THE EFFECT OF BEEF MATURITY AND MARBLING UPON RED-WHITE MUSCLE FIBER RATIOS AND RELATED PROPERTIES

by

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B.V.Sc., University of Madras (India), 1950

A Master's Thesis

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Husbandry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

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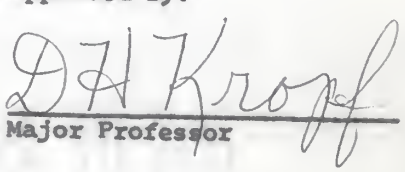

Major Professor

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INTRODUCTION

It has been known since a long time that there are two different types of skeletal muscle fibers in the mammals, the slow contracting red (dark) and fast contracting white (light). It is also known that muscles of the mammals are mixed, containing both dark and light fibers in varying proportions. Histological studies revealed that the dark fibers have a smaller diameter than the white fibers and they are of higher mitochondrial activity. Early work during the last century has established a firm relationship between the morphology of muscle and its function. It was shown that the red muscle fibers were slow contracting, but capable of sustained activity, and white fibers fast contracting, but short duration.

A number of authors have demonstrated a wide variation in the content of different enzymes in the individual muscle fibers in various animals. It has been speculated that the diversity of enzymes in different adult muscles mainly reflected various stages of development of one common fiber type. The variations in the staining reactions of the different fiber types depends upon the quantitative differences in lipid and fat content and their mitochondrial content.

The functional significance of this heterogeneity of skeletal muscle is not known precisely.

It has also been known that skeletal muscles of many animals vary in color over a wide range from white to red. Some of the red color is attributed to the blood supply of the muscle, but it has been shown that the difference in color between the muscles persisted even after exsanguinuation of the animal. The remaining color has been ascribed

to myoglobin, the oxygen storing pigment of the muscle, and to cytochromes in the muscle. It has also been established that the muscle color is dependent upon the proportion of red fibers which the muscle contains and the red fibers are believed to be rich in myoglobin.

Consumer preference for the desirable color in meat has initiated a research interest to investigate the factors that contribute to and are responsible for this color development and maintenance in fresh and frozen meats. Recently it has been observed in the developing fetus, and new born kittens, rabbits and rats that muscle is made up of red fibers entirely.

The percentages of red and white fibers in bovine muscles have not been studied extensively, nor has the effect of maturation and marbling been investigated.

The present study, which is a part of a major investigation, is an attempt to estimate (1) the percentages of different fibers in bovine longissimus dorsi muscle, (2) to find out the effect of maturity and marbling on red and white fiber ratios and (3) to ascertain the relationship of fiber types to the composition of muscle, fiber diameter and post rigor sarcomere length, and (4) to determine the contributing role of the different types of fibers to the ultimate color of the muscle.

REVIEW OF LITERATURE

Physiology of Red and White Skeletal Muscle Fibers

Physiological differences in red and white fibers in the rabbit were reported by Ranvier (1880). He correlated these differences with slow and fast contraction respectively. According to Grutzner (1884), the thin dark fibers in the gastrocnemius of the frog were analogous to the red fibers in the rabbit muscle. He concluded that the muscles of all vertebrates including man, were composed of two types of fibers; one thin and dark in color and the other larger and "clear". He attributed the dark color to granules in the sarcoplasm. No histological differences could be demonstrated by Lee, Guenther and Melenney (1916). However, they found chemical and physiological differences between the muscles studied. Bullard (1919), studying the same muscles histologically, reported light, dark and intermediate fibers of varying proportions and sizes in different muscles.

Two general categories of muscle fibers, dark and light, were visible in unstained preparations as described by Bullard (1912). Generally dark fibers were described as being of smaller diameter and containing more sarcoplasm and granules than the light fibers. As reviewed by Needham (1926), Knoll showed that most mammalian muscles are mixed, that is, they contain both dark and light fibers.

Biochemical studies have greatly helped in studying the enzymes in different muscles and different parts of the same muscle. Green (1957) commented that the red fibers had higher contents of enzymes associated with the citric acid cycle than did the white fibers.

Histochemical techniques are now being used to correlate functional activity of different muscle fibers with their morphology.

Succinic dehydrogenase activity of muscle fibers has been studied by many workers (Wachstein and Meisel, 1955; Buno and Germino, 1958; Nachmias and Padykula, 1958). In general, it was found that the smaller fibers were more reactive than the larger ones, and in the third group the activity was unrelated to size of the fibers. They suggested that the small fibers contained more mitochondria per unit area than large fibers.

Ogata (1958), studying the succinic dehydrogenase activity in the muscles of fish, frog, birds and mammals described three types of fibers; large or white, small or red, and fibers intermediate in size and color. Their proportion varied in different muscles. A similar variation in the muscles of the cat for diphosphopyridine nucleotide (DPN) diaphorase and triphosphopyridine nucleotide (TPN) diaphorase activity was demonstrated by Ogata (1958). DPN and TPN diaphorase activity was demonstrated by Bergman and Walker (1959) in frog sartorius muscle. The contrast in enzyme activity between different fibers was most strikingly shown by the DPN diaphorase and lactate dehydrogenase reactions by Dubowitz and Pearse (1960). The same workers noted a reciprocal activity of oxidative enzymes and phosphorylase in human, rat and pigeon muscle. In general they observed high oxidative enzyme content in small (red) fibers and high phosphorylase content in the large (white fibers). Their histochemical evidence supported the view that in mammalian and avian muscle, two distinct fiber types exist. This was also confirmed by the work of Pearse (1961). Engel (1962) stated that the DPNH dehydrogenase activity is greater in type I (red)

fibers and phosphorylase in type II (white) fibers. Thus the response of muscle sections to histochemical reactions confirms a functional heterogeneity of this tissue and is a major tool in classifying fibers from various muscles of the same or different species.

Distribution of Red and White Fibers in Various Skeletal Muscles

In the past decade, several reports have dealt with histological, biochemical and pharmacological observations regarding the differentiation of white and red muscle (Jewel and Zaimis, 1954; Blancher and Vanwijhe, 1962).

