THE RELATIONSHIP OF INTRAMUSCULAR FAT AND THE VASCULAR SYSTEM OF BEEF TENDERNESS

by

BALEMULA GURUVA REDDY

B.V. Sc. & A.H. Osmania University (INDIA), 1961

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1969

Approved by:

Major Professor

LD
2668
T4
1969
R32

TABLE OF CONTENTS

Page

INTRODUCTION 1

REVIEW OF LITERATURE 3

Morphological Changes in Fat Cells During Fat Deposition and Depletion 3
Lipolytic Enzyme Activity 7
Nerve Fibers 9
Blood Vessels 10
Marbling and Its Influence on Beef Quality 12
Maturity 16
Objective Techniques 17

MATERIALS AND METHODS 19

Sample Selection 20

RESULTS AND DISCUSSION 26

Marbling Effects 26
Maturity Effects 28
Position Effects 30
Side Effects 34
Correlations 35

SUMMARY 37

ACKNOWLEDGEMENTS 38

BIBLIOGRAPHY 39

APPENDIX 46

INTRODUCTION

One of the most important criteria for appraising beef quality is the amount of intramuscular fat. Historically, beef with a greater amount of intramuscular fat is considered to be higher in quality than muscle with limited fat. Skeletal muscles contain fat intra and extracellularly. The presence of intracellular fat (cytoplasmic) is frequently termed "fatty changes" by pathologists, whereas extracelluar deposition of fat is termed as muscular lipomorphosis. This intramuscular fat distribution is viewed differently by various diciplines. To a meat scientist, this type of fat deposition is very important in so far as meat quality is considered, whereas the same type of accumulation may be considered as a form of obesity by pathologists.

In the past, adipose tissue *per se* was considered to be more inert connective tissue. But, in view of recent literature (Rodahl and Issakutz 1964), it is a more complex structure, especially in relation to its origin and function. The importance of intramuscular fat to muscle tenderness has been studied, and conflicting findings exist. One group of workers found that marbling had an influence on tenderness, whereas the other group found no significant effect of marbling on tenderness.

Studies on the blood vessels of beef muscles and their relation to tenderness have received little attention. Conclusive evidence exists that well developed fat tissue is poor

in capillary bed compared to muscle tissue. The size and distribution of blood vessels also differ in white and red muscles.

As there is a paucity of information regarding fat distribution in *Longissimus dorsi* muscle, this investigation is designed to study the relationship of fat and vascular distribution to muscle tenderness of beef animals differing in marbling and maturity.

REVIEW OF LITERATURE

Morphological Changes in Fat Cells During Fat Deposition and Depletion

From the available literature, it is evident that there is little uniformity regarding the origin of fat cells or tissues. Texts on histology (Bloom and Fawcett 1964) mention that fat cells develop from undifferentiated mesenchymal cells, which are scattered singly or in groups in loose connective tissue of the intramuscular spaces. Wells (1940) viewed "it is a plant in active operation", producing some or all of its stored materials and probably conducting some other unknown functions. The autonomy of adipose tissue was further observed by Hauserberger (1955), who reported in rats, that when immature testicular fat bodies were transplanted subcutaneously into the abdominal wall; mature adipose tissue developed.

As early as 1909 (a), Bell observed in bovine fetuses and one year olds, the presence of fat droplets within and outside the muscle fibers. He thought that the presence of intracellular fat in aged animals was due to muscular atrophy. Similar findings were reported by Helander (1958) in man and animals. Increased lipomorphosis was associated with increase in age resulting in compression atrophy of the muscle bundles.

The processes of formation of fat cells from fibroblasts, which are indistinguishable from the preadipose tissue, were followed by Bell (1909b), Clark and Clark (1940) and Napolitano

(1963). According to Bell, the cells adjacent to blood vessels were first filled with fat, and then extended in all directions. The branched preadipose tissue cells became rounded after accumulation of fat. Finally the mass of adipose tissue increased with fattening by (a) increase in cell size (b) formation and filling of new cells in the interior of the lobule; and (c) formation of new lobules.

Clark and Clark (1940) observed that small refractile bodies appeared in the fibroblasts, and coalesced to form larger globules. The globules pushed membranes to the periphery of the cell with a thin rim of cytoplasm, giving the appearance of signet ring. Depletion of fat showed the reverse order of processes, characterized by breaking down of globules into smaller droplets. These ultimately disappeared leaving a cell only with granules. Napolitano (1963) noticed a change in size and shape of fat cells due to fat depletion which was manifested in an increased of cytoplasm and nonmembrane bound lipid. This was followed by a decrease in endoplasmicreticulum and mitochondrial shape with further depletion. During this differentiation glycogen appeared in the cells and there was no transport of lipid from the capillary or within the adipose tissue cells.

Hartroft (1950) observed small droplets of visible fat in central lobular liver cells of rats fed on a basal choline deficient diet. These coalesced to form large sperules in parenchyma cells with time. Finally the fat cells are

separated by septa which are formed as a result of stress and compression on the limiting membranes, due to continued fat accumulation.

Direct transfer of triglyceride particles seems to occur across the membrane. This is further confirmed by Ashworth et al (1960) in hepatic cells of rats, using an electron microscope. These particles in the cytoplasm of cells were considered for the genesis of larger droplets.

Much of the available literature on morphological changes in adipose tissue is from laboratory animals. Morphological changes were studied both with the light and electron microscope. The associated induced changes brought about by various factors like starving and adrenalectomy, have changed the shape and size of fat cells. Lever (1957) observed reduction in amount of lipid and size of the fat droplet in the adrenalectromized rat. Napolitano and Fawcett (1958), Williamson (1964) and Napolitano and Gagne (1963) reported somewhat similar findings. The later workers noticed pronounced cellular changes in lipid depleted cells. The cell membranes indented with the appearance of pinocytic vesicles and collagen fibers in intercellular spaces. The authors suggested these changes may be due to mechanical events associated with lipid loss from cells. In addition to indentations of base membrane, Williamson (1964) noticed microvesicles in cytoplasm and endothelium of blood vessels, which might be the possible mechanism for free fatty acid transport.

Barnett and Bell (1960) noticed in *in vitro* fat cells of the rat treated with insulin that the cytoplasm lost its normal appearance and small fat droplets appeared.

Simson and Williamson (1967), observed morphological changes in epididymal, pancreatic, hepatic, heart and diaphragmatic fat cells of chronic caloric deprived rats. The noticeable change was a thickened basement membrane and the presence of small foci in cytoplasm. These changes were considered as a generalized response to the catabolic rather than degenerative changes.

The association of mitochondria in fat synthesis is well known. Lever (1957), encountered mitochondria between the fat droplets, and these contained minute quantities of fat-like substances. In contrast, Napolitano and Fawcett (1958) observed that mitochondria were very often closely associated with lipid inclusions, and there was no conclusive evidence in favor of mitochondrial transformations into lipid droplets.

Carbohydrates play a fundamental role as a precursor in fat synthesis. Fawcett (1948), observed fat cells were devoid of stainable glycogen under "normal" conditions, but glycogen appeared in insulin treated cells or fasted and refed rats. Similar findings were reported by Hauserberger and Milstein (1955), however, a high fatty acid diet reduced glucose oxidation markedly. The rate of synthesis appeared to be regulated by the carbohydrate content of the diet, and glucose utilization increased with reduced amounts of fat.

Wang et al. (1954), observed the translocation and dispersion of fat in frozen cooked beef from Longissimus dorsi and Semitendinosus muscles. The fat released from the cells dispersed progressively along the path of collagen. The fat cell size decreased from the center of dispersion to the periphery. The authors theorized that this process was due to "emulsification" with collagen which acted as dispersing agent. In addition, heat accompanied by cooking, provided the necessary physical agitation to facilitate emulsifying processes.

Heruzaz and Jelinkova (1964) reported decreased fat catabolism with advancing age with catabolic stimulators like epinephrine, stress and somatotrophic hormone on fat.

Lipolytic Enzymes Activity

Lipid metabolism and lipolytic activity was studied histochemically by Leites (1964) in old animals. He noticed considerable difference in tissue metabolism of old animals as compared to one year olds; lipolytic enzymes decreased with increase in age. He suggested the decrease in enzymes was responsible for fat deposition in various tissues.

