437

February 22, 1973

To the Graduate Council:

I am submitting herewith a thesis written by Jimmy Ted Keeton entitled "A Histological Study of Bovine Longissimus Muscle Fiber Characteristics as Related to Performance and Carcass Traits." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Curtis C. Melton
Major Professor

We have read this thesis
and recommend its acceptance:

Robert R. Shrode

Wm. R. Backus

Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

# A HISTOLOGICAL STUDY OF BOVINE LONGISSIMUS MUSCLE FIBER CHARACTERISTICS AS RELATED TO PERFORMANCE AND CARCASS TRAITS

---

A Thesis

Presented to

the Graduate Council of

The University of Tennessee

---

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

Jimmy Ted Keeton

March 1973

## ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude and appreciation to the following individuals who have made this thesis possible.

To Dr. Curtis C. Melton, major professor, for his personal concern, guidance and assistance with problem areas in the study.

To Dr. William R. Backus for his knowledgable insight, reviewing of the manuscript and serving as a committee member.

To Dr. Robert R. Shrode for his influence and example of character, for reviewing the manuscript and serving as a committee member.

To Dr. Charles S. Hobbs and Dr. Samuel L. Hansard, deceased and present department head, respectively, for making this study possible.

To fellow graduate students James Cloar, Wayne Compton and Sam Winfree for their assistance in collecting performance and carcass data.

To Dr. R. L. Murphree, Dr. C. C. Collins, Dr. C. A. Shivers, Dr. J. D. Smalling, Dr. G. M. Merriman and Mrs. Gladis Reed for their technical assistance in various phases of histological tissue preparation.

To my parents for their encouragement to pursue a higher level of education.

To Jo Harriet, my wife, for her abiding love, understanding and patience throughout this endeavor.



1063567

ABSTRACT

Thirty-four bulls, 25 Angus and nine Hereford, ranging in age from 15 to 17 months and varying in weight from 362 to 485 kg were fed to a constant subcutaneous fat thickness of 10.0 mm. Longissimus dorsi muscle biopsies were removed from the 12th and 13th rib region and stained histologically for ATPase activity to study the relationships of muscle fiber characteristics as related to performance and carcass traits.

One-hundred muscle fibers from each sample were counted, measured and classified into type I, red, and type III, white, fibers. Breed muscle fiber means were compared using the T-test and observed to be non-significant ($P > .01$). Number of red fibers was significantly ($P < .05$) correlated with final fat thickness, initial weight, kidney fat and rib eye area per hundred weight of carcass. Number of white fibers was observed to be negatively correlated with the number of red fibers. Muscle fiber area measurements were not significantly correlated with performance traits. Mean red fiber areas increased significantly with rib eye area per day of age (0.43, $P < .01$) and mean white fiber areas increased significantly with rib eye area per hundred weight of carcass (0.40, $P < .05$). Muscle fiber area measurements significantly ($P < .05$) increased with increasing rib eye area. Yield grade was correlated ($P < .05$) with total red fiber area (-.35) and total white fiber area was correlated with dressing percent (0.52). Percent red and white fiber areas were significantly correlated with yield grade, kidney fat, rib eye area per hundred weight of carcass

and rib eye area per day of age. Muscle fiber characteristics as the independent variables were fitted in various regression models, but their predictive effectiveness, even though significant, was poor.

TABLE OF CONTENTS


| CHAPTER | | PAGE |
|---|---|---|
| I. | INTRODUCTION | 1 |
| II. | REVIEW OF LITERATURE | 3 |
| | Muscle Biopsy | 3 |
| | Muscle Relaxation | 6 |
| | Histological Characteristics of Skeletal Muscle | 8 |
| | Differentiation of muscle fiber types | 8 |
| | Muscle fiber size | 13 |
| | Capillary distribution | 16 |
| III. | EXPERIMENTAL PROCEDURE | 19 |
| | Source of Data | 19 |
| | Biopsy Technique | 19 |
| | Histological Technique | 20 |
| | Carcass Data | 22 |
| | Statistical Analysis | 23 |
| IV. | RESULTS AND DISCUSSION | 25 |
| | Performance Traits | 25 |
| | Number of fibers | 28 |
| | Fiber area | 31 |
| | Carcass Traits | 34 |
| | Number of fibers | 34 |
| | Fiber area | 35 |
| | Summary | 42 |
| LITERATURE CITED | | 44 |

CHAPTER PAGE

APPENDIXES . . . . . 50

Appendix A . . . . . 51

Appendix B . . . . . 53

Appendix C . . . . . 54

Appendix D . . . . . 55

Appendix E . . . . . 56

VITA . . . . . 58

LIST OF TABLES


| TABLE | | PAGE |
|---|---|---|
| 1. | Means and Standard Errors of Selected Performance Traits, Carcass Traits and Histological Muscle Fiber Characteristics | 26 |
| 2. | Coefficients of Correlation of Performance Traits and Histological Muscle Fiber Characteristics | 30 |
| 3. | Multiple Regression Equations - Partial Regression Coefficients Predicting Dependent Variables | 32 |
| 4. | Coefficients of Correlation of Carcass Traits and Histological Muscle Fiber Characteristics | 36 |
| 5. | Multiple Regression Equations - Partial Regression Coefficients Predicting Dependent Variables (Red Fiber Characteristics) | 38 |
| 6. | Multiple Regression Equations - Partial Regression Coefficients Predicting Dependent Variables (White Fiber Characteristics) | 40 |
| 7. | Coefficients of Correlation of Carcass Traits and Muscle Fiber Characteristics | 55 |
| 8. | Multiple Regression Equations - Partial Regression Coefficients Predicting Dependent Variables (Red Fiber Characteristics and White Fiber Characteristics) | 56 |

# CHAPTER I

## INTRODUCTION

One of the goals of current research in animal science is the development of efficient beef animals which yield carcasses containing a large proportion of primal retail cuts. Various aspects of bovine performance and cutability have been studied in an effort to predict the yield of palatable beef.

The selection of gentically superior sires, feeding balanced rations and improved herd management programs have contributed substantially toward improved performance and carcass traits. The generation interval of beef animals is lengthy and allows for only gradual herd improvement. Current evaluation programs of potential herd sires could be greatly enhanced if methods were available to predict selected bovine traits at an early age, thus reducing the generation interval.

Muscle biopsies have been shown to be an effective sampling technique for histochemical and histological examination of muscle tissue without apparent harm to an animal. Certain histological characteristics, such as, muscle fiber area, diameter and red-to-white fiber ratios have been investigated and observed to be associated with carcass quality and quantity.

Growth and palatability traits are considered to be appreciably heritable, and the potential possibility of using muscle biopsies as an additional criteria for selection of herd replacements is worthy of further investigation.

This study was undertaken to identify relationships between histological muscle fiber characteristics and selected quantitative and qualitative bovine traits.

# CHAPTER II

## REVIEW OF LITERATURE

### I. Muscle Biopsy

The biopsy technique has been used to examine subcutaneous adipose tissue and skeletal muscle samples of various species. Merkel et al. (1954) using porcine animals, devised a biopsy technique whereby muscle samples could be removed for histological and chemical analysis. The procedure was described as follows. Animals were restrained in lateral recumbency and anesthetized by an intraperitoneal injection of a 10% aqueous solution of magnesium sulfate and chloral-hydrate. An incision 1 1/2 to 3 inches long was made parallel to and 1 to 2 inches laterally from the vertebral column, posterior to the last rib. A tissue sample of the longissimus dorsi muscle 1 1/2 x 3/4 inch was removed, sulfa-urea power applied to the wound and the skin sutured with umbilical suture tape.

Schmidt, Zuidam and Sybesma (1972) employed a similar procedure to study the l. dorsi of swine and ultimately identify high quality pork carcasses prior to slaughter. Twenty Dutch Landrace pigs were anesthetized with an intravenous injection of thiopentone sodium (750 mg/animal) after being placed in a canvass sling. The biopsy site was shaved and disinfected prior to the surgical removal of a three-gram longissimus muscle sample in the area of the last thoracic vertebra. A second experiment involved only local anesthetization of the skin by placing a precooled container of liquid nitrogen on the

skin for approximately 10 seconds. A one centimeter incision through the skin was sufficient to remove a 200-mg muscle sample with "Koffler" tongs, a device used in nose surgery for humans. Resections of the biopsy sample required about five seconds with little or no apparent harm to the animal.

Muscle biopsy samples of the l. dorsi in the region of the 12th rib were taken from mature rams, yearling ewes and lambs by Spurlock, Bradford and Wheat (1962) with no infection or disability resulting from the operation.

Everitt and Carter (1961) used an instrument similar to a cork borer for extracting samples from the semitendinosus muscle of cattle. After the application of an epidural anesthetic, an incision was made through the skin and the cutting edge of the borer was pushed through the fat and into the muscle with a screwing action. The distal surface of the sample was then severed by a scalpel.

The longissimus and semitendinosus were successfully sampled using a muscle tissue extractor (MTE) designed and constructed by Harris and Bennett (1970). The MTE was attached to a Remington hand stunner and used to extract a core sample 5.03 to 6.30 x 1.78 to 1.02 cm with only a slight amount of bruising. Anesthetization of the biopsy area was accomplished by placing a container of liquid nitrogen on the skin for 20 to 25 seconds.

Wilson et al. (1955) obtained samples of semimembranosus muscle from extradurally anesthetized cattle by simply opening the skin and cutting out the desired muscle area. This biopsy method was shown to be practical and non-harmful to the beef animal. However,

Link et al. (1967) observed fatty degeneration within muscle tissue from which biopsies had previously been removed. The cause of fatty degenerated muscle was concluded to be accidental denervation during the biopsy operation using an instrument described by Everitt and Carter (1961). Fatty degeneration of muscle tissue was not observed when biopsy samples were removed with a scalpel.