Ogata (1958), in a series of histochemical studies of the red and white fibers of the muscles of fish, frogs, birds and mammals, observed that in the cat gastrocnemius the percentage of white, intermediate and red fibers was 33, 19, and 48, respectively. In the other muscles of the cat studied, he further observed that the distribution of these fibers was very different in each muscle. Generally the muscle which was located near the body surface was "rich" in white muscle fibers, while the deep muscle was "rich" in medium and red fibers and in the same muscle the external parts were richer in white muscle fibers, while internal parts were richer in red and medium ones. He also observed a close relationship between their enzymatic activity, sudanophillicity and size. Small fibers, namely red fibers, had the strongest enzymatic activity and showed strongest sudanophillicity. The large fibers, namely the white ones, had the weakest enzymatic reaction and weakest sudanophillicity. The medium sized fibers were intermediate in the histochemical reactions. He further observed the red fibers to have higher activity of cytochrome systems and by using DPN diaphorase reactions could distinguish between the three types of muscle fibers.

Jinnai (1960) reported that in the cat, gastrocnemius muscle spindle histological examination showed white fibers to exceed red by a ratio of 6:2. The soleus muscle, having a tonic function, contained a high proportion of red fibers, while gastrocnemius and other limb muscles of phasic function tended to contain more white fibers. The white fibers had a tendency to gather in the external part of the muscle and the red fibers in the internal part and near the tendon.

Sreter and Woo (1963) observed that the superficial layers of rat gastrocnemius were composed mostly of white fibers, while plantaris and deep layers of gastrocnemius contained more red fibers. The average dark fiber content of the soleus, deep layers of gastrocnemius, plantaris and superficial layers of gastrocnemius were 61.5, 55.5, 44.5 and 18 percent, respectively.

Histochemical reactions with various muscles from adult chickens, show breast muscles to be composed primarily of white (pale fibers) with strong phosphorylase reaction in contrast to weak phosphorylase activity of red cardiac fibers. A mixture of white, red and intermediate types were seen in leg muscles (Cosmos, 1965, 1966).

Beecher et al. (1965) observed that the white porcine muscles, semitendinosus (light portion), biceps femoris (outside), longissimus dorsi and gluteus medius, contained less than 30% red fibers, whereas red muscle, serratus ventralis, rectus femoris, biceps femoris (inside) semitendinosus (dark portion) and trapezius, contained more than 40% red fibers. The red fiber content of all muscles in their study ranged from 19.5 to 47.7 percent.

Fiber Diameter and Sarcomere Length

In frozen sections of mouse muscle, the fibers showing granular cytoplasm were as a rule smaller in diameter than those possessing relatively agranular cytoplasm (Bajusz, 1964).

Sudan black B positive fibers described by Norman (1965) and also by Beecher et al. (1965) in porcine muscle appeared to be somewhat smaller in size than the adjacent fibers.

Beaty et al. (1966) are of the opinion that classification of the redness of a muscle by percent of red fibers is open to the error associated with differences in relative sizes of the red and white fibers in some muscles. In the whitest portion of the brachioradialis of rhesus monkey, the reddest fibers were much smaller than the average white fibers, while in other muscles such as pectoralis and sartorius, the red and white fibers were more uniform in size.

Generally the post rigor sarcomere length of porcine muscle was slightly longer in red muscles than in white muscles. However, specific red muscles, semitendinosus (dark portion) and trapezius, had significantly longer post rigor sarcomeres than specific white muscles (gluteus medius and longissimus dorsi). Post rigor sarcomere length was significantly associated with the percent red fibers, succinic dehydrogenase and myoglobin content (Beecher et al., 1965).

Fat, Moisture and Maturity Relationships to Red and White Fibers

Denny-Brown (1929) noted that the dark fibers in cat muscle had a higher fat content than the light ones, but he could not demonstrate any correlation between histological features of the fibers and the speed of contraction. George and Bhakthan (1961) reported that red

(slow) muscles of cockroach, compared with white (fast) muscles, contained higher lipid concentration. Bilinski and Jonas (1964) have shown that the red fibers or dark muscles have a greater ability to oxidize lipids than light muscles.

The fat content of porcine muscles studied by Beecher et al. (1965) was variable and not significantly associated with the percent red fibers of the same muscles. However, significant but low correlations between lipid content and myoglobin values and also between lipid content and succinic dehydrogenase activity were observed. They also noted that the total lipid content (ether extractable) of the muscle (extra- and intracellular) had little effect on the efficiency of detecting red fibers by the Sudan black B method used in their study.

Intramuscular lipid content in the porcine longissimus dorsi muscle was greater in heavy weight animals than in light weight animals (Allen et al. 1967). They also observed fibers positive for Beta hydroxy butyric dehydrogenase in porcine muscle and are of the opinion that porcine muscle is more capable of utilizing lipids as a source of energy than the mouse muscles studied by Ogata and Mori (1964).

Dubowitz (1965) observed a striking difference in the maturation of skeletal muscle in different species of animals. In the guinea pigs, at birth, the muscle already showed a full differentiation into fiber types. The rat and mouse showed no differentiation of their muscle into fiber types at birth and the process was only complete in the rat by about two weeks of age. The hamster and rabbit showed differentiation at birth, but this was less striking than in the guinea pig. Thus there is some correlation between the presence of differentiation

in the muscle at birth and the general maturity and mobility of the animal and also the length of gestation.

Waldman (1967) observed a tendency in the red fibers of bovine longissimus dorsi muscle to decrease in number with increased live weight and age. He is of the opinion that as the animal develops and grows, there is a conversion of red fiber to white fiber which also indicates a change in functional activity and source of metabolizable energy.

Color

Color vision is perhaps the most valued gift of nature. To see a body in its true color, the body must be illuminated by light of the same color or of complete color.

Most objects owe their color to substances that absorb radiant energy within the visible spectrum. These substances are called colorants; if insoluble, pigments and if soluble, dyes. Objects around us are made visible by the radiant energy that comes from them to our eyes. The nature of radiant energy is such that it is hard to form even an approximate concept of it. Radiant energy is known to us under many names which refer simply to differences in wavelength and frequency (Judd and Wyszecki, 1963).

Color Measurement

The analysis of a beam of radiant energy into its spectral components may be accomplished either by means of a prism or by means of a diffraction grating. A spectrophotometer is an instrument used to measure the radiant flux of one beam compared to that of a standard beam.

The fundamental properties of an object responsible for its color are spectral transmittance for transparent objects and spectral reflectance for opaque objects. The spectral reflectance is the ratio of reflected to incident radiant flux for one narrow band of the spectrum (Judd and Wyszecki, 1963). Butler (1962) has shown that light in passing through a turbid sample may traverse an optical path which is many times the sample thickness. He also showed that the reflectivity and scattering coefficient can be determined absolutely without reference to a standard material from the optical density measurements.