The proportion of red and white fibers vary in different muscles, in species, with age and metabolic state. Beatty et al. (1963), demonstrated striking metabolic differences in white and red muscle fibers. The glycogen content of fresh muscle was higher in white than the red fibers, but this was

reversed after two hours of incubation. There was no difference in glucose uptake in these two types of fibers, however, the lactate formation was higher in the white fibers. The uptake by the red fibers of aceto-acetic acid was greater than the uptake of aceto-acetic acid by the laclate fraction of the white fibers. A reverse relationship existed between the glycogen fraction of the white fibers and the red fibers as more aceto-acetic acid was taken up by the glycogen fraction of the white fibers than by the red fibers.

Allen et al. (1967a) differentiated 4 types of porcine muscle fibers Type I, II, III & IV by histochemical tests which were based on enzyme activity. Varying enzyme content of the fibers is known to cause varying rates of fat synthesis in the tissues. The authors noticed a higher percentage of beta-hydroxy butyric dehydrogenase fibers in light weight boars and a higher percent of esterase positive fibers in heavy weight groups. Also the amount of intramuscular fat increased with slaughter weight. They postulated that barrows and gilts had a tendency for a greater accumulation of intramuscular lipid due to smaller percentage of fibers positive for esterase and β-hydroxybutyric dehydrogenese enzymes.

In another study Allen et al. (1967b) reported that weight and sex influenced fatty acid composition. Animal weight had a significant effect on amount of various fatty acids. A marked increase of C-18 fatty acid was noted in heavier barrows and gilts as contrasted to lighter barrows

and gilts. There was no weight effect in boars. It was suggested that the sex hormones might have changed the ratio of C-18-1 to C-18 in the 55 kg live weight group.

Allen et al. (1967c) also reported intrafascicular cells resembling fat in Longissiumus dorsi of pigs. The phospholipid fractions of some of the fibers had high content of C-16 and C-18-2 fatty acids. This difference in composition is suggestive of some change in membrane structures of the fibers.

Link et al. (1967), observed fatty degeneration of muscles from which previous biopsy samples have been collected. The degenerated muscle contained primarily fat cells with remnants of atrophied muscles cells and little connective tissue compared to normal tissue.

Nerve Fibers

The importance of nerve supply in health and disease which controls the normal functions of the body, have been well studied. In adipose tissue some of the nerve fibers terminate in fat itself, while others accompany the blood vessels. Sidman and Fawcett (1954) reported the metabolic sequences of denervation in mice and rats. In both animals, the denervated fat body deposited significantly more fat and during acute fast lost fat slowly.

Blood Vessels

Clark and Clark (1939), studied the new growth of blood capillaries in amphibian tail and rabbit, which is characterized by the following sequence of events: sprout formation, anastomosis of adjacent capillaries, lumen formation, and retraction of surplus capillaries. The fibroblasts surrounding the capillaries migrated into the growth space and became connective tissue fibers between the capillaries. Factors like rapid circulation and inflammatory conditions, which induced the growth of capillaries also favored the growth of lymphatics, nerves, and connective tissue cells present in the same region.

Gersh and Still (1945), in a study of the relationship of capillary bed to the volume of tissue, reported a ratio of 51.9 and 222.2 for fat rich and fat poor tissue respectively. During ordinary activity half of the capillaries of fat rich and one fourth of fat poor tissue were open. The total capillary bed of fat rich tissue had one third the number of capillaries as compared to muscle. The authors concluded that the capillary bed of adipose tissue is inadequate, compared to other tissues.

Shoshenko (1963), reported a correlation coefficient of 0.84 and average ratio of 1:14 between capillary surface to muscle fiber surface.

Romanul (1964), demonstrated the relationship of alkaline phosphatase and cytochrome oxidase activities to the distribu-

tion of capillaries in muscle fibers. The density of capillaries was found to parallel the cytochrome oxidase activity and there were as many as eight capillaries around some muscle fibers manifesting alkaline phosphatase activity in their endothelium.

Allen et al. (1967a) noted differences in distribution of blood vessels in light and dark muscles of pigs. Dark muscles and fibers received a richer blood supply than white muscles and fibers.

In the formation of adipose tissue, Bell (1909b) and Clark and Clark (1940) reported that cells adjacent to blood vessels with slow or moderate blood flow were filled first with fat. Helander (1958), Blumer et al. (1964) and Moody and Cassens (1968) noticed that main marbling deposits were close to or within the heavy vascular network.

Differences exist in the amount of fat deposition between species, breeds, planes of nutrition and locations of muscle. Histologial structure also plays an important role in fat accumulation. Kauffman and Safanie (1967) reported that loose textured Latissimus dorsi muscle contained more fat than the more compact distal muscles. In these muscles, fat content increased with age.

Moody and Cassens (1968) reported that with more extensive marbling in Longissimus muscle, large areas were occupied with fat cells. The number of fat cells increased with marbling within and as well as outside the fat deposit. The histologically estimated fat was significantly ($P < .01$)

different between marbling groups, and followed the same trends as ether extract but was higher.

Marbling and Its Influence on Beef Quality

Studies by Ramsbottom et al. (1945) did not show any relationship between fat content and tenderness in different muscles of the animals.

Bowman et al. (1954) reported highly significant correlation coefficients between juiciness and tenderness.

Palmer et al. (1954) using the Longissimus dorsi at 12th rib from 136 carcasses, found highly significant correlations between grade and marbling, ether extract and tenderness. A similar relationship between marbling and ether extract was also observed. The percentage of variability in panel tenderness attributable to grade ranged from 8% to 11%.

Cover et al. (1956), using yearling steers, reported that tenderness scores were closely related to ether extract, and postulated that the latter might be associated with juiciness and tenderness of meat.

Cover et al. (1958) summarized studies using 203 beef carcasses of known history and reported that marbling, when measured as ether extract of the Longissimus dorsi, was significantly correlated ($r = 0.33$) with tenderness. Wellington and Stouffer (1958) obtained non-significant correlations between ether extract and panel tenderness.

However, highly significant ($P < .01$) correlations (0.263) were reported for marbling scores and tenderness, and for ether extract with panel juiciness (0.296).

Doty and Pierce (1961) obtained correlation coefficients of 0.61 between tenderness and intramuscular fat, and 0.56 between tenderness and marbling respectively. The authors also observed that 7-8% of the variance in juiciness was closely associated with marbling or intramuscular fat. However further increases in the fat percentage did not improve juiciness. Goll et al. (1965), reported that fine texture and even distribution of intramuscular fat was significantly associated with tenderness.

Blumer (1963), in his review on the relationship of marbling to the palatability of beef, stated that intramuscular fat had little effect upon tenderness.

Tuma et al. (1962), working with 24 Hereford steers and heifers of 18, 42 and 90 months of age, found that marbling levels did not significantly ($p < .05$) influence the tenderness in Longissimus dorsi steaks. However steaks from carcasses with slightly abundant marbling were a little more tender than those from carcasses with a slight amount of marbling. Tenderness of muscles decreased significantly ($p < .05$) with advancing age, and the greatest difference in tenderness was noticed between the 18 and 42 month groups.

Blumer et al. (1962), reported that the highest amount of ether extract was associated with coarse marbling. The

marbling patterns appeared to be somewhat consistent with the heavy intramuscular fat deposits. The latter occured at more or less regular intervals within the rib eye muscle. In contrast, Carpenter et al. (1961), reported in pork studies that a higher percentage of extractable fat was associated with finely dispersed small sized fat cells in the muscles.

Goll et al. (1965), Walter et al. (1965), Romans et al. (1965) and McBee and Wiles (1967), reported that the higher marbling scores were associated with higher ether extract values.

Tuma et al. (1963), observed that the chemical fat content differed significantly among the age groups; it was low in the young and very old animals. However Helander (1958), did not notice any significant difference in fat content of Gastrocnemius and Biceps brachii from young and old horses, cattle and rabbits, but in human beings there was a significant increase from birth to 70 years.

Cook et al. (1964), noticed in the Longissimus dorsi muscle that the intramuscular fat varied significantly ($p<.01$) among anatomical locations. It was uniform between the 10th and 13th thoracic vertebrae and decreased towards both extremities. Highly significant differences ($p<.01$) were observed for marbling and ether extract, even though the uniformity of marbling distribution did not vary much among the marbling groups. Carpenter et al. (1961), concluded from pork studies at the 7th, 13th, thoraeic and 6th lumber vertebrae, that extreme variability in fat content existed

among the different locations of Longissimus dorsi muscle. Moody and Cassens (1968), reported in a study involving three levels of marbling that there was no overall marbling pattern from medial to lateral. However, within marbling groups, the traces group had less histologically estimated fat in the lateral position, while small and moderate marbling levels showed equal amounts in medial and lateral cores. Besides these inconsistencies, the moderate marbling group had more fat in the central core.

