	493	1	Good+	Slight+
6022	Bull	465	5	Good-	Slight
6023	Bull	465	5	Standard+	Trace-
6025	Steer	465	1	Choice-	Small+
6027	Steer	493	2	Good+	Slight+
6030	Steer	493	2	Choice-	Small+
6032	Bull	465	5	Good-	Trace+
6033	Bull	464	4	Good-	Slight-
6034	Bull	492	3	Good-	Trace+
6035	Bull	463	4	Choice-	Small+
6036	Steer	463	2	Good+	Slight+
6041	Steer	463	2	Good+	Slight+
6042	Steer	463	1	Choice-	Small+
6051	Bull	489	5	Standard+	Trace
6055	Bull	460	3	Good-	Slight-
6057	Bull	487	3	Standard	Trace-

Carcass No.	Sex	Age (Days) at Slaughter	Treatment Given	Carcass Grade	Marbling Level
6059	Steer	487	2	Good+	Slight+
6060	Steer	487	1	Good	Small
6064	Steer	459	1	Good-	Small-
6065	Bull	458	3	Standard+	Trace
6066	Bull	486	5	Good+	Slight+
6067	Bull	486	4	Choice-	Small+
6068	Bull	486	5	Choice-	Small+
6069	Bull	458	3	Good-	Slight-
6071	Bull	486	4	Good-	Slight-
6073	Steer	486	1	Choice-	Small+
6076	Bull	457	4	Good-	Slight+
6079	Bull	457	3	Standard	Trace-
6082	Steer	484	2	Good+	Slight+
6088	Bull	483	3	Standard	Practically devoid
6089	Bull	483	3	Good+	Trace+
6090	Bull	483	5	Choice-	Small
6093	Steer	454	2	Choice-	Small+
6101	Bull	480	4	Choice-	Small+
6108	Bull	452	3	Good-	Trace+
6109	Bull	452	3	Standard+	Trace
6110	Bull	480	4	Good-	Slight
6111	Bull	452	5	Good+	Slight+
6114	Bull	479	3	Standard	Trace
6116	Steer	451	2	Good+	Slight+

Carcass No.	Sex	Age (Days) at Slaughter	Treatment Given	Carcass Grade	Marbling Level
6122	Bull	477	3	Standard+	Trace
6127	Steer	477	2	Choice-	Small
6129	Bull	447	4	Good+	Small-
6134	Bull	475	3	Standard+	Trace
6142	Bull	445	5	Good-	Slight
6143	Bull	473	3	Good-	Slight
6144	Steer	444	2	Good+	Small-
6153	Bull	440	4	Standard	Trace
6155	Steer	439	1	Good-	Slight
6157	Bull	463	5	Good-	Slight-
6158	Bull	435	3	Standard	Trace
6160	Bull	460	3	Choice-	Small+
6164	Bull	431	3	Standard	Trace
6165	Bull	431	4	Good-	Trace+
6167	Steer	430	1	Good	Slight
6174	Steer	442	2	Good+	Slight+
6177	Bull	430	5	Good+	Slight+

INFLUENCE OF SEX ON THE HISTOLOGICAL AND TENDERNESS CHARACTERISTICS OF BEEF

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Longissimus dorsi muscle from the right 9-10-11 rib cuts of 24 steers and 39 bulls ranging in age from 430-496 days were used in this study. The grades ranged from average standard to low choice and the marbling level from practically devoid to small+. Stilbestrol implants were used on part of the bulls and steers. The treatments were as follows: Treatment 1 - steers without implant (1SU), Treatment 2 - steers with 48mg implant (2ST), Treatment 3 - bulls without implant (3BU), Treatment 4 - bulls with 96mg implant (4BT), and Treatment 5 - bulls with 192mg implant (5BTH). The histological and tenderness characteristics studied were fiber diameter, sarcomere length, percent waviness of fibers, fat distribution, and shear value (deep fat cooking and microwave cooking).

Fiber diameters were measured with an eyepiece micrometer in a Bausch and Lomb microscope at 150X. Sarcomeres were extracted from the fibers and measured with a Wild Heerburg phase contrast microscope using the oil immersion objective and an eyepiece micrometer at 1500X. For the measurement of fat distribution, muscle sample cores were sectioned at 10-12 microns on a cryostat microtome at -20°C, stained with Oil Red O for fat and with hematoxylin for nuclei. The stained slides were photographed in a negative carrier of an Omega D-2 enlarger. From these photographs, area of the fat deposits was calculated on the basis of the area covered by each and every fat deposit. The fat deposits were categorized as small (Category I, covering 0-0.15 sq. mm), moderate (Category II, covering 0.15-0.29 sq. mm), and large (Category III, covering more than 0.29 sq. mm). Shear values were recorded for the samples cooked in deep fat and in microwave oven. The experiment was a completely randomized design.

There was a significant ($P < .01$) increase in the fiber diameter

means from 65.05 to 69.07 microns with an increase in the animal age. Correlations between fiber diameter and sarcomere length and fat deposits of larger size were r=-.33 and r=-.24 respectively. Fiber diameter was not influenced by treatment.

Sarcomere length decreased significantly ($P<.01$) with increase in the age of the animal. No significant effect of sarcomere length was recorded on tenderness.

The wavy or contracted fiber means ranged from 46.32 to 58.44 percent and were not significantly influenced by age. Treatment with stilbestrol significantly ($P<.05$) decreased the proportion of wavy fibers in both steers and bulls. Sarcomere length was found to be negatively correlated (r=-.28) with percent wavy fibers.

There was a significant ($P<.01$) decrease in the number of fat deposits of Category I (small) and total number of fat deposits with advancing animal age. Total area of the fat and percent fat were not significantly influenced by age. No correlation existed between the fat deposits and tenderness.

Tenderness (shear, microwave cooking) decreased significantly ($P<.05$) with advancing age of the animal. This trend was not observed for shear, fat cooking.

A significant ($P<.05$) treatment effect was observed on tenderness. Means for the shear value due to the five different treatments ranged between 14.90 to 21.38. Treatment 1SU and 2ST had the lowest shear values comparing all treatments. Whereas, treatment 4BT and 5BTH recorded the lowest shear values for bulls.