Judd and Wyszecki (1963) stated that the layer of meat used for reflectance studies must be sufficiently thick so that no change in reflectance occurs upon further increasing the thickness. The reflectance of such infinitely thick samples has been termed "reflectivity" by them and given the symbol R_∞.

Color may be subjectively evaluated by the human eye or objectively measured by such instruments as the spectrophotometer, the Hunter Color Difference Meter or by the Munsell Spinning Disk System.

Two possible methods are available to measure the various amounts of myoglobin present at the surface of the meat. Broumand et al. (1958) used the absorption ratio method for the extracts of meat taken from the surface of beef cuts. The reflectance ratio method was used by Dean and Ball (1960) to analyze the myoglobin fractions on the surface of beef cuts. However, each method is not free from error. During extraction and analysis the myoglobin of meat changes to oxymyoglobin or to its reduced form, metmyoglobin. Similarly, there is no sound theoretical basis relating reflectance spectra and quantities of pigments (Snyder, 1965).

On the other hand Naughton et al. (1958) claimed that reflected light measured on an absorbency scale is directly proportional to amounts of myoglobin, oxymyoglobin and metmyoglobin at the surface of tuna samples.

Snyder (1965) demonstrated a non-destructive method for measuring the proportions of myoglobin derivatives in meat samples. The reflectance spectra were recorded on the absorbency scale for samples of beef containing predominantly myoglobin, oxymyoglobin or metmyoglobin at the surface. The spectra were so adjusted that the reflectance measured on the absorbancy scale (R_A) was 1.0 at 525 mu, the isobestic point for the three derivatives. With this kind of adjustment, he has shown that the isobestic point for metmyoglobin was 474 mu, and for myoglobin and oxymyoglobin was 571 mu and these values were reproducible.

Stewart et al. (1965) suggested an improved method for total raw pigments of meat, based on reflectivity of meat samples at 525 mu, the isobestic point for myoglobin, oxymyoglobin and metmyoglobin. The reflectivity data, when calculated as the corresponding ratios of the absorption coefficients (K) to the scattering coefficient (S), were linearly related. Lowering the pH of the meat decreased the K/S values

Snyder and Armstrong (1967) obtained data showing that K/S ratios (absorbency coefficient) per unit of sample thickness (K) divided by the scattering coefficient per unit of sample thickness (S) were best suited for quantitative analysis of myoglobin derivatives in intact meat samples.

Factors Affecting Muscle Color

Muscle color is dependent on the pH of the muscle and also on the concentration of myoglobin in it. Briskey et al. (1960) working with porcine muscle observed that muscle pH affected color and muscles of the same myoglobin concentration appeared darker at a higher pH.

It has been established (Denny-Brown, 1929; Ogata, 1958) that muscle color is dependent upon the proportion of red fibers which the muscle contains. Early work done by Mackintosh and Hall (1935) on beef and later by Craig et al. (1959) showed a significant correlation between the color of muscle and its fat content. Krebs (1950) indicated that the problem of relating myoglobin concentration to muscular activity is complicated by the fact that tissue respiration tends to increase with a decrease in the body size of the animal. Lawrie (1953) demonstrated a close correlation in the amount of succinic dehydrogenase, cytochrome oxidase and myoglobin present in the skeletal muscles of the horse.

Red muscles have high oxidative enzyme activity, whereas white muscles have higher glycolytic activity. Variations in these red and white muscle interrelationships have been noted by Lawrie (1953), and Tappel and Martin (1958). Lawrie (1953) observed a sigmoidal relation between the percent myoglobin and the QO_2 (QO_2 = calculated ul. O_2 uptake/mg. fat free dry enzyme solids/hr., at infinite cytochrome-C concentration) measured as cytochrome oxidase, in a variety of vertebrate muscles. Recent solubilization and purification of succinic dehydrogenase from beef and pork heart mitochondria (Neufeld, Scott and Stotz, 1954; Singer and Kearney, 1954; Singer, Kearney and Bernath, 1956) show

the primary enzyme to be a ferroflavoprotein. These observations suggest that the color or density differences of vertebrate muscle fibers are due partly to the concentration of myoglobin, cytochromes and flavin enzymes.

Recent observations on small laboratory animals by several workers (Ogata, 1958; Romanul, 1964; Dawson and Romanul, 1964) have also indicated a close relationship between the red fiber content of a muscle, the visual muscle color and succinic dehydrogenase activity. It has been reported in man that all normal muscles are dark red and vary less in color than the muscles of quadrupeds. The differences in color and speed of reaction in red versus white muscle were also reported to be less distinct in primate (human) muscle than in muscles of lower mammals (Adams et al., 1962). Tuma et al. (1962) observed that the bovine longissimus dorsi steaks were darker red as the animal's age advanced. Romanul (1964) noted that the cytochrome oxidase activity of a muscle fiber was closely parallel to the density of the capillaries around it. The small oxidative fibers were proportionately surrounded by many more capillaries than the larger fibers.

Myoglobin content of the porcine muscles studied, generally increased with an increase in the percentage of red fibers. Red muscles contained significantly higher concentrations of myoglobin than white muscles (Beecher et al., 1965).

Henry and Bratzler (1959) calculated a correlation coefficient of $-.69$ between index of fading by Munsell spinning disks and muscle myoglobin concentration. They reported that neither index of fading or myoglobin concentration were significantly related to ether extract

or total moisture content of porcine longissimus dorsi muscle. The same workers (1960) did not regard moisture of any importance in influencing porcine muscle color, contrary to the opinion of Craig et al. (1959), who considered it as an important factor influencing the muscle color in beef.

MATERIALS AND METHODS

Sampling

Sixty steer carcasses (500-900 lbs.) were selected from three maturity groups as defined by the U.S.D.A. consumer and marketing service; namely A-A^{o}, A+B^{-} and B^{o}B+ which correspond to chronological ages of approximately 12-18, 18-30 and 30-42 months, respectively. These will be referred to as A, AB, and B, respectively.

Each maturity group included two marbling levels, namely, small and moderate corresponding to 5 and 7, respectively, of U.S.D.A. grading standard (1965). These two marbling levels were represented equally within each maturity group, with ten carcasses sampled per marbling maturity group. Thus the experiment was a 2 x 3 factorial design as illustrated in Table I.