Knowledge of intramuscular fat which enhances the palatability traits of meat products is evident. Kauffman et al. (1964), summarized data from 439 pork carcasses for total extractible fat and its relationship to palatability characteristics. The percent intramuscular fat (E.E) was significantly ($p < .01$) correlated with flavor ($r = -.38$) juiciness ($r = 0.70$), tenderness score ($r = 0.44$) and shear force ($r = -.35$) respectively. Fat variation accounted for 50% of the variation in juiciness and to a lesser extent for tenderness and flavor.

Gladys et al. (1965), reported that steaks from the rib cut and eye of round from high marbled carcasses were scored slightly more tender, juicy and flavorful than steaks from low marbled carcasses, cooked to the same internal temperature. In both steaks, marbling was not associated with palatability traits, but 20% of the variation in juiciness and flavor was associated with ether extract of broiled steaks.

Maturity

Walter et al. (1965), using seventy two carcasses representing three maturity (A, B and F) and eight marbling (moderately abundant to practically devoid) levels, noticed that marbling did not significantly influence tenderness.

Field et al. (1966) stated that tenderness of beef from steers and heifers, decreased with increased maturity. At constant slaughter weight, age had no effect on tenderness. The palatability rating was not influenced by age up to 2 years, when marbling was held constant, however at constant age, higher marbling scores were associated with higher sensory ratings.

Romans et al. (1965), working with four levels of maturity (A, B, C & D standards of 1956) and two levels of marbling (moderate and slight) in Longissimus dorsi, observed that neither marbling nor maturity had any significant effect on tenderness. There was a trend, however, for steaks from more mature animals to be less tender, and steaks with moderate marbling were significantly ($p<.01$) more juicy than those with a slight level of marbling.

McBee and Wiles (1967), in their studies on three-hundred and thirty short loins, representing eight degrees of marbling and two levels of maturity, indicated that there was a non-significant linear increase of tenderness with additional degrees of marbling. Steaks from younger animals were ($p<.01$) less juicy and less flavorful than those from the older

maturity group. The percentage ether extract increased with marbling and maturity.

No consistancy in tenderness at various locations in the _Longissimus dorsi_ muscle was found. Conflicting evidence exists for the tenderness at medial, central and lateral locations. Walter _et al_. (1965), reported that medial and lateral cores were more tender than central, whereas Tuma _et al_. (1962) and McBee and Wiles (1967), noticed the medial core was more tender.

Objective Techniques

Stouffer and Wellington (1957) obtained correlation coefficients of 0.793 between the ether extract and marbling score by photographs taken by polarized light. The series of photographys represented 12 degrees of marbling and were used to formulate an objective measurement technique.

Blumer and Fleming (1959), obtained correlation coefficients of 0.65 between the ether extract and marbling measured by using a bacterial colony counter. Marbling deposits having a surface area of two square mm. or larger were measured with the aid of a compass and millimeter ruler and scale, whereas fat deposits measuring less than two sq. mm. were counted.

Lewis _et al_. (1958), reported no significant difference in fat estimation by photomicrograghic technique and numerical scoring. However, the photomicrographic method has an edge over numerical scoring, in that fewer slides were required

for estimation and human error is eliminated, simultanous fat quantity and distribution can be estimated.

Henderson et al. (1959), measured the projected images of cross sectional area of carcasses and cuts with a planimeter. Reproducibility of results were within a range of 0.06 sq. inches. The authors were of the opinion that this method could be used for both quantitative and qualitative study.

Cook and Bray (1961), obtained a correlation of 0.93 between the ether extract and light transmitted through a positive transparency. However, the technique was not sensitive enough to detect small variations in marbling.

In a recent study, Moody and Cassens (1968) estimated marbling using a micrometer disc, and the results were expressed as the percent of grid squares occupied by fat tissue sections. They obtained significant ($P < .05$) correlations ($r = 0.56$) between histologically estimated percent fat and ether extract values.

MATERIALS AND METHODS

Sixty beef carcasses were used to study fat and vascular distribution and their relationship to muscle tenderness. In this experiment, carcasses with two levels of marbling (small and moderate) and three levels of maturity (A^-A=youthful, A^+B^-=Intermediate, BB^+=approaching maximum maturity) were employed using the left and right Longissimus dorsi muscles from wholesale rib cuts. The two marbling levels were represented equally within each maturity group of 20 ribs, so as to get a two x three factorial design as given in Table 1.

Table I

Experimental design

Marbling Levels	Maturity Levels						Total
	A^-A R	A^-A L	A^+B^- R	A^+B^- L	BB^+ R	BB^+ L	
Approximate grades	Choice		Choice	Good	Good		
Small	10	10	10	10	10	10	60
Moderate	Choice		Choice		Choice	Good	
	10	10	10	10	10	10	60
Total	40		40		40		120

R = Right Side
L = Left Side

A total of 120 ribs were purchased from two meat packers (Maurer Neuer & Co. of Kansas City, Kansas and Armour & Co.

of Emporia, Kansas) and used for this study.

Sample Selection

Three areas on the ventral aspect of 9th rib, position C, (Plate I, Fig. 2) were selected. Cores 1.27 cm in diameter were taken from each selected area (position 'C); and immediately frozen in a blast freezer (-18°F) and kept frozen until sectioned.

Before sectioning, the tissues were thawed and samples approximately 1 cm x 1 cm x 2 mm cut and mounted on previously cooled metal specimen blocks. Frozen tissue sections eight microns thick were cut using a Tissue Tek cryostat, transferred to the precooled slides and stained by Oil Red O for fat deposits. (Lillie 1965, procedure in appendix VIII).

The stained slides were mounted in the negative carrier of an Omega D-2 photographic enlarger equipped with a 50 mm enlarging lens. A micrometer disc rule, containing 196 squares, with a total area of 57.76 sq. mm, (6593.44 sq. mm when enlarged 47x) was placed face down on the cover slip, so that both the disc etchings and specimen were in close proximity at the focal plane. Exposures were made on medium contrast enlarging paper. Development was carried out in a standard photographic paper developer.

The total muscle area covered by the photographed grid was determined by the number of squares (Plate II) overlapping

Explanation of Plate I

Fig. 1 - Position of samples for ether extract from the 12th thoracic rib of the _Longissimus dorsi_ muscle.

Fig. 2, C - Position of samples for fat and vascular distribution.

PLATE I

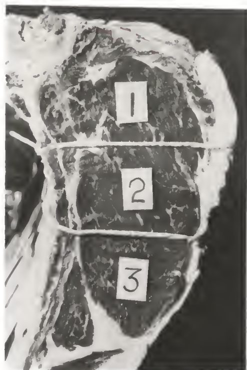


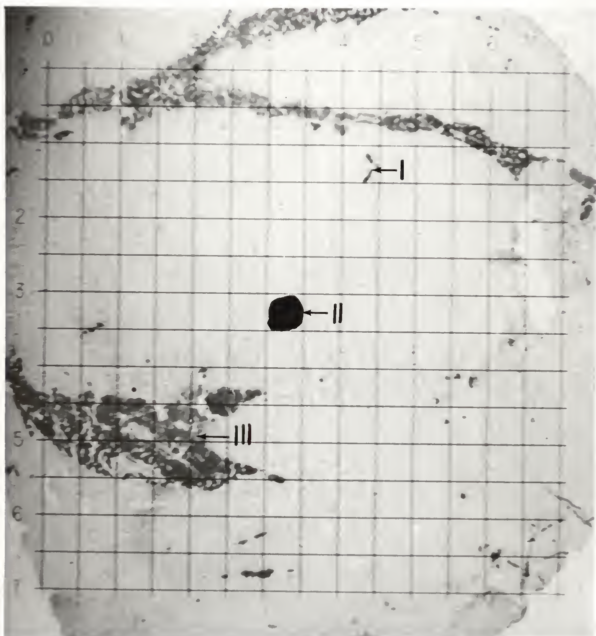
Fig. 1

Fig. 2

Explanation of Plate II

A photograph of a typical stained section with grid, showing fat deposits size I, II and III.

PLATE II



the section. Tissue section covering half or more of the little square were counted as one, whereas less than half s square were not counted. The total area covered by the grid was 6593.44 sq. mm and each little square covered 33.64 sq. mm. The grid was magnified 47 x.