Adaptation of the biopsy techniques discussed previously have been used by others to investigate the histological and histochemical aspects of muscle tissue. Melton (1970) and Dikeman et al. (1972) utilized the biopsy method to determine the usefulness of biopsy analysis in predicting bovine muscle quality. Twenty-one Hereford bulls representing five sire groups were biopsied according to the following procedure. An intramuscular tranquilizer was given 15 minutes prior to surgery and the surgical site sanitized. A 12-cm incision was made at the 13th rib, three-fifths of the distance from the medial to lateral edge of the longissimus muscle and parallel to the midline. Ten cubic centimeters of sodium pyrophosphate (0.05 M) was injected into the muscle as a relaxant, and two parallel incisions 7.0 x 2.0 cm were made. Two fixed Kelley forceps held the biopsy sample in place to prevent contraction of the muscle sample upon removal from the animal. A 7.0 x 2.0 x 2.5 cm tissue sample was removed and rinsed in sodium phrophosphate solution (10 seconds), and cross-sections were made for histological analysis. Samples were then cryogenically frozen in isopentane and stored on dry ice until sectioned. A bacteriocidal compound, topazone, was applied and the wound closed with interrupted sutures. No adverse post-operative effects were reported by these workers.

The muscle biopsy technique, according to published reports, appears to be a proven and feasible means of obtaining muscle tissue samples without adverse or harmful effects to the animal.

## II. Muscle Relaxation

The striated muscle fiber contractile structures have been described by Hanson and Huxley (1953) in terms of an "interdigitating filament model" consisting of thick myosin and thin actin myofilaments located in the A and I band regions of the myofibrils, respectively.

Straub (1942) extracted a protein complex, antomyosin, consisting of two complex proteins, action and myosin. Szent-Györgyi (1942) demonstrated that threads of actomyosin would contract in solutions containing adenosine triphosphate (ATP), indicating that actomyosin must be present along the whole length of the muscle for shortenings of 70 to 80 percent to occur. Huxley (1960) observed "cross-links" between primary and secondary myofilaments which were reported to be sites of adenosine triphosphatase (ATPase) activity and of actin-myosin combination.

Englehardt and Ljubimova (1939) isolated the enzyme, ATPase, contained in myosin, which catalyzes the dephosphorylation of ATP. In a medium containing ATP, Weber (1951) immersed glycerinated muscle fibers and observed the development of tension in all fibers. When the ATP was removed, the contractile structure "set" at a fixed length. Weber and Portzehl (1954) allowed muscle fibers to develop tension in ATP and then added Salyrgan, a substance known to inhibit myosin ATPase, with a large decrease in tension occurring. A similar experiment

was conducted by Marsh (1951, 1952) in which ATP plus a "relaxing" factor replaced an ATP solution, which previously had produced tension and, in effect, caused partial inhibition of ATPase activity. Relaxation of the muscle fibers resulted in the presence of ATP, indicating a dissociation of the actomyosin complex. Various substances such as $2.5 \times 10^{-2}$M sodium pyrophosphate, triphosphate, adenosine diphosphate (ADP) and others, which inhibit ATPase activity, cause fibers to relax in the presence of ATP (Weber and Portzehl, 1954). Huxley (1960) concluded that the "cross-bridges" in the interdigitating filament model represented sites of ATPase activity and of actin-myosin combination. Dephosphorylation of ATP caused the actin and myosin filaments to slide past one another until ATP splitting was stopped, thus causing contraction to occur. If ATP remained without splitting, the actomyosin complex dissociated, allowing relaxation of the fiber.

Studies by Maruyama and Gergely (1962), Levy and Ryan (1966) and Weiner, Pearson and Schweigert (1969) suggest that $Mg^{++}$ inhibits ATPase activity and assists ATP in keeping the actin-myosin complex dissociated. Levy and Ryan (1966) indicated that contraction requires the reaction of ATP and $Mg^{++}$ at two sites with only one of these being the site for ATP hydrolysis. Weiner et al. (1969) utilized eight rabbits to determine the turbidity, viscosity and ATPase activity of fibrillar protein extracts. The rate of ATP hydrolysis was decreased by the addition of $MgCl_2$, which inhibited the ATPase activity of the myosin moiety. Additional results suggested that actomyosin from muscle in rigor was more easily dissociated than actomyosin from pre-rigor muscle.

Weber and Portzehl (1954), Martonosi and Meyer (1964), Weiner et al. (1969, Melton (1970) and Dikeman et al. (1972) have shown sodium or potassium pyrophosphate to behave much like ATP in that pyrophosphate is not enzymatically split by myosin, and it possesses the property of being able to dissociate actomyosin. Muscle fibers, bathed in an ATP medium, developed tension and "set" upon ATP removal by washing (Weber and Portzehl, 1954). Immersion of the fibers into a medium containing $2.5 \times 10^{-2}$M sodium pyrophosphate reduced the tension to a low value near that of a relaxed fiber. Weiner et al. (1969) have shown 0.1 M potassium pyrophosphate in the presence of magnesium to be as effective as ATP in clearing actomyosin solutions. Pyrophosphate samples retained their low viscosity characteristics indefinitely, indicating the possibility of its use as an in vivo muscle relaxant. Melton (1970) anesthetized eight rabbits and excised the biceps femoris after injections of four treatments of magnesium salts. In a second trial the b. femoris of ten rabbits was injected with 0.025 M sodium pyrophosphate prior to biopsy. After a comparison of the two trials, sodium pyrophosphate was found to be effective in maintaining pre-rigor muscle in a relaxed state for an indefinite period of time. Ten cubic centimeters of 0.05 M sodium pyrophosphate were later used to relax bovine longissimus muscle prior to biopsy and histological observation.

## III. Histological Characteristics of Skeletal Muscle

Differentiation of muscle fiber types. Denney-Brown (1929) recognized a mixture of clear and dark muscle fiber types in the early

extra-uterine life of the kitten. Dubowitz (1960) studied abnormally large (100-200μ) dystrophic muscle fibers and noted that they reacted weakly for oxidative enzymes and strongly for phosphorylase, while small atrophic fibers (20-30μ) tended to react conversely. He concluded that normal human muscle was composed of two types of fiber, one of smaller diameter, strong in intramitochondrial oxidative enzymes and weak in phosphorylase, while the larger fiber was its complement in these respects. Fibers of the rat gastrocnemius and soleus were classified histochemically by Stein and Padykula (1962), using glycogen, succinic dehydrogenase (SDH), two kinds of ATPase and esterase constitutients. Three fiber types, A, B and C, were characterized by the distribution of SDH activity. Type C fibers showed heavy subsarcolemmal activity while the type A fibers were rich in glycogen, which did not appear in types B and C. Esterase and ATPase activities indicated that the type A fiber was the classical "white" fiber and that types B and C represented "red" fibers. Ogata and Mori (1964) identified three types of mammalian striated muscle fibers following a histochemical demonstration of oxidative enzyme reactions. The oxidative SDH, diphosphopyridine nucleotide (DPN), DPNH-diaphorase, triphosphopyridine nucleotide (TPN) and TPNH-diaphorase were used to study the muscles of humans, monkeys, cows, pigs, dogs, cats, rabbits, guinea pigs, rats, mice, whales, pigeons, lovebirds, chickens, snakes, lizards, geckos, tortoises, frogs and fish. Large white fibers showed lower activities of oxidative enzymes, while small red fibers showed higher activities, with a "medium fiber" showing activities between red and white muscle fibers. Romanul (1964), using enzymes, classified

eight different types of fiber from serial cross-sections of the gastrocnemius and plantaris of the rat. A comparative histochemical study by Ashmore and Doerr (1971) was performed on chick, mouse, bovine and porcine red and white muscles to determine whether the system of nomenclature for muscle fiber types, described for chick muscle, was applicable to other species. Species differences were apparent in α fiber ATPase reactions after alkali preincubation; however, it was concluded that the terms α red, α white and β red were adequate to describe the types and metabolic states of muscle fibers in all species examined. Research by Cooper et al. (1970) showed fiber types to be undetectable in the newborn pig, but red fibers were distinguishable in pigs one week old. White and intermediate fiber types were clearly detectable in pigs four weeks of age, followed by a mean fiber area increase in white fibers until maturity. Similar work by Ashmore, Tompkins and Doerr (1972), using piglets, lambs and calves, revealed two basic fiber types termed, α and β, based on myosin ATPase activity. Transformation from an initial α "red" (α red) aerobic state of metabolism to an anaerobic state (α white) was primarily concerned with changes in energy producing enzymes. The number of fibers in a muscle remained fixed at birth with β fibers remaining "red" throughout their lifespan. It was suggested that increased muscularity in domestic animals is the result of practices which favor transformation of α red fibers to α white. The previously cited research substantiates the existence of three skeletal muscle fiber types as found by Dubowitz (1963), Wirsen and Larson (1964), Moody and Cassens (1968), Cassens, Cooper and Morita (1969) and Melton (1970).

The metabolism of various muscles and muscle fibers has been studied in an effort to distinguish the interrelationships which would be of value in predicting carcass quality. Lawrie (1952) and Beatty, Peterson and Bocek (1963) and Cassens et al. (1969) demonstrated metabolic pathway differences between red and white muscle fibers of rats and swine. Glycogen concentrations in rat adductor were higher in white fibers immediately after sacrificing, but after two hours incubation, this relationship was reversed. Lactate production and creatine phosphate level was greater in white muscle fiber while aceto-acetic acid $^{14}C$ uptake was greater for the red fiber groups. Farrell and Fedde (1969) examined the structural uniformity throughout the length of four transversus abdominis muscles of male white Leghorn chickens. Myosin ATPase activity was either high or low for an entire fiber. They concluded that skeletal muscle fibers maintain uniform structural and histochemical characteristics along their entire length.