Table I

Experimental Design

Maturity Group	Degree of Marbling		
	Small	Moderate	Total
A	10	10	20
A B	10	10	20
B	10	10	20
Total	30	30	60

The beef ribs were purchased from two packers (Maurer-Neuer and Company, Kansas City, Kansas, and Armour and Company of Emporia, Kansas).

The ribs were cut into about two inch thick steaks (coinciding with length of vertebrae), wrapped in Kraft polyethylene laminate paper, frozen at -10°F and freezer stored at 0°F for about 6 months until used for this experiment. The longissimus dorsi muscle sample removed at the seventh thoracic vertebrae was used for this experiment. Only the right side was included in the present study.

The ribs were removed from freezer and thawed in a refrigerator overnight. A half inch core was cut at the medial position (Plate 1.A) from each of the sixty steaks. Each core was cut transversely approximately at the middle, at right angles to the fiber direction in the muscle. A 3-5 mm thick section of the muscle was removed from each core and pressed lightly between the folds of a paper towel to remove excess moisture. The sample was transferred to a metal specimen block kept on the freeze bars of the cryostat at -25 to -30° C, on which a few drops of OCT compound II was placed. Sufficient OCT compound was used so that the sample was completely surrounded by it. Immediately, the quick-freeze attachment was placed on the top of the muscle and held for about two and a half to three minutes. 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. Sections were transferred to the surface of the pre-cooled microscope slides kept in the cryostat and thawed immediately. The sections were dried at the room temperature for about half hour and this fixed the sections to the slides uniformly well. Within an hour after sectioning, the sections were stained.

Plate I-A

Tracing of the Longissimus Dorsi Muscle Showing the Medial Position of Sampling

Plate I-B

A Camera Lucida Drawing of the Outline of a Typical Stained Section Showing the Five Different Locations, where Photomicrographs were Taken

Plate I-A

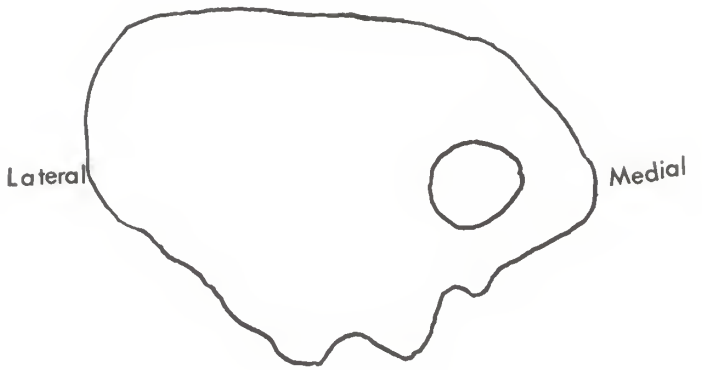
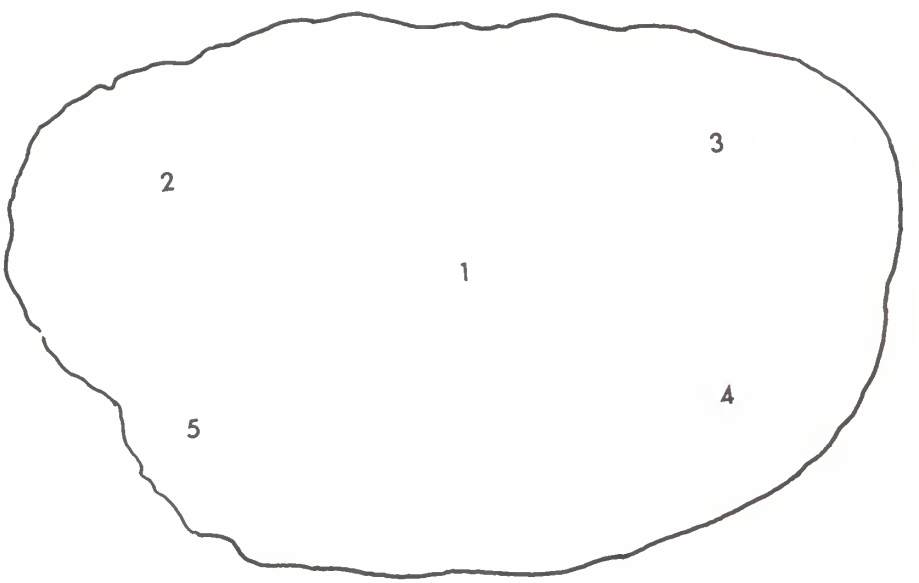


Plate I-B



Staining,Photographing and Counting Technique

A histochemical method utilizing the DPNH diaphorase reaction (a slight modification of the method described by Scarpelli, Hess and Pearse, 1958) was used to identify red, intermediate and white muscle fibers.

A stock solution of 0.2 molar tris (tris-hydroxymethyl amino methane) buffer pH 7.4 was made and stored in the refrigerator at 4° C. A stock solution of 0.5 molar cobaltous chloride was made and stored.

The incubating medium consisted of:

0.2 molar tris buffer pH 7.4	2.5 ml.
0.5 molar cobaltous chloride solution	0.5 ml.
Distilled water	7.0 ml.

To the above mixture was added 2.5 m.g. of M.T.T. (tetrazolium 3-(4.5-dimethyl thiazolyl -2) -2,5- diphenyl tetrazolium bromide) and 20 m.g. of DPNH (Beta -diphosphopyridine nucleotide - reduced form) and mixed well. The incubating medium was freshly prepared just before staining, since it changed color and precipitated within an hour at room temperature. The sections were incubated in the above mixture for one hour at room temperature. After the incubation was over, the reaction was immediately stopped by washing the sections with 10% formol saline for 1-2 minutes. Then the sections were washed in distilled water, dried at room temperature and mounted in glycerine jelly taking care to avoid any air bubbles. The glycerine jelly was made up as follows:

Glycerine	17.5 ml.
Gelatin	2.5 g
Phenol	A few crystals
Distilled water	15.0 ml.

The solids were dissolved by gentle warming in a water bath at 55° C. Before mounting the stock glycerin jelly was liquified in a water bath at 55° C.