To facilitate measurement, fat deposits were arbitrarily divided into three categories: Category I or small, covered less than half a square (16.82 sq. mm), Category II or moderate includes fat deposits between half and one square (16.82 to 33.64 sq. mm.), and Category III or large with fat deposits exceeding one square (33.64 sq. mm).

The total number of blood vessels were determined by examining the stained sections under the light microscope at 100x power and subsequently marking the photomicrographs.

The % ether extract used for histological and chemical comparison was from 12th rib analysis by Covington (1968). The sample positions (medial, central and lateral) for ether extract are shown in Plate I, Fig. 1.

Data were subjected to analysis of variance and Duncan's New Multiple Range test to test significant differences among the means. Finally all possible correlation coefficients were computed between the various physical and histological characteristics of Longissimus dorsi muscle.

RESULTS AND DISCUSSIONS

Marbling Effects

Quantitative histological (H) fat data for two levels of marbling (small and moderate) are presented in Table II. As was expected, there were fewer fat deposits of all sizes in the small than in the moderate marbled muscle. The differences in the number of fat deposits between the marbling levels were significant for the small (6.9 and 7.8) and large (1.2 and 1.7) but not for the medium size fat deposits.

Area of the three fat categories followed the same trends as number of fat deposits in both marbling levels (Table II). The moderate marbled muscle had significantly more fat area for small (6.0 sq. mm) and large (183.0 sq. mm.) fat deposits than the small marbled muscle. In addition, moderately marbled muscle had a greater quantity ($P < .05$) of histologically estimated total fat (268.6 to 461.0 sq. mm.) and percent fat (4.4 to 7.6%). The percent fat (H) was in close agreement with percent ether extract which was 4.8 in the small and 6.7 in the moderate marbling group respectively. The positive relationship between histological and chemical determination of fat is in agreement with Moody and Cassens (1968) although they reported a higher histological fat percent expressed on an area basis.

The effect of marbling on vascular distribution is shown in Table II. As was expected the moderate marbled muscle had a greater ($P < .05$) number of blood vessels (7.3) than the

Table II

Effect of bovine marbling on <u>Longissimus</u> <u>dorsi</u> fat and vascular distribution

	Small	Moderate
No. fat deposits size [a] I	6.9*	7.8
Fat deposit area[d] Size I	20.9*	26.9
No. fat deposits size[b] II	0.7	1.2
Fat deposit area[d] Size II	13.7	17.7
No. fat deposits size[c] III	1.2*	1.7
Fat deposit area size III	233.0*	416.0
Total No. fat deposits	8.8*	10.4
Total fat area	268.5*	461.0
Fat %	4.4*	7.6
Ether extract (%)	4.77*	6.65
No. Blood Vessels	6.1*	7.3
No. Blood Vessels/unit fat area	56.1*	37.8

a = Small (16.82 sq mm)

b = Moderate (16.82 to 33.64 sq mm)

c = Large (33.64 sq mm)

d = mm^2, significant at 5% level

* = $P < .05$

small marbled muscle (6.1). The number of blood vessels decreased as fat area increased for both moderate and small marbling. These findings suggest that well marbled muscle will have a greater number of functional blood vessels compared to less marbled. However, when considered on a fat area basis, the more highly marbled muscle will have fewer blood vessels.

Maturity Effects

The effect of maturity upon the fat and vascular distribution is shown in Table III. No maturity effect is noted for number and area of small, moderate and large fat deposits, although the B^-B^+ maturity group had the highest mean values for the fat area (H). These slight differences for the number and area of fat deposits, between the maturity groups, might be due to a decrease in lipolytic acitivity and fat catabolism with advancing maturity as reported by Hruzaz and Jelinkova (1964), and Leites (1964). The least fat area (H) in A^+B^- maturity group can not be explained.

Maturity had no effect on total fat (H) and percent fat as shown in Table III. The A^+B^- maturity had the smallest means for total fat area (H) and % fat, and the B^-B^+ maturity group the highest mean. The highest ($P < .05$) ether extract values for the three groups was indicated for the B^-B^+ group and the lowest values for the A^-A° group. These results are contrary to expectations and not in accord with ether extract

Table III

Effect of bovine maturity on <u>Longissimus dorsi</u> for fat and vascular distribution

	Maturity		
	AA^-	A^+B^-	BB^+
No. fat deposits size I	7.0	7.7	7.4
Fat area size I	21.0	25.0	25.3
No. fat deposits size II	0.7	0.7	0.8
Fat area size II	14.9	15.3	16.8
No. fat deposits size III	1.6	1.4	1.5
Fat area size III	331.9	278.6	363.6
Total fat area	368.2	319.8	406.3
Fat %	6.0	5.3	6.6
Ether extract %	5.5^a	5.6^a	6.0^b
No. Blood vessels	7.3^c	6.0^a	6.8^b
No. Blood vessels/unit fat area	50.9	48.6	41.4

Fat area in mm^2 ,

AA^- = Youthful, A^+B^- = Intermediate, BB^+ = approaching maximum maturity.

All means with same or no superscript are not significantly (P<.05) different.

values. However McBee and Wiles (1967), keeping quality constant, reported higher ether extract values for B maturity compared to the A maturity group.

The maturity group means for blood vessels are significantly ($P < .05$) different (Table III). The largest number of blood vessels was observed in the young (7.3) and the least in the intermediate (6.0) maturity group. Similarly, as mentioned earlier, the number of blood vessels per unit area decreased as fat area increased. The differences in number of blood vessels in the maturity groups may be due to metabolic status of the animal. It is reasonable to assume that the number of functional blood vessels decreases as age and fat content increases. It is difficult to explain why the intermediate maturity group (A^+B^-) had fewer blood vessels than young (A^-A) and approaching maximum (BB^+) maturity classes. An interesting feature in this was that even though the amount of fat in the young group exceeded the amount of fat in the intermediate group, the young group still had greater number of blood vessels per unit fat area. This suggests that the young animals are metabolically more active and have the potential for increased marbling and muscle mass compared to other two groups.

Position Effects

The effect of marbling on fat distribution within the muscle is given in Table IV. There was no consistency in the

Table IV

Effect of bovine marbling and *Longissimus dorsi* sample position on fat and vascular distribution

	Small			Moderate		
	Medial	Central	Lateral	Medial	Central	Lateral
No. fat deposits Size I	7.6^c	6.3^a	6.9^b	6.9^a	7.9^b	8.8^c
Fat area Size I	21.8^b	17.4^a	23.6^c	24.9	26.5	28.6
No. fat deposits Size II	0.7	0.6	0.7	0.8	0.6	1.0
Fat area Size II	14.9	10.9	15.2	17.2	13.6	22.1
No. fat deposits Size III	1.4^c	1.1^a	1.3^b	1.9	1.6	1.7
Fat area Size III	221.9	216.0	261.3	442.7	324.7	481.4
Total fat area	359.0^c	241.7^a	305.0^b	484.2^b	366.0^a	532.9^c
Fat %	4.3^b	3.6^a	5.2^c	7.9^b	5.9^a	8.9^c
Ether extract %	5.1	5.4	4.2	7.0	6.9	5.6
No. Blood vessels	6.2^b	5.6^a	6.6^c	7.2^b	6.9^a	7.7^c
No. Blood vessels/ unit fat area	58.5^b	58.0^b	51.7^a	28.7^a	45.9^b	38.9^b

Fat area in mm^2

All means with same or no superscript are not significantly ($P < .05$) different

distribution of the number of medial compared to lateral fat deposits for both marbling levels. The small marbled steaks had a greater number of fat deposits of all sizes in the medial and less in the central position; however, the moderate marbled muscle had the largest number of fat deposits in the lateral and least number of fat deposits in the medial position. These differences in number and size of fat deposits in the Longissimus dorsi muscle might be due to an increase in both cell numbers and size as reported by Allen et al. (1967a), and Moody and Cassens (1968).