Sreter, Seidel and Gergely (1966) concluded that there were structural differences between red and white muscle myosin as suggested by the higher apparent activation energy of the calcium-activated ATPase reaction catalyzed by red muscle myosin, by the higher rate of inactivation at pH 7.5 to 9.5 and by the slower rate of tryptic digestion of myosin from red muscle. Beecher et al. (1965, 1968) used various muscles from swine (sus domesticus) and determined a significant multiple correlation among myoglobin content, succinic dehydrogenase activity and percent red fibers. Red muscle fibers were found to be aerobic in nature with the reverse being true of the white fibers. The

appearance of myoglobin positive fibers (red) in one-week-old to six-month-old swine was found to be in agreement with Beecher et al. (1968). Moody and Cassens (1968) and Kastenschmidt, Hoekstra and Briskey (1968) postulated that the reciprocal enzyme activities of red and white fibers may represent different phases in a cycle of activity associated with different states of contraction which could explain fibers of intermediate size and activity.

Investigations of skeletal muscle fibers necessitates a method for distinguishing or identifying the various fiber types. In 1939 Gomori and Takamatsu (cited by Barka and Anderson, 1963) independently published a method for the histochemical demonstration of alkaline phosphatase. The sites of ATPase activity were easily identified by the formation of a black to brown precipitate localized generally in the white muscle fibers.

Padykula and Herman (1955a,b) and Padykula and Gauthier (1963), after using cardiac and skeletal muscle fiber and the mitochondria of the kidney tubules, suggested that alkaline phosphatase at pH 9.4 participates in the dephosphorylation of ATP.

Brooke and Kaiser (1969) and Melton (1970) demonstrated the myosin ATPase system associated with type I red fibers to be relatively alkali-labile (pH 8.0 to 10.3) when compared to that associated with type III white fibers, which was acid-labile (pH 4.3 to 5.0). In effect, the characteristic dark stain of white fibers achieved by preincubation in alkali medium, could be reversed simply by incubating in an acid medium.

Muscle fiber size. Muscle fibers, fundamental units of muscle structure, have been studied by various workers in an effort to determine their significance as predictors of carcass traits. Hiner et al. (1953), Joubert (1956), Tuma et al. (1962), Romans, Tuma and Tucker (1965) and Covington et al. (1970) noted that muscle fiber diameter increases with increasing animal maturity. Muscle bundle size of the l. dorsi was found to increase measurably by Cooper et al. (1968) when carcasses of A and B maturity groups were compared to those of E maturity.

Joubert (1956) measured the diameter of 16,450 individual fibers from rabbits, pigs, sheep and cattle and concluded the mean diameter of 100 fibers per sample to be a reliable value and a representative indicator of dispersion in a given muscle at a specific age. Post-natal m. l. dorsi and m. gastracnemius muscle growth was noted to be primarily a function of physiological age rather than chronological age, with the late maturing l. dorsi suggested as being a suitable muscle for estimating carcass muscularity. Melton (1970) measured 100 fibers in five areas of each muscle sample and observed no significant area variation. It was concluded that 100 muscle fibers per sample were representative of a particular muscle.

A large amount of variation in muscle fiber size was found by Swanson, Kline and Goll (1965) to exist among different positions and among different locations within each position of the l. dorsi muscle. The smallest fibers were found over the 12th rib in the lateral position, and fiber size increased anteriorly and posteriorly from there. The largest fibers were located between the 13th

rib and first lumbar vertebra. Low, non-significant correlations (-.15 to -.53) indicated that fiber size was not a good indicator of l. dorsi area. This work was not in agreement with Hiner et al. (1953) who found no significant differences among fiber sizes along the length of the loin. Tuma et al. (1962) noted earlier a significant "position" effect on fiber diameter which decreased from the dorsal to lateral position of the l. dorsi muscle.

Tenderness (resistance to shearing) was shown to be related linearly (0.83) to fiber diameter in the psoas major, chuck, round, neck and foreshank muscles (Hiner et al., 1953). Similar results were obtained by Herring et al. (1967), using stretched l. dorsi muscle, and Cooper et al. (1968) who reported correlations of 0.82 and 0.47, respectively, between tenderness and fiber diameter. Fiber diameter of the l. dorsi was shown by Tuma et al. (1962) to be a poor indicator of tenderness when the effect of animal age was removed. Results of Romans et al. (1965), Covington et al. (1970), Field et al. (1970) and Melton, Dikeman and Tuma (1971) also support this conclusion.

Romans et al. (1965) studied 80 beef ribs representing four maturity levels (A, B, C, D) and two marbling levels (slight and moderate) and concluded that muscle fiber diameters were significnatly ($P < .01$) larger in the moderate marbling group. A trend toward larger fiber diameters was noted for the muscle fibers from the more mature carcasses.

Longissimus muscles from 60 steer carcasses were used by Covington et al. (1970) to study small and moderate marbling groups. Fiber diameters tended to be greater for the moderate marbling level

and increased significantly in size as maturity increased. Percent wavey fibers was significantly correlated (0.45) with shear value. but not with marbling level or maturity group.

Eisenhut et al. (1965) determined the fiber arrangement in l. dorsi of 12 horizontally placed and vertically suspended bovine carcass sides. Fiber angles, from transverse processes, increased from anterior to posterior positions (7th rib to 3rd lumbar) along the loin muscle. The converse was true for fiber angles from the spinous processes. Vertical suspension, compared to horizontal placement, released some of the tension on the l. dorsi and produced shorter sarcomeres.

Moody and Cassens (1968) compared trapezius and l. dorsi muscle samples over the 4th thorscic and 5th lumbar vertebrae of 10 Chester Whites. The trapezius was observed to have a larger proportion (62 percent) of red fibers due to its tonic function as compared to the l. dorsi (30 percent), a typical white muscle with phasic function. Red fibers were noted to be in clumps and surrounded by white fibers. Fibers intermediate for DPNH-TR and amylophosphorylase were observed. Similar work by Cooper et al. (1970) has shown the characteristic clump distribution of red fibers to be present at the same time that differentiation was detectable in young porcine animals. Initial fibers were indistinguishable in the newborn pig longissimus, however, red fibers were detectable in muscles from one-week-old pigs and all fibers were detectable in those from pigs four weeks of age. White fiber area percent gradually increased throughout development

with a corresponding decline in area percent of both red and intermediate fibers.

Mean fiber diameters of six high gaining rats were shown to be larger (44.9μ) than those of six low gaining rats (38.9μ), by Swatland and Cassens (1972), with muscle enlargement in high gaining rats attributed to type III (white) fiber hypertrophy.

Melton et al. (1971) studied histological traits of the longissimus muscle from 21 Hereford bulls representing five sire groups and observed cross-sectional fiber area means for type I (red), type II (intermediate), and type III (white) fibers to be $3060\mu^2$, $4040\mu^2$ and $5170\mu^2$, respectively. They found significant intra-sire coefficients of correlation of type I fiber area with live weight (0.59), with external fat thickness (0.75), with yield grade (0.67), with marbling score (0.49) and with U.S.D.A. quality grade (0.54). Also found were coefficients of correlation of mean fiber areas of type II and III muscle fibers with flavor score (0.45 and 0.45, respectively) and with percent protein (0.62 and 0.56, respectively). Fiber area, loin eye area and taste panel juiciness also showed significant sire effects.

The histological differentiation of muscle fiber types determined from muscle biopsies could be useful in predicting cutability of carcasses of young beef bulls.

Capillary distribution. Capillary distribution throughout the longissimus muscle has been shown to be significantly associated with certain carcass and palatability traits. Martin, Woolley and Miller (1932) studied the capillary counts of resting and active gracilis

muscle of dogs. The number of open capillaries relative to that of resting capillaries approximately doubled, on the average, during exercise after the stoppage of circulation. Carrow, Brown and Van Huss (1967) compared fiber sizes and capillary-to-fiber ratios of rat gastrocnemius muscle with forced and voluntary exercise programs. A third sedentary group was used as a control. The number of capillaries per fiber was greater for white than for red fibers, and a greater increase in cross-sectional area of red fibers was observed with forced and voluntary exercise.

A directly proportionate relationship was found between the density of the capillary network around cardiac muscle fiber and the cytochrome oxidase and SDH activity of the fiber (Romanul, 1965). Capillary density differences were noted also in various portions of the muscle. Reddy et al. (1970) investigated the influence of marbling and vascular distribution of beef longissimus muscle. They observed a significant increase in number of blood vessels as marbling increased. However, blood vessel numbers per unit of fat area decreased.

Cooper, Cassens and Briskey (1969) showed porcine capillaries to be associated more with red fibers than with white fibers. A greater capillary-to-fiber ratio was observed in red muscle than in white muscle.

Numbers of capillaries per unit area (C/A) and number of capillaries per fiber (C/F) of beef longissimus biopsies were studied by Melton et al. (1971). C/A was negatively correlated with panel tenderness, flavor and overall acceptability (-.55, -.58 and -.58, respectively). C/F was correlated with live weight (0.47), with hot carcass weight

(0.49), with fat thickness (0.61), with yield grade (0.44), with marbling score (0.63) and with U.S.D.A. quality grade (0.66).

# CHAPTER III

## EXPERIMENTAL PROCEDURE

### I. Source of Data

A two-part study, involving 34 beef bulls, was conducted at the conclusion of the 1971 Performance Testing Program. The first part consisted of comparing the performance and economy of bulls utilizing three different rations of various silage and concentrate mixtures. The second portion of this study related selected histological muscle fiber characteristics to performance and carcass traits. This thesis was restricted to the latter phase of the study.

Twenty-five Angus bulls, eight Hereford bulls and one Angus X Hereford bull were randomly allotted into six treatment groups. Individual animals were removed from the treatment and slaughtered when they attained a subcutaneous fat thickness (estimated ultrasonically) of 10.0 mm over the longissimus dorsi in the region of the 12th and 13th rib. Animals reaching 515 days of age were slaughtered irrespective of fat thickness. Feed was withheld 24 hours prior to slaughter, at which time the bulls were transported to the University of Tennessee Meats Laboratory for slaughtering. Live weights, taken immediately prior to slaughter, ranged from 362 to 485 kg.