Photomicrographs of the stained sections were taken on a 35 mm black and white Kodak Panatomic-X film, ASA 32. A 35 mm Honeywell Pentax H2V camera was attached to a Bausch and Lomb Dynazoom photo-binocular microscope with built-in illumination. Using a 40X objective and a 10X eyepiece, photomicrographs of five different areas (plate I-B) selected at random, were taken. A 1/60 sec. exposure time was used. The area photographed was calibrated with a stage micrometer and was found to be 1.8 x 0.7 mm. Enlarged prints were made using a Simmon Omega enlarger.

The red, intermediate and white fibers (plate II) were counted and recorded. A thumb tack was soldered to a mechanical counter. Each individual fiber counted within a type was punched with the thumb tack. This ensured that all the fibers in each of the categories were counted and also eliminated the possible error of recounting an already counted fiber. Percentages of red, intermediate and white fibers of the total number of fibers were calculated.

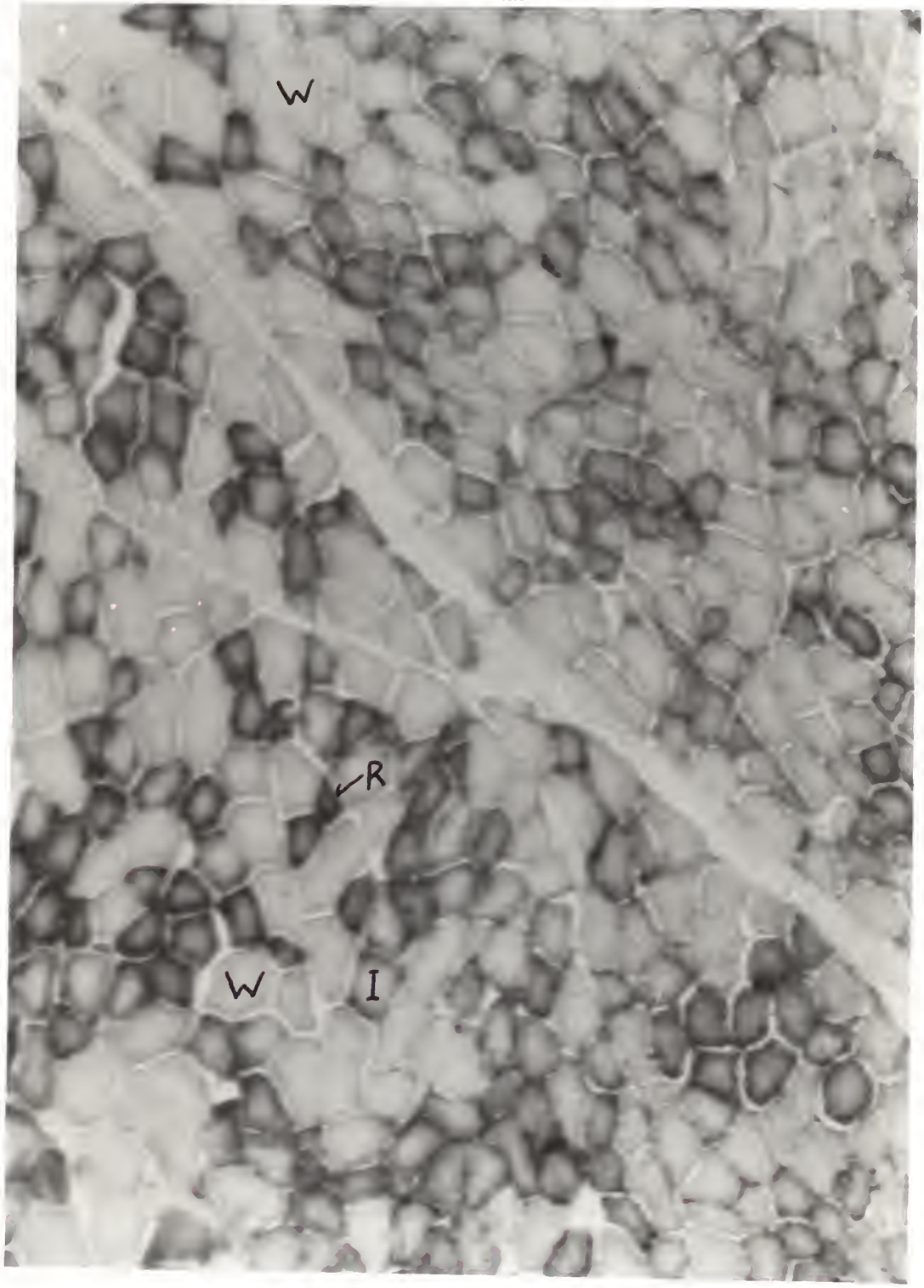
Composition and Reflectance Data

Data regarding the protein content, moisture, ether extract, fiber diameter and sarcomere lengths of the

Plate II

A Photomicrograph of a Typical Stained Section of the Bovine Longissimus Dorsi Muscle Showing Red (R), Intermediate (I) and White (W) Fibers

PLATE II



longissimus dorsi muscle of the same steers was available (Covington, 1967). The samples of longissimus dorsi muscle for the above study were collected from the right side at the ninth thoracic vertebra. Standard AOAC (1960) methods were used, to determine nitrogen (KJeldahl), ether extract (Soxhlet) and dry matter (vacuum oven). Sarcomere length was measured after extracting fibers with 0.02 M KCl solution in a waring blender at slow speed and measuring with an eyepiece micrometer using a Bausch and Lomb phase contrast microscope with the oil immersion objective. Fiber diameter was measured, after separating the fibers from the formol fixed sample by slow blending in a waring blender for 30 seconds, using a Bausch and Lomb microscope and an eyepiece micrometer.

Percent reflectance values of the longissimus dorsi muscle of the same carcasses were obtained from another parallel study (Allen, 1967). The muscle samples were obtained from the left side at the eight thoracic vertebra. The longissimus dorsi muscle was cut transversely and the surface was used to measure the percent reflectance in a Bausch and Lomb spectronic 600 recording reflectance spectrophotometer. Zero time reflectance values were obtained by placing a freshly sliced sample in the instrument and recording the scans at 474 mu. The samples were wrapped in cellophane paper and sealed. The percent reflectance values after 48 hours were recorded at wavelengths of 525 mu, 571 mu and 610 mu.

Statistical Analysis

Data on percentages of red, intermediate and white fibers were analyzed by analysis of variance. If a significant variance ratio was

calculated, Duncans Multiple Range Test was used to determine where the difference existed.