The histologically estimated fat of the three fat categories is shown in Table IV. As mentioned earlier, there was no consistency in fat distribution on an area basis from medial to lateral in both marbling levels. The small marbling had significantly less total fat area (H) in the central position (241.7 sq mm) and more ($P<.05$) in the medial (359.0 sq mm) position, whereas the moderate marbled lateral core (532.9 sq mm) had the highest amount of total fat area. The percent fat (H) followed the same trends as total fat area except in the small marbling level which had the highest percent fat in the lateral core. In addition, the combined means for percent fat (H) also revealed that the lateral core (7.1%) had a greater quantity ($P<.05$) of fat than the central (6.1%) and medial position (4.8%) (Table V). In contrast the ether extract values revealed the least amount of fat in the lateral core (4.2 and 5.6%) for both marbling levels. These

Table V

Position means for the total fat and ether extract of the vobine *Longissimus dorsi* muscle

Position	Percent Histological Fat	Percent Ether Extract Means
Medial	6.1 ± 0.5^{b}	20.99^{b}
Central	4.8 ± 0.5^{a}	21.23^{b}
Lateral	7.1 ± 0.7^{c}	17.56^{a}

All means with same or no superscript are not significantly ($P < .05$) different.

differences in the estimation of fat by histological and chemical methods might be due to the basic difference in the two procedures. In the histological evaluation, Oil Red O stains both unbound and bound neutral lipid, where as ether extract estimates only unbound neutral lipids. In addition, differences in estimation may also be due to differences in sample size, sample location and to great variation in the quantity and distribution of fat in *Longissimus dorsi* muscle (Wang *et al.*, 1954; Doty *et al.*, Blumer *et al.*, 1962; Cook *et al.*, 1964).

Sample position has significant ($P < .05$) effect on the number of blood vessels (Table IV). In both marbling levels the lateral position (small = 6.6, moderate = 7.7) had a greater number of blood vessels than the central position

(small = 5.6, moderate = 6.9). However, the number of blood vessels decreased with increased fat area (lower number in lateral position).

Side Effects

The data in Table VI indicates the effect of side on fat deposits. Large fat deposits were significantly greater in the right (1.7) than the lest (1.3) side. These differences in the fat deposits in the right and left Longissimus dorsi muscle, might be due to biological variance, such as the possibility that rumen pressure due to fill might make it more comfortable for the animal to lie on his right side.

Table VI

Effect of side upon histological characteristics of bovine *Longissimus dorsi* muscle

	Left	Right
No. fat deposits Size I	7.28	7.46
Fat deposits area Size I	23.90	23.67
No. fat deposits Size II	0.70	0.75
Fat deposits area Size II	15.27	16.06
No. fat deposits Size III	1.32*±0.075	1.66±.085
Fat deposits area Size III	310.88	338.50
Total fat area	350.68	378.89
Fat percent	5.63	6.33
No. Blood vessels	6.53	6.86
No. Blood vessels/per unit fat area	45.99	47.91

* Significant at 5% level

Correlations

Correlation coefficients are presented in Table VII. Positive, but low correlations were obtained between histologically estimated fat and ether extract values. The large fat deposits and number of squares with fat were significantly correlated with percent ether extract (r = 0.327 and 0.269 respectively). As expected these factors may have added to the bulk of ether extract.

Table VII

Correlation coefficients of various bovine <u>Longissimus dorsi</u> histological characteristics with ether extract and shear force value

	Ether Extract	Shear Force lbs
No. fat deposits Size I	0.044	-.040
No. fat deposits Size II	0.021	-.020
No. fat deposits Size III	0.327*	-.050
Total No. of sqs with fat	0.269*	-.050
Total fat area[a]	0.201	-.017
% fat	0.196	-.024
No. of blood vessels	-.020	0.050
No. of blood vessels per unit fat area	-.213	0.076

a = sq mm.

Extremely low correlation coefficients were obtained between the shear force and all the histological parameters of the muscle.

These results indicate that histological evaluation of fat and vascular distribution may not be a single reliable tool for predicting beef tenderness. The drawback in this method may be due to either a lack of variation within the marbling and maturity groups or to the variable nature of the *Longissimus dorsi* muscle as well as histologically unaccountable fat. At this stage of study it can not be stated that there is an association between fat distribution to muscle tenderness, unless the contribution from other histological characteristics like muscle fiber diameter, sarcomere length, red and white fiber ratio and the proportion of connective tissue is evaluated.

SUMMARY

The influence of marbling and vascular distribution on tenderness characteristics of beef was investigated. The right and left Longissimus dorsi muscle from 60 beef carcasses of unknown history were used in this study. Two marbling levels (small and moderate) and three maturity levels (AA^- = youthful, AB^- = Intermediate, BB^- = approaching maturity), were used.

The number and area of small (16.82 sq. mm.) and large (33.64 sq. mm.) fat deposits increased significantly with marbling. Within marbling groups, there was no consistency for distribution of number of fat deposits from medial to lateral positions. However, the general trend was for less total fat area in the central cores and greater fat area in the lateral cores. The number and area of small, moderate and large fat deposits had no significant effect on tenderness.

The number of blood vessels increased significantly with marbling but decreased significantly per unit fat area. Neither blood vessel number or number per unit fat area had a significant effect on tenderness.

Maturity had little influence on number and area of fat deposits, although the most mature group had the highest fat content. The number of blood vessels were significantly different in the three maturity levels with the highest number present in the young group and least number present in the intermediate maturity group.

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Dr. H. J. Tuma for his guidance in planning this study and preparing the manuscript.

He, also wishes to thank Dr. D. H. Kropf for his advice and assistance in completion of this manuscript, and also Dr. B. E. Brent for his advice and aid for photographic technique used in this study.

The author is greatful to his family members and wife, Kusuma, for their sacrifice and making it all worthwhile.

LITERATURE CITED

Allen, E.; R.W. Bray and R.G. Cassens. 1967a. Histochemical observations of Porcine Muscles as related to lipid accumulation. J. Food Sci. 32:20.

Allen, E.; R.W. Bray and R.G. Cassens. 1967b. Changes in fatty acid composition of Porcine muscle lipid associated with sex and weight. J. Food Sci. 32:26.

Allen, E.; R.G. Cassens, and R.W. Bray. 1967c. Atypical intrafascicular accumulation of fat in Porcine striated muscle. J. Food Sci. 32:146.

Alsmeyer, R.H.; A.Z. Palmer, M. Koger, and W.G. Kirk. 1959. The relative significance of factors influencing and/or associated with beef tenderness. Meat Industry Research Conference, American Meat Institute Foundation Circular. 50:85.

Alsmeyer, R.H.; R. Kulwich, and R.L. Hiner. 1962. Loin-eye tenderness variations measured by the STE. J. Animal Sci. 54:977.

Ashworth, C.T.; Stembridge, V.A.; and Sanders, E. 1960. Lipid absorption transport and hepatic assimilation studied with electron microscope. Am. J. Physiol. 198:1326.

Barnett, R.J.; and E. G. Ball. 1960. Metabolic and Ultrastructural changes induced in adipose tissue by Insulin. J. Biophy. Biochem. Cyto. 8:83.

Beatty, C.H.; Peterson, R.D. and R.M. Beck. 1963. Metabolism of red and white muscle fiber groups. Am. J. Physiol. 204:939.

Bell, E.T. 1909a. III. On the occurence of fat in the epithelium, cartilage and muscle fibers of the Ox. Am. J. Anat. 9:401.

Bell, E.T. 1909b. II. On the histogenesis of the adipose tissue of the Ox. Am. J. Anat. 9:412.

Bloom, W. and D.W. Fawcett. 1964. "A Textbook of Histology". W.B. Saunders Co., Philadelphia.

Blumer, T.N. and H.P. Fleming. 1959. A method for the quantitative estimation of marbling in beef rib eye muscles. J. Animal Sci. 18:959.

Blumer, T.N.; H.B. Craig, E.A. Pierce, W.W.G. Smart, Jr. and M.B. Wise. 1962. Nature and variability of marbling deposits in Longissimus dorsi muscle of beef carcasses. J. Animal Sci. 21:935.

Blumer, T.N. 1963. Relationship of marbling to the palatability of beef. J. Animal Sci. 22:771.

Bowman, F.; E. Page; M.H. Hazaleus, and H.H. Stonaker. 1958. Factors related to tenderness and juiciness. J. Animal Sci. 17:1153.

Branaman, G.A.; O.G. Hankins, and L.M. Alexander. 1936. The relation of degree of finish in cattle to production and meat factors. Proc. Am. Soc. An. Prod. 29: 295.

Carpenter, Z.L.; R.W. Bray, E.J. Briskey, and D.H. Traeder. 1961. Intramuscular fat distribution in the Longissimus dorsi of paired pork loins. J. Animal Sci. 20:603.

Carpenter, Z.L.; and G.T. King. 1965. Tenderness of Lamb rib chops. Food. Tech. 19:102.

Clark, E.R.; and E.L. Clark. 1939. Microscopic observation on the growth of blood capillaries in the living mammal. Am. J. Anat. 64:251.