### II. Biopsy Technique

Immediately following exsanguiation, a 12.0-cm incision was made parallel to and 7.0 cm laterally from the vertebral column of

the right side, exposing the l. dorsi in the 12th- and 13th-rib area. The parallel incisions, 8.0 x 1.5 cm, were made with a scalpel along the midline of the longissimus muscle. Two fixed Kelley forceps were inserted into the parallel cuts and clamped to reduce the excitability of the muscle tissue and facilitate its removal. Securing the nuscle eliminated gross contraction of the tissue. A muscle sample, 7.0 x 2.0 x 2.5 cm, was removed by cutting along the tips and outer edge of the forceps. The tissue sample was placed in a 0.05 M sodium pyrophosphate solution and infiltrated to sustain a relaxed pre-rigor state. Complete relaxation required approximately 30 to 45 seconds. After removing the tissue sample from the pyrophosphate solution, eight cross-sectional blocks, 1.0 x 1.0 x 1.0 cm, were cut from the central core of the muscle. Sectioned samples were placed on small cork squares and submerged in a bath of isopentane (2-methyl butane, m.p.-160° C), which had previously been cooled in liquid nitrogen (b.p.-195.8° C). Cryogenic freezing in isopentane required approximately 10 seconds and reduced the thermal shock to the muscle tissue. Frozen samples were placed in plastic bags and stored on dry ice (b.p.-78.5° C) until examined histologically.

Similar biopsy techniques, as described previously, have been used on live bovine animals by Wilson et al. (1955), Everitt and Carter (1961), Link et al. (1967, 1970), Melton (1970) and Harris and Bennett (1970) without apparent harm to the animals.

## III. Histological Technique

Muscle tissue samples were sectioned 12 μ thick with a cryostat held at -20° C. After mounting on cover slips, cross-sectioned

samples were fixed for five minutes in a cold formalin solution containing six parts 4 percent formalin and four parts 0.9 percent NaCl (pH 7.0). Sections were incubated for a period ranging from 15 to 60 minutes at 37° C in a freshly prepared medium (pH 9.4) containing adenosine triphosphate (ATP). Staining for adenosine triphosphatase (ATPase) activity was accomplished by a modification of a method described by Padykula and Herman (1955a,b) (Appendix A). Following incubation, samples were rinsed in 1 percent $CaCl_2$ and treated with 2 percent $CoCl_2$ for 3 to 5 minutes. Sites of enzymatic activity were visualized immediately upon treatment with dilute $(NH_4)_2S$. Type I (red) and type III (white) fibers appeared as light and dark areas, respectively, in each tissue cross-section. Only a small portion of fibers with intermediate color intensity were observed. Type II (intermediate) fibers have been reported previously, but were combined with the white fibers.

One-hundred muscle fibers per animal were selected to determine fiber areas and distribution of fiber types. Micro slides of stained muscle tissue from each animal were photographed using a Ziess Ultraphot II microscope at 80 X magnification. Photographic slides of samples were projected onto poster board and individual fibers traced. Fiber areas were determined to the nearest 0.01 square-inch by a compensating polar planimeter. An ocular micrometer grid was photographed and measured for conversion of fiber areas into square-millimeter units.

## IV. Carcass Data

After they were eviscerated, hot carcasses were weighed and chilled at 2 to 4° C. They then were aged seven days prior to breaking into retail cuts. Twenty-four hours post-mortem, the left side of each carcass was ribbed between the 12th and 13th ribs. The surface outline of the l. dorsi was traced on acetate paper and the area determined to the nearest 0.01 square-inch with a compensating polar planimeter. Subcutaneous fat thickness over the l. dorsi was measured to the nearest millimeter at a point three-fourths the length of the longissimus muscle from the chine end as described by Ramsey et al. (1962).

Sex condition and staginess were discounted when carcass quality grades were determined. Quality and yield grades of the carcasses were determined by competent personnel of the University of Tennessee Meats Laboratory.

Carcasses were broken into wholesale cuts as described by Wellington (1953) with the round, loin, rib and chuck cut into partially boneless retail cuts (Brungardt and Bray, 1963). All cuts were weighed to the nearest 10th of a pound, and excess subcutaneous fat was trimmed to .95-cm. Chine bones were removed from the blade pot roasts, and sections of ribs and sternum were removed from the arm pot roasts. Rib ends and chine bones were removed from the rib roasts. The sacral vertebrae were removed from the sirloin steaks, and all bones were removed from the rump roasts. Subcutaneous fat color was scored using Munsell color paddles. Muscle firmness, texture, color and marbling texture also were observed (Appendix C). Warner-Bratzler shear data were obtained from a 2.54 cm thick club steak taken at

the 13th rib surface. Frozen steaks were allowed to thaw overnight at room temperature. The steaks were grilled to an internal temperature of 68° C on a rotating gas grill. The first side of each steak was cooked to 54° C, turned and cooked to 62° C and turned again until an internal temperature of 68° C was attained. After steaks were allowed to cool to room temperature, three 2.54-cm cores were sheared on a Warner-Bratzler shear device.

## V. Statistical Analysis

Simple correlation coefficients and least square means were calculated using performance traits, carcass traits and muscle fiber characteristics. Breed means were subjected to the t-test (Steel and Torrie, 1960). Data from breeds whose means were observed to be non-significantly different were pooled in the subsequent analysis. The following regression models were fitted to determine the prediction value of muscle fiber characteristics:

$$\hat{Y} = \mu + NRED$$

$$\hat{Y} = \mu + MRFAREA$$

$$\hat{Y} = \mu + NRED + RFAREA$$

$$\hat{Y} = \mu + NRED + RFAREA + PRFA$$

$$\hat{Y} = \mu + NRED + RFAREA + PRFA + MWFAREA$$

$$\hat{Y} = \mu + NWHITE$$

$$\hat{Y} = \mu + MWFAREA$$

$$\hat{Y} = \mu + NWHITE + WFAREA$$

$$\hat{Y} = \mu + NWHITE + WFAREA + PWFA$$

$$\hat{Y} = \mu + NWHITE + WFAREA + PWFA + MWFAREA$$

Where $\hat{Y}$ = estimated performance or carcass trait.

$\mu$ = the population mean of the observed dependent variable.

NRED;NWHITE = the number of red and white fibers counted per 100 fibers.

MRFAREA;MWFAREA = the mean red and white fiber areas.

RFAREA;WFAREA = the total red and white fiber areas of 100 fiber samples.

PRFA;PWFA = the percent red and white fiber areas.

# CHAPTER IV

## RESULTS AND DISCUSSION

### I. Performance Traits

One-hundred randomly selected L. dorsi muscle fibers were measured after histological differentiation by the ATPase method of Padykula and Herman (1955a,b). Mean muscle fiber number or percent of type I, red, and type III, white, fibers were 33.03 and 66.97, respectively, (Table 1).

Stein and Padykula (1962), Ogata and Mori (1964), Romanul (1964) and Ashmore and Doerr (1971) classified muscle fibers as type I (red), type II (intermediate) and type III (white), according to their ATPase fiber types, α and β "red," based on myosin ATPase activity, and noted the majority of fibers to be α. Transformation from initially α "red" to α "white" fibers was primarily concerned with changes in energy-producing enzymes and accompanied by a rapid increase in fiber size. β fibers remained "red" throughout their lifespan. Type I, II and III fibers correspond to β "red," α "red" and α "white," respectively. Brooke and Kaiser (1969) and Melton (1970) observed the staining of muscle fibers to be pH dependent. Type I, red, fibers were alkali-labile at pH, 8.0 to 10.3, as compared to the acid-labile muscle fibers of intermediate staining intensity which were noted at a pH of 5.0 to 8.0 and type III, white, fibers at pH 4.3 to 5.0. These results indicate that muscle fibers designated as type II, intermediate, as observed by other workers, are actually "red" fibers in a state of transition to "white"

Table 1. Means and Standard Errors of Selected Performance Traits, Carcass Traits and Histological Muscle Fiber Characteristics

| | Mean | Standard Error |
|---|---|---|
| **Performance Traits** | | |
| Initial adj. ADG, lb./day | 1.76 | .03 |
| Overall ADG, lb./day | 2.38 | .04 |
| Initial fat thickness, mm | 2.35 | .15 |
| Final fat thickness, mm | 9.79 | .43 |
| Initial weight, lb. | 517.79 | 12.54 |
| Final weight, lb. | 918.53 | 14.78 |
| Final age in days | 438.18 | 7.04 |
| Days on test | 169.41 | 6.28 |
| **Carcass Traits** | | |
| Carcass weight, lb. | 539.26 | 9.81 |
| Estimated kidney fat, % | 3.20 | 0.09 |
| Carcass fat, mm | 9.47 | 0.46 |
| Carcass quality grade[a] | 11.32 | 0.16 |
| Rib eye area, $in.^2$ | 11.07 | 0.23 |
| Marbling[b] | 4.80 | 0.16 |
| Warner-Bratzler shear, lb. | 17.83 | 0.59 |
| Tenderometer, lb. force | 16.99 | 0.46 |
| Conformation[c] | 14.38 | 0.22 |
| Maturity[d] | 1.97 | 0.05 |
| Fat color[e] | 2.74 | 0.09 |
| Muscle color[f] | 5.32 | 0.17 |
| Muscle firmness[g] | 5.35 | 0.12 |
| Muscle texture[h] | 5.47 | 0.10 |
| Marbling texture[i] | 2.12 | 0.07 |
| Dressing percent | 58.69 | 0.42 |
| Yield grade | 2.15 | 0.09 |
| Kidney fat, lb. | 9.42 | 0.37 |
| Carcass length, cm | 111.11 | 0.82 |
| Leg length, cm | 67.56 | 0.58 |
| Loin length, cm | 57.49 | 0.39 |
| Loin weight, lb. | 37.54 | 0.66 |
| Loin weight/100 lb. carcass weight | 30.28 | 0.66 |
| Percent loin | 14.41 | 0.19 |
| Percent cuts | 50.66 | 0.21 |
| Rib eye area/100 lb. carcass weight | 2.14 | 0.04 |
| Rib eye area/day of age | 0.02 | 0.00 |
| Carcass weight/day of age | 1.19 | 0.02 |
| **Muscle Fiber Characteristics** | | |
| Number of red fibers/100 fibers | 33.03 | 1.14 |
| Number of white fibers/100 fibers | 66.97 | 1.14 |