Simple correlation coefficients were calculated between percentage of red, intermediate and white fibers with protein, ether extract moisture, fiber diameter, sarcomere length and reflectance values used.

RESULTS AND DISCUSSION

Effects of Maturity and Marbling on the Percentages of Red, Intermediate and White Fibers

A summary of marbling-maturity effects on bovine longissimus dorsi muscle red, intermediate and white fiber percentages is given in Table I.

Table I

The effect of maturity and marbling on percentages of red, intermediate and white fibers in bovine longissimus dorsi muscle

Marbling	Maturity	Group means of percentages of skeletal muscle fibers counted		
		Red fibers	Intermediate fibers	White fibers
Small	A	28.91[a]	19.95	50.78
Small	AB	33.79[ab]	20.36	45.93
Small	B	34.05[ab]	19.33	46.85
Moderate	A	36.39[b]	16.03	47.76
Moderate	AB	30.92[ab]	20.30	48.96
Moderate	B	29.65[a]	18.76	51.84
F value (analysis of variance)		6.08**	2.38	2.03
	A	32.65	17.99	49.27
	AB	32.35	20.33	47.45
	B	31.85	19.05	49.34
F Value (analysis of variance)		0.10	2.97	0.54
Small		32.24	19.88	47.85
Moderate		32.32	18.36	49.52
F Value (analysis of variance)		0.00	3.71	0.97

** $P < .01$.
All means with identical superscript are not significantly different.

The results showed that there was no significant difference between the small and moderate marbling groups in the mean percentage of different fiber types. The difference between marbling level in percent intermediate fibers approached significance, with a tendency toward a higher percentage of intermediate fibers in the small marbling group.

No significant difference was found between the maturity groups in the mean percentages of different fiber types. This disagrees with the information presented by Waldman (1967) who found a lower proportion of red fibers in bovine muscle from animals with greater weight or age. However, his study probably encompassed a greater range in animal maturity. Perhaps his results are misleading since number of carcasses sampled was very small.

A highly significant interaction was calculated between maturity and marbling with respect to percentage of red fibers. The mean percentage of red fibers showed a tendency to increase with increasing maturity in the small marbling level. Within the moderate marbling group, and contrary to findings within the small marbling group, the mean percentage of red fibers had a tendency to decrease with increasing maturity. No significant interaction was calculated in the mean percentages of intermediate and white fibers.

Diphosphopyridine nucleotide (DPN) diaphorase activity of the muscle fibers was used to differentiate and identify the red, intermediate and white fibers in the bovine longissimus dorsi muscle in this study.

DPN diaphorase is a flavo-protein enzyme capable of transferring hydrogen and electrons from the reduced coenzyme (DPNH) to the tetrazolium salt. DPNH, a coenzyme, was used as the substrate for the DPN

diaphorase. The mechanism involved in the staining reaction was the direct transfer of electrons and hydrogen from the reduced pyrridine nucleotide to the diaphorase which in turn transferred them to the tetrazolium salt (MTT was used in this study) reducing it to an insoluble formazan. The localization of this formazan is predominantly intramitochondrial. This DPNH diaphorase reaction was used to indicate mitochondrial activity in the muscle fibers. Since the red fibers are known to be high in mitochondrial activity and possess high activity of the tri carboxylic acid cycle (TCA) than the white fibers, this particular reaction was selected to differentiate the total red and white fibers in the muscle in preference to Sudan Black B Method used by various workers. Sudan Black B is a selective lipid stain, staining phospholipids preferentially to neutral lipids (Pearse, 1960). In fact, Sudan Black B staining method described by many workers was tried, but in the samples preserved for about six months, no reaction was noticed, while DPNH stain made clear distinction between the different fiber types in the muscle.

It is suggested from the results of this study, that the red (smaller) fibers contain more mitochondria and enzymes of the TCA cycle and are capable of utilizing lipids for energy than the white fibers. This characteristic is reflected histochemically by greater DPNH activity of the red fibers as evidenced by darker staining of smaller fibers.

The results of this study point out that the two marbling levels and the maturity groups have no effect on the red to white fiber ratios of the bovine longissimus dorsi muscle. On the other hand, within the small marbling level, the percentage of red fibers had a tendency to

increase with increasing maturity and also had a tendency to decrease with increasing maturity in the moderate marbling. Cosmos (1966) speculated on the basis of the diversity of enzymatic responses of adult muscles, that the different types of muscle fibers merely reflected the stages of development of one common fiber type. With maturation, some continued to differentiate into fibers specialized for a specific function, others continued to grow with no alteration in a specific enzyme response. Thus he argued that the white fibers are highly specialized cells adapted to anaerobic functions.

The results of this study are in partial agreement with the results of Waldman (1967) who observed that the red fibers tended to decrease with increasing age. However, his results are based on only ten carcasses but a wider range of maturity, that is, from birth to a live weight of 1300 lbs.

The percentage of red, intermediate and white fibers in the bovine longissimus dorsi muscle of individual carcasses of different maturity groups and marbling levels included in this study are presented in Table I of the appendix.

The red fibers appeared dark in color and stained more uniformly while the white fibers did not take any stain. The intermediate fibers had a staining reaction intermediate between the red and white fibers. These results substantially agree with the reports of Bullard (1912), Ogata (1958), Nachmias and Padykula (1958), Jinnai (1960), Dubowitz and Pearse (1960) and Engel (1962).

Relationships to Composition

Simple correlation coefficients between red, intermediate and white fibers and muscle composition, fiber diameter and sarcomere length of

the longissimus dorsi muscle are presented in Table II.

Table II

Correlation coefficients between red, intermediate and white fibers of bovine longissimus dorsi muscle, and the composition, fiber diameter and sarcomere length

Variable	Percent red fibers	Percent intermediate fibers	Percent white fibers
Protein	0.009	0.222	-0.115
Ether extract	-0.043	-0.170	0.120
Moisture	-0.042	0.159	-0.038
Fiber diameter	-0.119	-0.068	0.139
Sarcomere length	-0.069	-0.279*	0.200

* Significant at $P < .05$.

No significant relationship was noted between protein content of the muscle and percentage of different fiber types studied.

No significant correlation was observed between the percentages of red fibers ($r = .043$), intermediate fibers ($r = -.170$) or white fibers ($r = .120$) and the ether extract of the muscle. This is in general agreement with the work of Beecher et al. (1965).

The relationship between moisture percentage and percentage of each type of fiber was not significant.