Clark, E.R.; and E.L. Clark. 1940. Microscopic studies of the formation of the fat in living adult rabbits. Am. J. Anat. 67:255.

Cole, J.W.; W.R. Backus, and L.E. Orme. 1960. Specific gravity as a measure of beef eating quality. J. Animal Sci. 19:167.

Cook, C.F.; and R.W. Bray. 1961. A microphotographic technique for the quantitative determination of marbling and its relative distribution in meats. Food Tech. 15:540.

Cook, C.F.; R.W. Bray, and K.G. King. 1964. Variation in the quantity and distribution of lipid in the bovine Longissimus dorsi. J. Animal Sci. 23:329.

Cooper, C.C.; B.B. Breidenstein, R.G. Cassens, G. Evans, and R.W. Bray. 1967. Histological aspects of the influence of marbling and maturity on the palatability of the beef muscle. J. Animal Sci. 26:893.

Crobin, J.E.; J.L. Williamson, H.B. Geurin, and H.L. Wilcke. 1959. Photographic method of measuring loin eye area. J. Animal Sci. 18:1485.

Cover, S.; O.D. Butler, and T.C. Cartwright. 1956. The relationship of fatness in yearling steers to juiciness and tenderness of broiled and braized steaks. J. Animal Sci. 15:464.

Cover, S.; King, G.T.; and Butler, O.D. 1958. Effect of carcass grades and fatness on tenderness of meat from steers of known history. Texas Agr. Exp. Sta. Bul. 889.

Covington, R.C. 1968. A study of various chemical and histological characteristics of beef muscle and their relationships to palatability. Master's thesis. Kansas State University.

Dean, R.J.; L.J. Bratzler, and D.C. Price. 1959. The photometric measurement of carcass cross sectional areas directly from projected polaroid transparancies. J. Animal Sci. 18:1485.

Doty, D.M.; and J.C. Pierce. 1961. Beef muscle characteristics as related to carcass grade, carcass weight, and degree of using. U.S.D.A. Tech. Bul. 1231.

Fawcett, D.W. 1948. Histological observation on the relation of insulin to the deposition of glycogen in adipose tissue. Endocrinology. 42:454.

Fawcett, D.W. 1952. A comparison of histological organization and cytochemical reactions of brown and white adipose tissue. J. Morph. 90:363.

Field, R.A.; G.E. Nelms, and C.O. Schoonover. 1966. Effects of age, marbling and sex on palatability of beef. J. Animal Sci. 25:360.

Gersh, I. and M.A. Still. 1945. Blood vessels in fat tissue: Relation to problem of gas exchange. J. Exptl. Med. 81:219.

Gladys, G.L.; Butcher, O.M. and Deary, P.A. 1965. Influence of marbling and final internal temperature on quality characteristics of broiled rib and eye of round steaks. Food Tech. 19:152.

Goll, D.E.; A.F. Carlin, L.P. Anderson, E.A. Kline, and M.J. Walter. 1965. Effect of marbling and maturity on beef muscle characteristics. II. Physical, chemical, and sensory evaluation of steaks. Food Tech. 19:845.

Ham, A.W. 1965. "Histology". J.B. Lippincott Company, Philadelphia.

Hartroft, W.S. 1950. Accumulation of fat in liver cells and in lipodiastemata preceeding experimental dietary cirrhosis. Anat. Rec. 106:61.

Hausberger, F.X. 1955. Quantitative studies on the development of autotransplants of immature adipose tissue of rat. Anat. Rec. 122:507.

Hausberger, F.X. and S.W. Milstein. 1955. Dietary effect on lipogenesis in adipose tissue. J. Biochem. 214:483.

Hedrick, H.B.; W.C. Stringer, R.J. Epley, and H.D. Naumann. 1967. Comparison of factors affecting Warner-Bratzler Shear values of beef steaks. J. Animal Sci. 26:895.

Helander, E. 1959. Fat content of skeletal-muscular tissue. Acta Morphol. Neurl. Scand. 2:230.

Henderson, D.W.; D.E. Goll, M.H. Stromer, M.J. Walter, E.A. Kline, and R.E. Rust. 1966. Effects of different measurement techniques and operations on bovine Longissimus dorsi area. J. Animal Sci. 25:334.

Howard, R.D. 1966. Comparison of Sarcomere length to other preditors of beef tenderness. Master's thesis. Purdue University.

Hruzaz and M. Jelinkova. 1964. Decreased activity of the fatty tissue with repeated stress and artheriosclerosis. Vitalist Ziuilis Ationkran Kheiten 9:99.

Kauffman, R.G.; Z.L. Carpenter, R.W. Bray, and W.G. Hoekstra. 1964. Biochemical properties of pork and their relationship to quality. II Intramuscular fat. J. Food Sci. 29:70.

Kauffman, R.G. and A.H. Safanie. 1967. Influence of porcine muscle structure on its lipid accumulation during growth. J. Food Sci. 32:283.

Leites, F.L. 1964. Histochemical features of lipid metabolism and lipolytic activity in old animals. Federation Proc. 23:T747.

Lever, J.D. 1957. The fine structure of brown adipose tissue in the rat with observations on the cytological changes following starvation and adrenalectomy. Anat. Rec. 128:361.

Lewis, B.L.; D.L. Harrison, and D.S. Folse. 1958. A Photomicrographic method of estimating the quality of distribution of fat in muscle tissue. Food Research. 21:1.

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

Link, B.A.; R.G. Cassens, R.W. Bray and T. Kowalcyk. 1967. Fatty degeneration of bovine longissimus. J. Animal Sci. 26:694.

McBee, J.L.; and J.A. Wiles. 1967. Influence of marbling and carcass grade on the physical and chemical characteristics of beef. J. Animal Sci. 26:701.

Moody, W.G. and R.G. Cassens. 1968. A quantitative and morphological study of bovine Longissimus fat cells. J. Food Sci. 33:47.

Napolitano, L; 1963. The differentiation of white adipose cells, an electron microscopic study. J. Cell. Biol. 18:663.

Napolitano, L.; and H.T. Gagne. 1963. Lipid depleted white adipose cells: An electron microscopic study. Anat. Rec. 147:273.

Palmer, A.Z.; J.W. Carpenter, R.L. Alsmeyer, H.L. Chapman, and W.G. Kirk. 1958. Simple correlations between carcass grade, marbling, ether extract of loin eye and beef tenderness. J. Animal Sci. 17:1153.

Ramsbottom, J.M.; E.J. Strandine, and C.H. Koonz. 1945. Comparative tenderness of representative beef muscles. Food Res. 10:497.

Rodahl, K. and B. Issekutz, Jr. 1964. "Fat as a tissue." McGraw-hill Publishers.

Romans, J.R.; H.J. Tuma and W.L. Tucker. 1965. Influence of carcass maturity and marbling on the physical and chemical characteristics of Beef. I. palatability, fiber diameter and proximate analysis. J. Animal Sci. 24:681.

Romanul, F.C.A. 1964. Distribution of capillaries in relation to oxidative metabolism of skeletal muscle fibers. Nature. 201(4916):307.

Schilling, E. 1966. Muscle structure and meat quality. Z. tierzueht Zuchtungs boil. 82:219.

Sheldon, H., C.H. Hollenberg and A.I. Winegrad. 1962. Observation on the morphology of adipose tissue. I. The fine structure of cells from fasted and diabetic rats. Diabetes. 11:378.

Shoshenko, K.A. 1963. On the number of capillaries in the skeletal muscles of the frog. Izvest. Sibirsk. Otd. Akad. nauk. SSSR. 4:86.

Sidman, R.L. and Fawcett, D.W. 1954. The effect of peripheral nerve section on some metabolic responses of brown adipose tissue in mice. Anat. Rec. 118:487.

Simon, T.L. and Williamson, J.R. 1967. The fate of lipid depleted fat cells. An ultrastructural study of the effects of prolonged calorie deprivation on fat cells and other tissues of the rat. Arch. Path. 83:162.

Stouffer, J.R. and G.H. Wellington. 1957. Photographs for determining the degree of marbling in beef. J. Animal Sci. 16:1073.

Tuma, H.J.; R.L. Henrickson, D.F. Stephens and Ruby Moore. 1962. Influence of marbling and animal age on factors associated with beef quality. J. Animal Sci. 21:848.