Table 1. (continued)

| | Mean | Standard Error |
|---|---|---|
| Mean red fiber area, $mm^{2j}$ | 2.28 | 0.01 |
| Mean white fiber area, $mm^{2j}$ | 3.64 | 0.01 |
| Total red fiber area, $mm^{2j}$ | 75.90 | 0.45 |
| Total white fiber area, $mm^{2j}$ | 241.69 | 0.81 |
| Percent red fiber area | 23.65 | 0.89 |
| Percent white fiber area | 76.35 | 0.89 |

[a]High Good = 11; Low Choice = 12

[b]Slight = 4; Small = 5

[c]Average Choice = 13; Low Prime = 15

[d]$A^{-} = 1$, $A^{0} = 2$, $A^{+} = 3$

[e]Munsell Color Paddle Scores

[f]Slight Dark Red = 5; Cherry Red = 6

[g]Moderately Firm = 5; Firm = 6

[h]Moderately Fine = 5; Fine = 6

[i]Medium = 2; Fine = 3

[j]Mean X $10^{-3}mm^{2}$

fibers. Melton (1970) compared the three classical fiber types and observed that the addition of type II to type III fibers increased the magnitude of most correlations between percent fiber type and carcass traits, palatability traits and proximate analysis. This indicated that type II fibers were more like type III. Because pH change influences the characteristic staining type, muscle samples were incubated in a medium at pH 9.4 which stains type II fibers identically to type III. Type I and III fiber types were classified in this study to reduce the subjective error of determining type II fibers (Figure 1).

Number of fibers. Initial weight of the bulls was significantly ($P < .05$) correlated with the number of red and white fibers (-.34 and 0.34), respectively, as shown by correlation coefficients in Table 2. Red and white muscle fibers were observed to be complements, thus inverse correlation coefficients exist for some fiber measurements. These data appear to be in agreement with Ashmore et al. (1972) in that certain red fibers have been shown to be initially smaller in young bovine animals, but possess the capability of transforming into larger white fibers with chronological and physiological maturity of the animal.

Final fat thickness at the 12th rib was estimated ultrasonically to the nearest 0.1 mm and was significantly ($P < .05$) correlated with the numbers of red (-.40) and white (0.40) fibers.

Initial adjusted average daily gain, initial fat thickness (12th rib), off-test live weight, days on test, final age in days

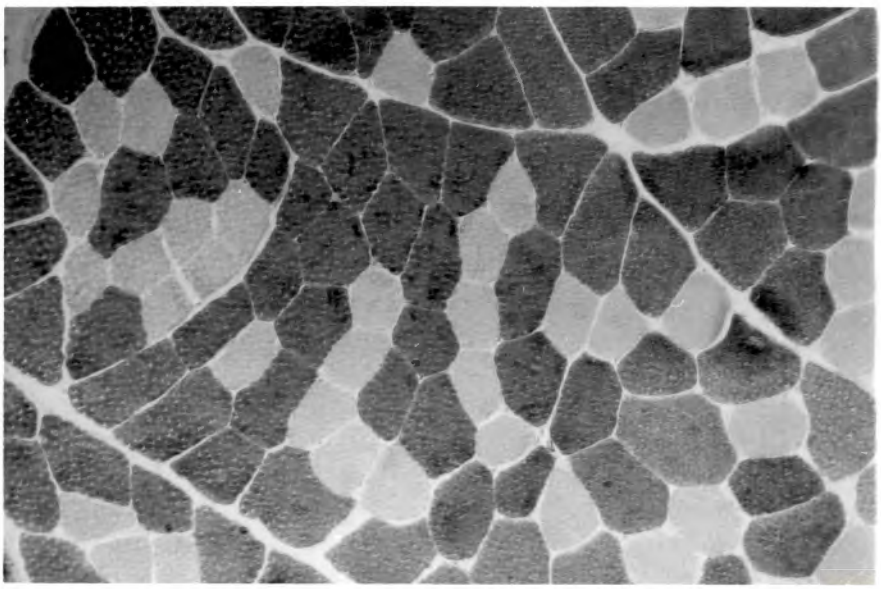


Figure 1. Type I, red (light) and Type III, white (dark), muscle fibers of *longissimus dorsi* as demonstrated by ATPase staining (Padykula and Herman, 1955a,b).

Table 2. Coefficients of Correlation of Performance Traits and Histological Muscle Fiber Characteristics

| Performance Traits | Fiber Characteristics[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NRED | NWHITE | MRFA | MWFA | TRFA | TWAF | PRFA | PWFA |
| Initial adj. ADG | 0.07 | -0.07 | 0.13 | 0.13 | 0.15 | 0.11 | 0.08 | -0.08 |
| Overall ADG | 0.11 | -0.11 | 0.28 | 0.17 | 0.24 | 0.15 | 0.22 | -0.22 |
| Initial fat thickness[b] | -0.21 | 0.21 | -0.24 | -0.16 | -0.29 | -0.09 | -0.27 | 0.27 |
| Final fat thickness[b] | -0.40* | 0.40* | -0.12 | -0.23 | -0.28 | -0.08 | -0.33 | 0.33 |
| Initial weight | -0.34* | 0.34* | -0.18 | -0.31 | -0.32 | -0.18 | -0.23 | 0.23 |
| Final weight | -0.11 | 0.11 | 0.01 | -0.05 | -0.07 | 0.00 | -0.04 | 0.04 |
| Final age in days | -0.16 | 0.16 | -0.14 | -0.08 | -0.21 | -0.01 | -0.20 | 0.20 |
| Days on test | 0.13 | -0.13 | 0.04 | 0.13 | 0.09 | 0.07 | 0.06 | -0.06 |

[a]Fiber areas measured in $mm^2$; NRED = number of red fibers; NWHITE = number of white fibers; MRFA = mean red fiber area; MWFA = mean white fiber area; TRFA = total red fiber area; TWFA = total white fiber area; PRFA = percent red fiber area; PWFA = percent white fiber area.

[b]Measured ultrasonically, mm.

*Significant, $P < .05$ level.

and overall average daily gain (ADG) were not significantly correlated with the numbers of red or white fibers (Table 2).

Various regression models were fitted using muscle fiber characteristics as independent variables to determine their usefulness in predicting performance and carcass traits. Criteria for selecting the best fit models were the $R^2$ value and level of significance of the F-test. Table 3 presents a summary of the most effective models fitted for predicting performance traits.

Partial regression coefficients of the dependent variable initial weight and fat thickness were significant ($P < .05$) with the average red or white fiber number as the independent variable (Table 3). Coefficient of determination values for initial weight and final fat were 12.0 and 16.0 percent, respectively. Only a small portion of the total variation could be accounted for by the regression on number of red or white fibers, indicating these variables to be poor predictors of initial weight and final fat at this stage of maturity. By combining number of fibers, area of fibers and percent fiber area, the predictive value of the regression models studied was not improved as compared to the number of fibers alone. R's at additional regression models in Table 3 were non-significant ($P > .05$).

Fiber area. Mean fiber areas of Angus and Hereford bulls were not significantly different ($P > .01$) when compared using the T-test for two sample means of unpaired observations with pooled variance (Steele and Torrie, 1960). Also, total area of all fibers, mean fiber area and percent fiber area for red and white muscle fiber

Table 3. Multiple Regression Equations - Partial Regression Coefficients Predicting Dependent Variables[a]

| Dependent Variable | Red Fiber Characteristics[b] | | | | | |
|---|---|---|---|---|---|---|
| | Constant | NRED ($X_1$) | MRFA ($X_2$) | TRFA ($X_3$) | PRFA ($X_4$) | $R^2$ |
| Performance Traits | | | | | | |
| Initial adj. ADG | 2.34 | -0.02 | -0.52 | -0.00 | -0.00 | 0.04 |
| Overall ADG | 1.26 | 0.02 | 0.84 | -0.02 | 0.01 | 0.11 |
| Initial fat thickness | 4.64 | -0.03 | -1.18 | 0.01 | -0.03 | 0.09 |
| Final fat thickness | 14.71 | -0.15 | - | - | - | 0.16* |
| Initial weight | 643.22 | -3.80 | - | - | - | 0.12* |
| Final weight | 584.43 | 7.08 | 314.09 | -9.01 | 3.51 | 0.05 |
| Final age in days | 268.51 | 6.36 | 169.27 | -4.99 | -1.87 | 0.08 |
| Days on test | 12.70 | 5.56 | 115.52 | -2.96 | -2.04 | 0.05 |

| Dependent Variable | White Fiber Characteristics[b] | | | | | |
|---|---|---|---|---|---|---|
| | Constant | NWHITE ($X_1$) | MWFA ($X_2$) | TWFA ($X_3$) | PWFA ($X_4$) | $R^2$ |
| Performance Traits | | | | | | |
| Initial adj. ADG | 2.12 | -0.00 | -0.10 | 0.00 | -0.00 | 0.02 |
| Overall ADG | 4.19 | -0.00 | -0.51 | 0.01 | -0.02 | 0.12 |
| Initial fat thickness | -5.12 | 0.05 | 2.12 | -0.04 | 0.06 | 0.10 |
| Final fat thickness | -0.17 | 0.15 | - | - | - | 0.16* |
| Initial weight | 263.48 | 3.80 | - | - | - | 0.12* |
| Final weight | 765.33 | 6.54 | 83.60 | -1.21 | -3.83 | 0.03 |
| Final age in days | 253.23 | 0.47 | 30.83 | -0.53 | 2.12 | 0.04 |
| Days on test | 55.41 | 0.27 | 58.56 | -0.82 | 1.14 | 0.03 |

Table 3. (continued)

[a]Models were selected on the basis of the highest $R^2$ value and level of significance. For example, the equation for predicting initial adjusted ADG in conventional form would be:

$$\hat{Y} = 2.34 - 0.02\ (X_1) - 0.52\ (X_2) + 0.02\ (X_3) - 0.00\ (X_4).$$

[b]NRED = number of red fibers; MRFA = mean red fiber area; TRFA = total red fiber area; PRFA = percent red fiber area; NWHITE = number of white fibers; MWFA = mean white fiber area; TWFA = total white fiber area; PWFA = percent white fiber area.