Protein, fat or moisture content in this study did not appear to be related to the ratios of different fibers in the longissimus dorsi muscle.

Relationships to Fiber Diameter and Sarcomere Length

Fiber diameter was not significantly related to percentages of red, intermediate and white fibers. However, a tendency was noted for a slight decrease in fiber diameter with increase in the percentage of red fibers ($r = -.119$).

A significant, but low correlation was calculated between sarcomere length and percentage of intermediate fibers. This is not in agreement with the results of Beecher et al. (1965) in porcine muscle. They observed a slight increase in post rigor sarcomere length of "red" muscles compared to "white" muscles. Since longissimus dorsi muscle is classified as a "white" muscle, the percentage of white fibers was greater than the percentage of red fibers in this muscle. In the present study, a slight decrease in sarcomere length was observed as the percentage of red fibers increased. Until more "red" and "white" bovine muscles are studied, it is not possible to generalize any statements regarding the influence of red and white fiber ratios on the sarcomere length. The ideal technique would be to determine type of fiber on the same fibers measured for sarcomere length rather than sample the muscle grossly for both sarcomere length and fiber type.

Relationships to Color Reflectance

Simple correlations between red, intermediate and white fibers and color reflectance values of bovine longissimus dorsi muscles are presented in Table III.

Table III

Correlation coefficients between percent red, intermediate and white fibers of bovine longissimus dorsi muscle and color reflectance

Percent reflectance at	Percent red fibers	Percent intermediate fibers	Percent white fibers
Zero time, 474 mu	-0.167	0.047	0.126
48 hours, 525 mu	-0.257*	0.092	0.186
48 hours, 571 mu	-0.219	0.092	0.152
48 hours, 610 mu	-0.327*	0.005	0.290*

* $P < .05$.

At zero time, the percent reflectance of the surface of longissimus dorsi muscle at the 474 mu wave length was not significantly related to the percentages of different fibers. However, there was a slight nonsignificant decrease in percent reflectance ($r = -.167$) with increase in percent red fibers. At zero time, myoglobin would be in the non-oxygenated and non-oxidized state. Poor relationships may be partially due to selection of wrong wave length. Perhaps those in red portion of visible spectrum are more meaningful.

At 48 hours, there was a significant ($P < .05$) decrease in the percent reflectance at 525 mu wave length, the isobestic point for myoglobin, metmyoglobin and oxymyoglobin, with an increase in percent red fibers ($r = -.257$). As the proportion of red fibers increased, the percent reflected light at this wavelength decreased, indicating a greater darkness of color. The relationship between the percentages of intermediate and white fibers and the percent reflectance at this wave length was not significant.

At 48 hours, the percent reflectance at 571 mu, the isobestic point for myoglobin and oxymyoglobin, was not significantly associated with the percentages of different fibers in the muscle. However a slight nonsignificant decrease in percent reflectance with an increase in percent red fibers (r = -.219) was observed.

At 48 hours, a significant decrease in percent reflectance at 610 mu wave length, the orange region, was noted as the percentage of red fibers increased (r = -.327) and also there was a simultaneous significant increase in percent reflectance value with increase in percent white fibers (r = .290). This would indicate that reflected light at 610 mu was increased as the proportion of white fibers increased, and decreased as red fiber percentage increased.

Myoglobin is the oxygen storing pigment primarily responsible for the red color in muscle (Lawrie, 1953). Barly work by Ran Vier (1880) showed that the red muscles of rabbit had a higher density of capillaries than light ones. It has also been established by Denny-Brown (1929), and Ogata (1958), that the muscle color is dependent upon the proportion of red fibers which the muscle contains. Muscles darker in color are shown to have more red fibers. Beecher et al. (1965) have shown that the red muscles contained significantly higher concentration of myoglobin, than the white muscles.

High concentration of this pigment myoglobin is usually found in muscles of high physiological activity (Lawrie, 1950). The physiologically active muscles are known to contain more red fibers and these red fibers are shown to be high in oxidative enzymes and cytochrome systems (Lawrie, 1953). This suggests that the red fibers also contain

more myoglobin. These points emphasize that the color of vertebrate muscles is caused in part by the concentration of myoglobin, cytochromes and flavin enzymes, such as DPNH diaphorase, besides the influence of pH, ether extract, moisture and water holding capacity of muscle.

As the myoglobin content increases, the muscles become darker in color and therefore the percent color reflectance decreases. Generally it was expected that more reflectance would indicate lighter color and less myoglobin and also related directly to proportion of white fibers and inversely to proportion of red fibers. In the present study, the results indicated that as the percentage of red fibers increased, the percent color reflectance at the wave lengths studied decreased, showing that the red fibers definitely contributed to the color of the muscle.

SUMMARY

Longissimus dorsi muscle samples of sixty bovine carcasses were used in this study. Two levels of marbling, small and moderate, and three maturity groups, $A^{-}A^{o}$, $A^{+}B^{-}$, and $B^{o}B^{+}$, which correspond to chronological ages of approximately 12-18, 18-30 and 30-42 months respectively were included in this experiment. The two marbling levels were represented equally within each maturity group.

DPNH diaphorase staining method was used to distinguish the fiber types in the muscle.

The two marbling levels and maturity groups, independently, had no effect on the red to white fiber ratio of the muscle.

A significant interaction was calculated between marbling and maturity with respect to proportion of red fibers. Red fibers had a tendency to increase within the small marbling with increasing maturity and had a tendency to decrease with increasing maturity in the moderate marbling group.

Protein, fat or moisture did not appear to be related to the ratios of different fibers.

Fiber diameter was not very much associated with the percentage of fiber types, but a very slight decrease in fiber diameter with increase in percent red fibers was noticed ($r = -0.119$).

A significant but low correlation was calculated between sarcomere length and percentage of intermediate fibers ($r = -0.279$).

At time of exposure to oxygen the percent reflectance of the surface of the longissimus dorsi muscle at 474 mu wave length was not significantly associated with the percentages of different fibers. A

slight nonsignificant decrease in percent reflectance ($r = -0.167$) with increase in percent red fibers was noticed.

At 48 hours after exposure to oxygen in the air, a significant decrease in the percent reflectance at 525 mu was observed with increase in percent red fibers ($r = -0.257$).

At 48 hours the percent reflectance at 571mu was not significantly related to the percentages of different fibers in the muscle.