Tuma, H.J.; R.L. Henrickson, G.V. Odell and D.F. Stephens. 1963. Variation in the physical and chemical characterisitcs of the Longissimus dorsi muscle from animals differing in age. J. Animal Sci. 22:354.

Walter, M.J.; D.E. Goll, E.A. Kline, L.P. Anderson and A.F. Carlin. 1965. Effect of marbling and maturity on beef muscle characteristics. Food Tech. 19:841.

Wang, H.; E. Rasch, V. Bates, F.J. Beard, J.C. Pierce and O.G. Hankins. 1954. Histological observation on fat loci and distribution in cooked beef. Food Res. 19:314.

Wells, H.G. 1940. Adipose tissue: a neglected subject. JAMA. 114:2177.

Williamson, J.R. 1964. Adipose tissue: Morphological changes associated with lipid mobilization. J. Cell Biol. 20:57.

Wellington, G.H.; and J.R. Stouffer. 1959. Beef marbling its estimation and influence on tenderness and juiciness. Cornell Univ. Agri. Exptl. Station. Bul. 941.

APPENDIX TABLE I

Effect of Marbling on Histological Characteristics of Bovine Longissimus Dorsi Muscle

	Small Marbling	Moderate Marbling
Total muscle area, sq cm	59.7± 5.1*	61.1± 3.5
No. of fat deposits size I	6.9± 0.3*	7.8± 0.3
No. of squares with fat size I	13.5± 0.5*	15.2± 0.6
Area of fat size I[a]	20.9± 1.2*	26.7± 1.4
No. of fat deposits size II	0.7± 0.1	1.2± 0.1
No. of squares with fat size II	3.4± 0.4	3.8± 0.4
Area of fat size II[a]	13.7± 1.4	17.7± 1.7
No. of fat deposits size III	1.2± 0.1*	1.7± 1.1
No. of squares with fat size III	19.5± 1.4*	32.0± 1.7
Area of fat size III[a]	233.1±24.1*	416.3±30.8
Total area of fat[a]	268.6±24.0*	461.0±30.4
Total no. of squares with fat	36.3± 1.4*	51.0± 1.5
Total no. of fat deposits	8.8± 0.3*	10.4± 0.3
Ave. size of fat deposit[a]	37.9± 4.5*	60.7± 6.6
% fat	4.4± 0.4*	7.6± 0.5
No. of squares of fat/unit muscle area	1.4± 0.1*	2.3± 0.2
No. of blood vessels	6.1± 0.2*	7.3± 0.3
No. of blood vessels/ unit muscle area	1.1± 0.1	1.2± 0.1
No. of blood vessels/unit fat area	56.1± 4.7	37.8± 4.8*

a area sq. mm.

* significant at 5% level.

APPENDIX TABLE II

Effect of Marbling on Sample Position, Amount of Fat and Blood Vessels of Bovine Longissimus Dorsi

	Small Marbling			Moderate Marbling		
	Medial	Central	Lateral	Medial	Central	Lateral
Total Muscle area, sq cm	61.1	58.7	58.9	61.4	62.0	60.0
No. of fat deposits size I	7.6^c	6.3^a	6.9^b	6.9^a	7.9^b	8.8^c
No. of squares with fat size I	13.6^b	12.7^a	14.3^c	13.2^a	15.6^b	16.8^c
Area of fat size I, sq. mm.	21.8^b	17.4^a	23.6^b	24.9^a	26.5^a	28.6^a
No. of fat deposits size II	0.7	0.6	0.7	0.8	0.6	1.0
No. of squares with fat size II	3.4	2.8	4.1	3.5	3.4	4.5
Area of fat size II, sq. mm.	14.9	10.9	15.2	17.3	13.6	22.1
No. of fat deposits size III	1.4^c	1.1^a	1.3^b	1.9^c	1.6^a	1.7^b
No. of squares with fat size III	20.0	17.9	20.5	34.7	26.8	34.6
Area of fat size III, sq. mm.	221.9	216.0	261.3	442.7	324.7	481.5
Total area of fat, sq. mm.	259.0^b	241.7^a	305.0^c	484.2^b	366.0^a	533.0^c
Total no. of squares with fat	36.8^b	33.5^a	38.7^c	51.4^b	45.7^a	56.0^c
Total no. of fat deposits	9.6^c	7.9^a	8.8^b	9.7^a	10.1^a	11.5^b
Ave. size of fat deposit, sq. mm.	35.7^a	37.9^b	40.1^c	62.4^b	42.8^a	76.9^c
% fat	4.3^b	3.6^a	5.2^c	7.9^b	5.9^a	8.9^c
No. of squares of fat/unit muscle area	1.3^a	1.4^b	1.5^c	2.3^b	1.8^a	2.7^c
No. of blood vessels	6.2^b	5.6^a	6.6^c	7.3^b	6.9^a	7.7^c
No. of blood vessels/unit muscle area	1.1	1.0	1.1	1.2	1.1	1.3
No. of blood vessels/unit fat area	58.5^b	58.0^b	51.7^a	28.7^a	46.0^b	38.9^b

All means with the same or no superscript are not significantly different ($P < .05$)

APPENDIX TABLE III

Effect of Sample Position on Amount of Fat and Blood Vessels of Bovine *Longissimus Dorsi* Muscle

	Medial	Central	Lateral
Total muscle area, sq cm	61.2 ± 4.0	60.3 ± 6.2	59.4 ± 5.7
No. of fat deposits size I	7.2 ± 0.3	7.1 ± 0.3	7.8 ± 0.4
No. of squares with fat size I	13.4 ± 0.6[a]	14.1 ± 0.7[b]	15.5 ± 0.7[c]
Area of fat size I, sq. mm.	23.3 ± 1.6[b]	21.9 ± 1.4[a]	26.1 ± 1.6[c]
No. of fat deposits size II	0.7 ± 0.1	0.6 ± 0.1	0.9 ± 0.1
No. of squares with fat size II	3.4 ± 0.4	3.1 ± 0.4	4.3 ± 0.5
Area of fat size II, sq. mm.	16.1 ± 1.8	12.2 ± 1.6	18.7 ± 2.1
No.of fat deposit size III	1.7 ± 0.1[c]	1.3 ± 0.1[a]	1.5 ± 0.1[b]
No. of squares with fat size III	27.3 ± 1.8	22.4 ± 1.9	27.5 ± 2.2
Area of fat size III, sq. mm.	332.3 ± 32.0	270.4 ± 31.0	371.4 ± 40.4
Total area of fat, sq. mm.	371.6 ± 32.0[b]	303.8 ± 30.3[a]	419.0 ± 40.1[c]
Total no. of squares with fat	44.1 ± 1.8[b]	39.6 ± 1.9[a]	47.3 ± 2.1[c]
Total no. of fat deposits	9.6 ± 0.3	9.0 ± 0.3	10.2 ± 0.4
Ave. size of fat deposit, sq. mm.	49.1 ± 6.4	40.4 ± 4.6	58.5 ± 9.1
% fat	6.1 ± 0.5[b]	4.8 ± 0.5[a]	7.1 ± 0.7[c]
No. of squares of fat/unit muscle area	1.8 ± 0.2	1.6 ± 0.2	2.1 ± 0.2
No. of blood vessels	6.7 ± 0.3	6.3 ± 0.3	7.1 ± 0.4
No. of blood vessels/unit muscle area	1.1 ± 0.1	1.0 ± 0.04	1.2 ± 0.1
No. of blood vessels/unit fat area	43.6 ± 5.4	52.0 ± 5.7	45.3 ± 6.6

All means with the same or no superscript are not significantly different ($P < .05$)

APPENDIX TABLE IV

Effect of Maturity and Marbling upon Histological Characteristics of Bovine Longissimus Dorsi Muscle