*Significant, $P < .05$ level.

types showed no significant breed effect. This is in agreement with Ramsey, Cole and Tendick (1967) who found no significant differences in muscle fiber diameter between Angus and Hereford steers.

Mean cross-sectional areas of red and white fibers were 2.28 x $10^{-3}$ and 3.64 x $10^{-3}$ $mm^2$, respectively, (Table 1, page 26). Melton (1970) noted a similar size pattern of red, 3.06 x $10^{-3}$ $mm^2$, and white, 4.61 x $10^{-3}$ $mm^2$, fibers. Field et al. (1970) observed the average area of all fibers to be 3.04 x $10^{-3}$ $mm^2$ from bovine longissimus taken at nine days post-mortem. Average fiber area of all fibers in the present study was 2.96 x $10^{-3}$ $mm^2$ which is comparable to the values previously published. Joubert (1956) and Tuma et al. (1962) attributed variation in fiber size to breed and to age, respectively.

Correlation of performance traits and muscle fiber area measurements are presented in Table 2, page 30. Mean, total and percent red and white fiber areas were not significantly ($P > .05$) correlated with any of the performance traits studied.

No significant ($P > .05$) R's were found for equations predicting performance using muscle fiber area measurements as independent variables (Table 3).

## II. Carcass Traits

Number of fibers. Mean red fiber number was significantly correlated with rib eye area per hundred weight of carcass (0.44, $P < .01$) and with kidney fat (-.34, $P < .05$). Correlations of mean white fiber number with the above traits were opposite in sign to those

involving red fiber number for the previous traits (Table 4). Melton (1970) observed a significant correlation ($P < .01$) between percent type I (red) reversal fibers and percent kidney fat. None of the other carcass traits in Table 4 were significantly correlated with mean red or white fiber number.

Equations with kidney fat as the dependent variable were the only ones yielding significant $R^2$ values when mean red or white fiber number was an independent variable (Tables 5 and 6). The predictive effectiveness of muscle fiber number, even though significant, was poor, practically speaking, as indicated by the $R^2$ value (0.11).

Fiber area. Correlations of muscle fiber area with carcass traits are presented in Table 4. Mean red fiber area was significantly ($P < .05$) correlated with rib eye area per day of age (0.43, $P < .01$), and ($P < .05$) with rib eye area (0.36), with dressing percent (0.38) and with carcass weight per day of age (0.34). Mean white fiber area was significantly ($P < .05$) correlated with rib eye area (0.38), dressing percent (0.41), rib eye area per hundred weight (0.38) and rib eye area per day of age (0.40).

Total red fiber area was significantly ($P < .01$) correlated with eye area per hundred weight. Correlations of this value also were significant ($P < .05$) with rib eye area (0.34), yield grade (-.35) and rib eye area per day of age (0.46). Total white fiber area was significantly correlated only with dressing percent (0.52, $P < .01$) and with rib eye area (0.34, $P < .05$).

The significant positive correlations between rib eye area and fiber areas suggest that heavier muscled animals possess larger fibers.

Table 4. Coefficients of Correlation of Carcass Traits and Histological Muscle Fiber Characteristics

| Carcass Traits | Fiber Characteristics[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NRED | NWHITE | MRFA | MWFA | TRFA | TWFA | PRFA | PWFA |
| Carcass weight | -0.16 | 0.16 | 0.14 | 0.11 | -0.00 | 0.19 | -0.11 | 0.11 |
| Estimated kidney fat | -0.16 | 0.16 | -0.17 | -0.11 | -0.17 | -0.08 | -0.23 | 0.23 |
| Carcass fat | -0.20 | 0.20 | -0.04 | -0.00 | -0.11 | 0.07 | -0.25 | 0.25 |
| Carcass quality grade | 0.05 | -0.05 | -0.05 | 0.00 | 0.00 | -0.04 | -0.01 | 0.01 |
| Rib eye area | 0.20 | -0.20 | 0.36* | 0.38* | 0.34* | 0.34* | 0.23 | -0.23 |
| Marbling | -0.18 | 0.18 | -0.21 | -0.22 | -0.21 | -0.20 | -0.20 | 0.20 |
| Warner-Bratzler shear | 0.14 | -0.14 | 0.23 | 0.23 | 0.23 | 0.20 | 0.16 | -0.16 |
| Tenderometer | -0.13 | 0.13 | -0.19 | -0.18 | -0.22 | -0.14 | -0.15 | 0.15 |
| Muscle firmness | -0.31 | 0.31 | -0.18 | -0.21 | -0.33 | -0.06 | -0.30 | 0.30 |
| Muscle texture | -0.21 | 0.21 | -0.08 | 0.05 | -0.16 | 0.16 | -0.32 | 0.32 |
| Marbling texture | 0.08 | -0.08 | 0.05 | 0.31 | 0.08 | 0.34 | -0.11 | 0.11 |
| Dressing percent | -0.16 | 0.16 | 0.38* | 0.41* | 0.18 | 0.52** | -0.18 | 0.18 |
| Yield grade | -0.33 | 0.33 | -0.29 | -0.29 | -0.35* | -0.18 | -0.38* | 0.38* |
| Kidney fat | -0.34* | 0.34* | -0.20 | -0.17 | -0.31 | -0.05 | -0.38* | 0.38* |
| Loin weight | -0.26 | 0.26 | 0.03 | -0.09 | -0.14 | 0.02 | -0.16 | 0.16 |
| Loin weight/100 lb. carcass wt. | -0.16 | 0.16 | 0.08 | -0.00 | -0.05 | 0.08 | -0.10 | 0.10 |
| Percent loin | 0.00 | -0.00 | -0.03 | -0.11 | -0.02 | -0.12 | 0.05 | -0.05 |
| Rib eye area/100 lb. carcass wt. | 0.44** | -0.44** | 0.33 | 0.38* | 0.46** | 0.23 | 0.44** | -0.44** |
| Rib eye area/day of age | 0.28 | -0.28 | 0.43** | 0.40* | 0.46** | 0.31 | 0.34* | -0.34* |
| Carcass weight/day of age | -0.06 | 0.06 | 0.34* | 0.22 | 0.22 | 0.26 | 0.06 | -0.06 |

[a]Fiber areas measured in $mm^2$; NRED = number of red fibers; NWHITE = number of white fibers; MRFA = mean red fiber area; MWFA = mean white fiber area; TRFA = total red fiber area; TWFA = total white fiber area; PRFA = percent red fiber area; PWFA = percent white fiber area.

*Significant, $P < .05$ level.

**Significant, $P < .01$ level.

Percent red fiber area was correlated significantly ($P < .05$) with kidney fat (-.38), yield grade (-.38) and rib eye area per day of age (0.34). A significant ($P < .01$) correlation was observed between rib eye area per hundred weight and percent red fiber area (0.44). Correlations of percent white fiber area and the previously mentioned carcass traits are shown in Table 4.

Combinations of muscle fiber characteristics were fitted in various regression models to determine their usefulness as indicators of carcass traits. Partial regression coefficients predicting carcass traits are summarized in Tables 5 and 6. Muscle fiber characteristics alone accounted for only a small portion of the total variation associated with carcass traits as evidenced by the low $R^2$ values.

Data obtained in this study conflict with that of a similar study by Melton (1970). Animals used in the previously cited study were Hereford bulls, fed for growth, ranging in age from 13 to 15 months and variable in subcutaneous fat. Bulls used in the present study were predominatly Angus, fed a high energy ration for greater fat deposition, ranging in age from 15 to 17 months and slaughtered at a constant fat thickness. Melton's (1970) animals were more variable in performance and carcass traits and had not attained their growth potential. Lack of variation in subcutaneous fat thickness, age and stage of maturity was reflected in the low correlations observed in this study.

Further investigation of muscle fiber characteristics is indicated to identify relationships among muscle fiber characteristics and performance and carcass traits. Prediction equations involving performance and carcass traits may be enhanced by the inclusion