At 48 hours a significant decrease in percent reflectance at 610 mu, the orange region, was noticed as the percentage of red fibers increased ($r = -0.327$) and also there was a simultaneous significant increase in percent reflectance with increase in percent white fibers ($r = -0.290$). Thus it was shown that the proportion of red fibers contributed towards the color of the muscle, since when red fiber percentage increased, reflectance decreased indicating that muscle color was a darker red.

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to Dr. D. H. Kropf, major professor, for his guidance and assistance in planning this study and preparing this manuscript, also to Dr. H. J. Tuma and Dr. D. C. Kelley for their assistance in completion of this manuscript. I also wish to thank Dr. G. H. Cardinet, Department of Anatomy for his advice on the staining technique; and Dr. B. E. Brent for his advice and assistance with photographic techniques used in this study.

Appreciation is also extended to Dr. A. D. Dayton, Department of Statistics for his help with the statistical analysis, to Mr. C. Allen and Mr. D. Covington for making available some of their data, and to Mr. D. L. Grant and other graduate students for their help and interest in this study.

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APPENDIX

Percentages of the three types of fibers in different maturity and marbling groups

Maturity	Marbling	ID No.	% red fibers	% intermediate fibers	% white fibers
A	Small	005	24.7	18.8	56.5
		006	25.0	17.9	57.1
		013	36.2	20.5	43.3
		027	28.9	21.8	49.3
		028	23.8	18.3	57.9
		029	30.7	21.8	47.5
		030	27.7	22.1	50.2
		031	26.8	19.3	53.4
		033	39.1	18.9	42.0
		034	29.1	20.2	50.8
A	Moderate	032	27.9	20.9	51.3
		035	30.6	16.4	53.0
		047	34.3	12.7	53.0
		048	34.4	23.9	41.7
		049	36.5	14.1	49.4
		050	35.5	13.7	50.8
		051	34.8	17.8	47.5
		058	41.2	13.5	45.3
		059	41.0	12.6	46.4
		060	46.5	12.9	40.6

APPENDIX--Continued

Percentages of the three types of fibers in different maturity and marbling groups--Continued

Maturity	Marbling	ID No.	% red fibers	% intermediate fibers	% white fibers
AB	Small	001	37.4	20.0	42.7
		004	32.2	22.3	45.5
		008	28.3	17.8	53.9
		019	50.3	22.6	27.1
		020	27.7	17.2	55.1
		024	33.4	21.2	45.4
		025	33.0	24.9	42.1
		026	27.6	20.9	51.6
		044	34.0	19.6	46.4
		045	34.0	16.3	49.7
AB	Moderate	002	25.0	16.4	58.6
		003	26.4	17.5	56.1
		007	27.8	21.4	50.8
		015	30.7	21.4	47.9
		017	33.3	21.3	45.4
		018	33.1	18.0	48.8
		036	30.2	23.0	46.8
		037	32.3	24.3	43.4
		043	36.5	18.0	45.6
		046	33.1	21.3	45.6

APPENDIX--Continued

Percentages of the three types of fibers in different maturity and marbling groups--Continued

Maturity	Marbling	ID No.	% red fibers	% intermediate fibers	% white fibers
B	Small	011	26.0	17.0	57.0
		016	29.9	22.0	48.0
		021	21.8	16.9	61.3
		039	32.6	28.1	39.3
		040	37.9	17.9	44.1
		041	37.4	21.0	41.6
		042	36.8	23.9	39.4
		052	35.5	16.4	48.1
		053	38.1	13.4	48.5
		054	42.0	17.0	41.0
B	Moderate	009	31.1	19.4	49.4
		010	28.7	18.1	53.2
		012	34.5	20.9	44.7
		014	29.4	17.0	53.5
		022	20.9	17.0	62.1
		023	22.7	18.4	59.0
		038	39.3	23.3	37.3
		055	34.4	16.1	49.5
		056	31.8	20.9	47.3
		057	21.4	16.1	62.6

THE EFFECT OF BEEF MATURITY AND MARBLING UPON RED-WHITE MUSCLE FIBER RATIOS AND RELATED PROPERTIES

by

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An Abstract of a Master's Thesis

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Husbandry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1968

The effect of beef maturity and marbling upon the red-white fiber ratios and other related properties of bovine longissimus dorsi muscle was studied.

Sixty beef steer carcasses of three maturity groups and two marbling levels were selected for this study. The right side longissimus dorsi from the seventh thoracic vertebra was used. Half inch cores at the medial position of the muscle were cut from each of the sixty steaks.

The samples of the muscle were sectioned in a cryostat at -25° C. They were stained for DPNH reaction. The red and white fibers were identified as dark and clear fibers respectively. The intermediate fibers were intermediate in size and staining reaction between the red and white fibers. Thus it was shown that three types of fibers; red, intermediate and white existed in bovine longissimus dorsi muscle.

The two marbling levels and maturity groups, independently, had no effect on the red to white fiber ratio of the muscle.

A significant interaction was calculated between marbling and maturity with respect to proportion of red fibers. Red fibers had a tendency to increase within the small marbling with increasing maturity and had a tendency to decrease with increasing maturity in the moderate marbling group.

Protein, fat or moisture did not appear to be related to the ratios of different fibers.

Fiber diameter was not very much associated with the percentage of fiber types, but a very slight decrease in fiber diameter with increase in percent red fibers was noticed ($r = -0.119$).

A significant but low correlation was calculated between sarcomere length and percentage of intermediate fibers ($r = -0.279$).

At time of exposure to oxygen the percent reflectance of the surface of the longissimus dorsi muscle at 474 mu wave length was not significantly associated with the percentages of different fibers. A slight nonsignificant decrease in percent reflectance ($4 = -0.167$) with increase in percent red fibers was noticed.

At 48 hours, after exposure to oxygen in the air, a significant decrease in the percent reflectance at 525 mu was observed with increase in percent red fibers ($r = -0.257$).

At 48 hours the percent reflectance at 57 mu was not significantly related to the percentages of different fibers in the muscle.

At 48 hours a significant decrease in percent reflectance at 610 mu, the orange region, was noticed as the percentage of red fibers increased ($r = -0.327$) and also there was a simultaneous significant increase in percent reflectance with increase in percent white fibers ($r = -0.290$). Thus it was shown that the proportion of red fibers contributed towards the color of the muscle, since when red fiber percentage increased, reflectance decreased indicating that muscle color was a darker red.