	Small Marbling			Moderate Marbling		
	AA^-	A^+B^-	BB^+	AA^-	A^+B^-	BB^+
Total muscle area, sq. cm.	60.2^b	56.9^a	61.9^b	61.4	61.0	61.0
No. of fat deposit size I	6.9	7.0	6.8	7.2	8.4	7.9
No. of squares with fat size I	12.9^a	13.4^a	14.1^b	13.4^a	16.5^c	15.8^b
Area of fat size I, sq. mm.	19.1^a	20.4^b	23.3^c	22.9^a	29.7^b	27.4^b
No. of fat deposits size II	0.6	0.7	0.7	0.8	0.8	0.9
No. of squares with fat size II	3.2	3.6	3.6	4.0	3.6	3.8
Area of fat size II, sq. mm.	11.9	14.3	14.8	17.8	16.2	18.9
No. of fat deposits size III	1.2^b	1.1^a	1.4^c	2.0^c	1.8^b	1.5^a
No. of squares with fat size III	17.7	14.9	25.9	36.5^b	29.9^a	29.5^a
Area of fat size III, sq.mm.	204.2^b	160.4^a	334.6^c	459.6	396.7	392.6
Total area of fat, sq. mm.	236.1^b	195.4^a	374.2^c	500.4^b	444.2^a	438.5^a
Total no. of squares with fat	33.6^b	31.8^a	43.6^c	54.0^b	50.0^a	49.1^a
Total no. of fat deposits	8.8^b	8.7^a	8.9^c	10.0^a	10.9^c	10.3^b
Ave. size of fat deposit, sq. mm.	30.8	26.2	56.8	71.1	55.7	55.5
% fat	3.8^b	3.4^a	5.9^c	8.3^b	7.2^a	7.2^a
No. of squares of fat/unit muscle area	1.3^b	1.0^a	1.8^c	2.5	2.1	2.2
No. of blood vessels	6.4^b	5.2^a	6.8^c	8.3^b	6.8^a	6.9^a
No. of blood vessels/unit muscle area	1.0	0.9	1.2	1.3	1.1	1.1
No. of blood vessels/unit fat area	68.0^c	57.9^b	42.1^a	33.7	39.2	40.6

All means with same or no superscript are not significantly different $(P<.05)$

APPENDEX TABLE V

Effect of Maturity on Fat Deposits, Total Fat and Blood Vessels of Bovine Longissimus Dorsi Muscle

	AA^-	A^+B^-	BB^+
Total muscle area, sq cm	60.8 ± 4.7	59.0 ± 6.9	61.4 ± 4.0
No. of fat deposits size I	7.0 ± 0.3	7.7 ± 0.4	7.4 ± 0.3
No. of squares with fat size I	13.2 ± 0.6	14.9 ± 0.7	14.9 ± 0.7
Area of fat size I, sq. mm.	21.0 ± 1.3	25.0 ± 1.7	25.3 ± 1.7
No. of fat deposits size II	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
No. of squares with fat size II	3.6 ± 0.6	3.6 ± 0.5	3.7 ± 0.5
Area of fat size II, sq. mm.	14.9 ± 1.8	15.3 ± 1.8	16.8 ± 2.0
No. of fat deposits size III	1.6 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
No. of squares with fat size III	27.1 ± 2.0	22.4 ± 1.9	27.7 ± 2.0
Area of fat size III, sq. mm.	331.9 ± 35.2	278.6 ± 32.2	363.6 ± 36.7
Total area of fat, sq. mm.	368.2 ± 35.0	319.8 ± 31.8	406.3 ± 36.5
Total No. of squares with fat	43.8 ± 2.1	40.9 ± 1.8	46.3 ± 1.9
Total no. of fat deposits	9.4 ± 0.4	9.8 ± 0.4	9.6 ± 0.3
Ave. size of fat deposit, sq. mm.	50.9 ± 7.8	40.9 ± 5.6	56.1 ± 7.3
% fat	6.1 ± 0.6	5.3 ± 0.3	6.6 ± 0.6
No. of squares of fat/unit muscle area	1.9 ± 0.2	1.6 ± 0.2	2.0 ± 0.2
No. of blood vessels	7.3 ± 0.4[c]	6.0 ± 0.3[a]	6.8 ± 0.3[b]
No. of blood vessels/unit muscle area	1.2 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
No. of blood vessels/unit fat area	50.9 ± 5.5	48.6 ± 7.0	41.4 ± 5.00

All means with the same or no superscript are not significantly different ($P < .05$)

APPENDIX TABLE VI

Effect of Side upon Histological Characteristics of Bovine Longissimus Dorsi Muscle

	Left	Right
Total muscle area in sq. cm.	6.07	6.00
No. of fat deposits size I	7.28	7.46
No. of squares with fat size I	14.03	14.65
Area of the fat size I, sq. mm.	23.90	23.67
No. of fat deposits size II	0.70	0.75
No. of squares with fat size II	3.60	3.60
Area of the fat size II, sq. mm.	15.27	16.06
No. of fat deposits size III	1.32*±.075	1.66±.085
No. of squares with fat size III	24.38	27.07
Area of the fat size III, sq. mm.	310.88	338.50
Total area of the fat, sq. mm.	350.68	378.89
Total no. of squares with fat	41.98	45.34
Total no. of fat deposits	9.38	9.86
Ave. size of the fat deposit, sq. mm.	46.53	52.10
% fat	5.63	6.33
No. of squares of fat/unit muscle area	1.76	1.88
No. of blood vessels	6.53	6.86
No. of blood vessels/unit muscle area	1.10	1.13
No. of blood vessels/unit fat area	45.99	47.91

* Significant at 5% level.

APPENDIX TABLE VII

Simple Correlations Between Histological Variations and Chemical and Physical Components of Bovine Longissimus Dorsi Muscle

	E.E.	Shear Force lb.
Total muscle area, sq. cm.	0.067	-.012
No. of fat deposits size I	0.044	0.045
No. of squares with fat size I	0.043	0.029
Area of fat size I[a]	0.071	0.020
No. of fat deposits size II	0.021	-.023
No. of squares with fat size II	-.021	-.025
Area of fat size II[a]	-.052	-.053
No. of fat deposits size III	0.327*	-.047
No. of squares with fat size III	0.248	-.056
Area of fat size III[a]	0.195	-.016
Total area of fat[a]	0.201	-.017
Total no. of squares with fat	0.269*	-.054
Total no. of fat deposits	0.149	0.018
Ave. size of fat deposit[a]	0.053	0.030
% fat	0.196	-.024
No. of squares of fat/unit muscle area	0.178	-.011
No. of blood vessels	-.020	0.057
No. of blood vessels/unit muscle area	0.011	0.031
No. of blood vessels/unit fat area	-.213	0.076

a area--sq. mm.

* Significant at 5% level.

APPENDIX TABLE VIII

Staining procedure for fat

1. Air dry the sections for 30 min.

2. Fix in 4% neutral formalin for 10 min.

3. Rinse in cold distilled water.

4. Stain the rinsed sections for 20 min. in oil Red O.

5. Rinse in cold distilled water.

6. Counter stain with Harris hematoxylin for 30 sec.

7. Blue in cold tap water, until the nuclei are distinct, clean and mount in phenol-glycerol-jelly.

THE RELATIONSHIP OF INTRAMUSCULAR FAT AND THE VASCULAR SYSTEM OF BEEF TENDERNESS

by

BALEMULA GURUVA REDDY

B.V. Sc. & A.H. Osmania University (INDIA), 1961

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1969

The effect of beef maturity and marbling upon the fat and vascular distribution of bovine Longissimus dorsi muscle at 9th rib was studied.

Sixty beef carcasses with two levels of marbling (small and moderate) and three maturity groups (AA^-, A^+B^-, and BB^+ which correspond to chronological ages of approximately 12-18, 18-30 and 30-42 months) were used in this study. The two marbling levels were represented equally within each maturity group. Three 1.27 cm. cores (medial, central and lateral) from the ventral aspect of both right and left Longissimus muscle were taken.

The muscle samples were sectioned at 6 to 8 thickness on the Cryostat (-20° C), stained with Oil Red O for fat and with hematoxylin for blood vessels. The stained slide was photographed in a negative carried with a micrometer disc. Area of fat was determined, based on the area covered by each single deposit, as Category I or small (16.82 sq. mm), Category II or moderate in size (16.82 to 33.64 sq. mm) and Category III or large (33.64 sq. mm), respectively. The number of blood vessels were determined under a light microscope at 100 magnification.

The marbling had a significant ($p < .05$) effect on the number and area of small and large fat deposits. Within marbling groups no consistency was noted for distribution of number of fat deposits from medial to lateral positions. However, the general trend was for less total fat area in the Central cores and greater fat area in the lateral cores.

The number and area of small, moderate and large fat deposits had no significant effect on taste panel tenderness scores or Warner-Bratzler shear values.

The total number of blood vessels increased significantly with the higher marbling level but decreased significantly per unit fat area. Neither blood vessel number or number pe unit fat area had a significant effect on tenderness.

Maturity had little influence on number and area of fat deposits, although the most mature group had the highest fat content. The number of blood vessels in areas observed histologically were significantly different in the three maturity levels with the highest number present in the young group and least number present in the intermediate maturity group.