Table 5. Multiple Regression Equations - Partial Regression Coefficients Predicting Dependent Variables (Red Fiber Characteristics)[a]

| Dependent Variable | Red Fiber Characteristics[b] | | | | | |
|---|---|---|---|---|---|---|
| | Constant | NRED ($X_1$) | MRFA ($X_2$) | TRFA ($X_3$) | PRFA ($X_4$) | $R^2$ |
| Carcass Traits | | | | | | |
| Carcass weight | 315.15 | 5.63 | 234.46 | -5.47 | -1.16 | 0.08 |
| Estimated kidney fat | 9.23 | -0.15 | -4.70 | 0.13 | -0.02 | 0.22 |
| Carcass fat | 31.02 | -0.52 | -15.43 | 0.46 | -0.18 | 0.14 |
| Carcass quality grade | 15.32 | -0.09 | -3.51 | 0.10 | -0.02 | 0.04 |
| Rib eye area | 8.99 | - | 1.66 | - | - | 0.13* |
| Marbling | 14.57 | -0.26 | -7.58 | 0.20 | 0.10 | 0.18 |
| Warner-Bratzler shear | -4.90 | 0.60 | 17.62 | -0.42 | -0.08 | 0.10 |
| Tenderometer | 15.25 | 0.10 | 2.65 | -0.12 | -0.00 | 0.05 |
| Conformation | 1.14 | 0.34 | 10.62 | -0.27 | -0.00 | 0.19 |
| Maturity | 2.01 | 0.01 | 0.47 | -0.00 | -0.03 | 0.21 |
| Muscle firmness | 2.96 | 0.08 | 2.86 | -0.09 | -0.01 | 0.15 |
| Muscle texture | 7.60 | -0.02 | -1.09 | 0.04 | -0.08 | 0.14 |
| Marbling texture | 1.18 | 0.07 | 0.82 | -0.01 | -0.08 | 0.19 |
| Dressing percent | 62.16 | -0.04 | - | 0.14 | -0.33 | 0.30* |
| Yield grade | 9.20 | -0.18 | -5.19 | 0.14 | -0.01 | 0.33* |
| Kidney fat | 13.06 | -0.11 | - | - | - | 0.11* |
| Loin weight | 28.44 | 0.13 | 11.75 | -0.32 | 0.14 | 0.10 |
| Rib eye area/100 lb. carcass weight | 0.16 | 0.05 | 1.28 | -0.03 | -0.00 | 0.30* |
| Rib eye area/day of age | 0.02 | - | 0.01 | - | - | 0.19** |
| Carcass wt./day of age | 1.05 | - | 0.11 | - | - | 0.11* |
| Percent cuts | 35.16 | 0.39 | 11.24 | -0.30 | 0.03 | 0.34* |

Table 5. (continued)

[a]Models were selected on the basis of the highest $R^2$ value and level of significance. For example, the equation for predicting carcass weight in conventional form would be:

$$\hat{Y} = 315.15 + 5.63\ (X_1) + 234.46\ (X_2) - 5.47\ (X_3) - 1.16\ (X_4).$$

[b]NRED = number of red fibers; MRFA = mean red fiber area; TRFA = total red fiber area; PRFA = percent red fiber area.

*Significant, $P < .05$ level.

**Significant, $P < .01$ level.

Table 6. Multiple Regression Equations - Partial Regression Coefficients Predicting Dependent Variables (White Fiber Characteristics)[a]

| Dependent Variable | White Fiber Characteristics[b] | | | | | |
|---|---|---|---|---|---|---|
| | Constant | NWHITE ($X_1$) | MWFA ($X_2$) | TWFA ($X_3$) | PWFA ($X_4$) | $R^2$ |
| Carcass Traits | | | | | | |
| Carcass weight | 166.93 | 7.23 | 144.65 | -1.74 | -2.22 | 0.08 |
| Estimated kidney fat | -4.55 | 0.08 | 3.06 | -0.05 | 0.04 | 0.14 |
| Carcass fat | -29.28 | 0.43 | 14.41 | -0.21 | 0.13 | 0.12 |
| Carcass quality grade | 4.72 | 0.07 | 3.17 | -0.05 | 0.03 | 0.03 |
| Rib eye area | 8.77 | - | 1.15 | - | - | 0.14* |
| Marbling | -14.03 | 0.26 | 8.59 | -0.14 | 0.03 | 0.25 |
| Warner-Bratzler shear | 45.27 | -0.32 | -11.25 | 0.20 | -0.13 | 0.09 |
| Tenderometer | 14.35 | -0.05 | -1.11 | 0.00 | 0.11 | 0.05 |
| Conformation | 17.35 | 0.04 | -0.01 | 0.01 | -0.09 | 0.09 |
| Maturity | 0.56 | 0.01 | - | 0.00 | - | 0.19* |
| Muscle firmness | 9.68 | -0.09 | -3.53 | 0.05 | 0.02 | 0.16 |
| Muscle texture | 2.91 | -0.04 | -0.51 | 0.01 | 0.06 | 0.14 |
| Marbling texture | 0.82 | -0.04 | -0.22 | 0.01 | 0.05 | 0.20 |
| Dressing percent | 48.37 | 0.06 | - | 0.05 | - | 0.30** |
| Yield grade | -6.26 | 0.09 | 2.93 | -0.05 | 0.04 | 0.25 |
| Kidney fat | 2.04 | 0.11 | - | - | - | 0.11* |
| Loin weight | 4.28 | 0.72 | 14.38 | -0.21 | -0.21 | 0.11 |
| Rib eye area/100 lb. carcass weight | 5.10 | -0.03 | -0.91 | 0.02 | -0.01 | 0.30* |
| Rib eye area/day of age | 0.04 | 0.00 | - | 0.00 | -0.00 | 0.24* |
| Carcass wt./day of age | 0.73 | 0.02 | 0.29 | -0.00 | -0.01 | 0.16 |
| Percent cuts | 55.07 | -0.06 | - | - | - | 0.13* |

Table 6. (continued)

[a]Models were selected on the basis of the highest $R^2$ value and level of significance. For example, the equation for predicting carcass weight in conventional form would be:

$$\hat{Y} = 166.93 + 7.23\ (X_1) + 144.65\ (X_2) - 1.74\ (X_3) - 2.22\ (X_4).$$

[b]NWHITE = number of white fibers; MWFA = mean white fiber area; TWFA = total white fiber area; PWFA = percent white fiber area.

*Significant, $P < .05$ level.

**Significant, $P < .01$ level.

of histological muscle measurements. Muscle biopsies are a usable tool in selection of herd sires or dams only if muscle fiber characteristics can be shown to be associated with economically important traits. Future studies should be designed to permit appreciable variation in all variables recorded.

## III. Summary

Thirty-four bulls were biopsied at 15 to 17 months of age at a constant fat thickness of 10.0 mm. Longissimus muscle samples were taken from the 12th- and 13th-rib region and stained histologically for ATPase activity. One-hundred muscle fibers from each sample were counted, measured and classified into type I, red, and type III, white, fibers. Breed means were compared using the T-test and observed to be non-significant.

Number of red fibers was significantly correlated with final fat thickness, initial weight, kidney fat and rib eye area per hundred weight of carcass. Number of white fibers was observed to be negatively correlated with the number of red fibers. Muscle fiber area measurements were not significantly correlated with any of the performance traits studied. Red and white muscle fiber areas increased significantly with rib eye area, rib eye area per hundred weight of carcass and rib eye area per day of age. Yield grade was correlated significantly with total red fiber area (-.35). Total white fiber area was correlated with dressing percent (0.52). Percent red and white fiber areas were significantly correlated with yield grade, kidney fat, rib eye per hundred weight of carcass and rib eye area per day of age.

Variation among performance and carcass traits was minimal since the animals were held at a constant fat thickness and within a small weight range. Histologically differentiated muscle fibers were observed to differ only to a small degree, which, in the light of results of other workers, could be attributable to the relatively small amount of variation in the traits studied.

# LITERATURE CITED

## LITERATURE CITED


Ashmore, C. R. and L. Doerr. 1971. Comparative aspects of muscle fiber types in different species. Exp. Neurology 31:408.

Ashmore, C. R., G. Tompkins and L. Doerr. 1972. Postnatal development of muscle fiber types in domestic animals. J. Anim. Sci. 34:37.

Barka, T. and P. J. Anderson. 1963. Histochemistry: Theory, Practice and Bibliography. Harper and Row, Publishers, Inc. New York, Evanston and London.

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

Beecher, G. R., R. G. Cassens, W. G. Hoekstra and E. J. Briskey. 1965. Red and white fiber content and associated post-mortem properties of seven porcine muscles. J. Food Sci. 30:969.

Beecher, G. R., L. L. Kastenschmidt, R. G. Cassens, W. G. Hoekstra and E. J. Briskey. 1968. A comparison of the light and dark portions of a striated muscle. J. Food Sci. 33:84.

Brooke, M. H. and K. K. Kaiser. 1969. Some comments on the histochemical characterization of muscle adenosine triphosphatase. J. Histochem. Cytochem. 17:431.

Brungardt, V. H. and R. W. Bray. 1963. Variation between sides in the beef carcass for certain wholesale and retail yields and linear carcass measurements. J. Anim. Sci. 22:746.

Carrow, R. E., R. E. Brown and W. D. Van Huss. 1967. Fiber sizes and capillary to fiber ratios in skeletal muscle of exercised rats. Anat. Rec. 159:33.

Cassens, R. G., C. C. Cooper and S. Morita. 1969. Differentiation of muscle fibers during growth and development. Proc. 22nd Annual Rec. Meat Conf. 101.

Cooper, C. C., B. B. Breidenstein, R. G. Cassens, G. Evans and R. W. Bray. 1968. Influence of marbling and maturity on the palatability of beef muscle. II. Histological considerations. J. Anim. Sci. 27:1542.

Cooper, C. C., R. G. Cassens and E. J. Briskey. 1969. Capillary distribution and fiber characteristics in skeletal muscle of stress-susceptible animals. J. Food Sci. 34:299.

Cooper, C. C., R. G. Cassens, L. L. Kastenschmidt and E. J. Briskey. 1970. Histochemical characterization of muscle differentiation. Dev. Biol. 23:169.

Covington, R. C., H. J. Tuma, D. L. Grant and A. D. Dayton. 1970. Various chemical and histological characteristics of beef muscle as related to tenderness. J. Anim. Sci. 30:191.

Denney-Brown, D. E. 1929. The histological features of striped muscle in relation to its functional activity. Proc. Roy. Soc. London Ser. B. 104:371.

Dikeman, M. E., C. C. Melton, H. J. Tuma and G. R. Beecher. 1972. Biopsy sample analysis to predict bovine muscle palatability with emphasis on tenderness. J. Anim. Sci. 34:49.

Dubowitz, V. 1960. Reciprocal relationship of phosphorylase and oxidative enzymes in skeletal muscle. Nature 185:701.

Dubowitz, V. 1963. Enzymatic maturation of skeletal muscle. Nature 197:1215.

Eisenhut, R. C., R. G. Cassens, R. W. Bray and E. J. Briskey. 1965. Fiber arrangement and micro-structure bovine longissimus dorsi muscle. J. Food Sci. 30:955.

Engelhardt, V. A. and M. N. Ljubimova. 1939. Myosine and adenosine-triphosphatase. Nature 144:668.

Everitt, G. C. and A. H. Carter. 1961. Growth and muscle development of steers implanted with hexoestrol. J. Agr. Sci. 57:213.

Farrell, P. R. and M. R. Fedde. 1969. Uniformity of structural characteristics throughout the length of skeletal muscle fibers Anat. Rec. 164:219.

Field, R. A., A. M. Pearson, W. T. Magee and R. A. Merkel. 1970. Chemical and histological characteristics of the M. longissimus in young bulls selected for tenderness or leanness. J. Anim. Sci. 30:717.

Hanson, J. and H. E. Huxley. 1953. Structural basis of the cross-striations in muscle. Nature 172:530.

Harris, R. M. and J. A. Bennett. 1970. Mechanism for biopsy sampling. J. Anim. Sci. 31:451.

Herring, H. K., R. G. Cassens, G. G. Suess, V. H. Brungardt and E. J. Briskey. 1967. Tenderness and associated characteristics of skeletal and contracted bovine muscles. J. Food Sci. 32:317.

Hiner, R. L., O. G. Hankins, H. S. Sloane, C. R. Fellers and E. E. Anderson. 1953. Fiber diameter in relation to tenderness of beef muscle. Food Res. 18:364.

Huxley, H. E. 1960. Muscle cells. In J. Brachet and A. E. Mirsky (Ed.) The cell. Vol. IV. Academic Press, New York and London.

Joubert, D. M. 1956. Postnatal growth and development of the muscle fiber. J. Agric. Sci. Cambridge 47:59.

Kastenschmidt, L. L., W. G. Hoekstra and E. J. Briskey. 1968. Glycolytic intermediates and co-factors in "fast-" and "slow-glycolyzing" muscles of the pig. J. Food Sci. 33:151.

Lawrie, R. A. 1952. Biochemical differences between red and white muscle. Nature 170:122.

Levy, H. M. and E. M. Ryan. 1966. Evidence that the contraction of actomyosin requires the reaction of adenosine triphosphate and magnesium at two different sites. Biochem. Z. 345:132.

Link, B. A., R. W. Bray, R. G. Cassens and R. G. Kauffman. 1970. Lipid deposition in bovine skeletal muscle during growth. J. Anim. Sci. 30:6.

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

Marsh, B. B. 1951. A factor modifying muscle fiber synaeresis. Nature 167:1065.

Marsh, B. B. 1952. The effects of adenosine triphosphate on the fibre. Volume of a muscle homogenate. Biochem. et Biophys. Acta 9:247.

Martin, E. G., E. C. Woolley and M. Miller. 1932. Capillary counts in resting and active muscles. Am. J. Physiol. 100:407.

Martonosi, A. and H. Meyer. 1964. The binding of pyrophosphate and adenosine triphosphate to myosin. J. Biol. Chem. 239:640.

Maruyama, K. and J. Gergely. 1962. Interaction of actomyosin during the clear phase. J. Biol. Chem. 237:1095.

Melton, C. C. 1970. Histological and histochemical characteristics of bovine muscle biopsies as related to composition and quality. Ph.D. Thesis. Kansas State University, Manhattan, Kansas.

Melton, C. C., M. E. Dikeman and H. J. Tuma. 1971. Histological evaluation of bovine longissimus muscle biopsies as related to meat quality and cutability. J. Anim. Sci. 33:221. (Abstr.).

Merkel, R. A., D. K. Sorensen, T. Kowalezyk and R. W. Bray. 1954. A technique for securing tissues to study muscular fat deposition in swine. The Cornell Vet. 44:4.

Moody, W. G. and R. G. Cassens. 1968. Histochemcial differentiation of red and white muscle fibers. J. Anim. Sci. 27:961.

Morita, S., C. C. Cooper, R. G. Cassens, L. L. Kastenschmidt and E. J. Briskey. 1970. A histological study of myoglobin in the developing muscle of the pig. J. Anim. Sci. 31:664.

Ogata, T. and M. Mori. 1964. Histochemical study of oxidative enzymes in vertebrate muscles. J. Histochem. Cytochem. 12:171.

Padykula, H. A. and G. F. Gauthier. 1963. Cytochemical studies of adenosine triphosphatases in skeletal muscle fibers. J. Cell Biol. 18:87. (Abstr.).

Padykula, H. A. and E. Herman. 1955a. Factors affecting the activity of adenosine triphosphatase and other phosphatases as measured by histochemical techniques. J. Histochem. Cytochem. 3:161.

Padykula, H. A. and E. Herman. 1955b. The specificity of the histochemical method for adenosine triphosphatase. J. Histochem. Cytochem. 3:170.

Ramsey, C. B., J. W. Cole and C. S. Hobbs. 1962. Relation of beef carcass grades, proposed yield grades and fat thickness to separable lean, fat and bone. J. Anim. Sci. 21:193.

Ramsey, C. B., J. W. Cole and E. W. Tendick. 1967. Effect of breed of steer on muscle fiber diameter and its relationship to palatability. Tenn. Agr. Exp. Sta. Bull. 424.

Reddy, G. B., H. J. Tuma, D. L. Grant and R. C. Covington. 1970. Relationship of intramuscular fat and the vascular system to bovine tenderness. J. Anim. Sci. 31:837.

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. Anim. Sci. 24:681.

Romanul, F. C. A. 1964. Enzymes in muscle. Arch. Neurology 11:355.

Romanul, F. C. A. 1965. Capillary supply and metabolism of muscle fibers. Arch. Neurology 12:497.

Schmidt, G. R., L. Zuidam and W. Sybesma. 1972. Biopsy technique and analysis for predicting pork quality. J. Anim. Sci. 34:25.

Spurlock, G. M., G. E. Bradford and J. D. Wheat. 1962. The biopsy technique for estimation of meat quality in lambs. Proc. Rec. Meat Conf. 15:178.

Sreter, F. A., J. C. Seidel and J. Gergely. 1966. Studies on myosin from red and white skeletal muscles of the rabbit. J. Biol. Chem. 241:5772.

Steel, R. G. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.

Stein, J. M. and H. A. Padykula. 1962. Histochemical classification of individual skeletal muscle fibers of the rat. Am. J. Anat. 110-111:103.

Straub, F. B. 1942. Studies Inst. Med. Chem. Univ. Syeged 3:23.

Swanson, L. A., E. A. Kline and D. E. Goll. 1965. Variability of muscle fiber size in bovine longissimus dorsi. J. Anim. Sci. 24:97.

Swatland, H. J. and R. G. Cassens. 1972. A brief study of muscle enlargement in the rat. J. Anim. Sci. 34:21.

Szent-Györgyi, A. 1942. Studies Inst. Med. Chem. Univ. Syeged 1:17.

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

Weber, A. 1951. Muskelkontraktion und modellkontraktion. Biochem. et Biophys. Acta 7:214.

Weber, H. H. and H. Portzehl. 1954. Prog. in Biophys. and Biophys. Chem. 4:60.

Weiner, P. D., A. M. Pearson and B. S. Schweigert. 1969. Turbidity, viscosity and ATPase activity of fibrillar protein extracts of rabbit muscle. J. Food Sci. 34:303.

Wellington, G. H. 1953. Recommended procedure for cutting beef. Proc. Recip. Meat Conf. 6:73.

Wilson, G. D., W. E. Batterman, D. K. Sorensen, T, Kowalczyk and R. W. Bray. 1955. A biopsy technique and its use in study changes in muscle during fattening. J. Anim. Sci. 14:398.

Wirsen, C. and K. S. Larson. 1964. Histochemical differentiation of skeletal muscle in foetal and newborn mice. J. Embryol. Exp. Morph. 12:759.

# APPENDIXES

## APPENDIX A

## ATPASE STAINING PROCEDURE

Modification of Calcium Method (Padykula and Herman, 1955a,b).

1. Freeze tissue in isopentane that has been pre-cooled in liquid nitrogen and store frozen tissue on dry ice.
2. Cut cross-sections 6 to 8μ on a cryostat held at -20° C.
3. Fix in a cold formalin solution, 60 ml of 4 percent formalin and 40 ml of 0.9 percent NaCl, for five minutes and adjust pH to 7.0.
4. Wash 10 times with distilled water for a total time of two minutes.
5. Leach out the sodium pyrophosphate, 0.05 M, by soaking tissue in a solution containing 0.04 $CaCl_2$ in 0.02 M sodium barbitol buffer for 15 minutes (this step may not be required).
6. Incubate a series of sections for 15-minute time periods up to 60 minutes at 37° C and select the incubation period which yields the best stain. Prepare the incubating medium freshly as follows:

| | |
|---|---|
| 0.1 M sodium barbituate ($NaC_8H_{11}N_2O_3$) | 20.0 ml |
| 2 percent calcium chloride ($CaCl_2$) | 25.0 ml (62.5 ml)[a] |
| distilled water | 55.0 ml (17.5 ml) |
| ATPase (sodium salt) | 152.0 mg |

Adjust pH to 9.4 with 0.1N NaOH and 3 percent HCl.

7. Rinse in three changes of 1 percent $CaCl_2$.
8. Transfer into 2 percent cobalt salt solution (chloride or nitrate) for 3 to 5 minutes.
9. Wash in four changes of distilled water for a total period of one minute.

10. Treate with dilute ammonium sulfide, $(NH_4)_2S$, for 3 to 5 minutes.

11. Wash in tap water 3 to 5 minutes.

12. Dehydrate, clear in xylene and mount in a synthetic medium.

[a]Two percent $CaCl_2$ was increased 2 1/2 times to aid in preventing surface precipitation and wrinkling artifacts (Brooke and Kaiser, 1969).

## APPENDIX B

## STOCK SOLUTIONS

### 4% Neutral Formalin

| | |
|---|---|
| 40% Formalin | 10.00 ml |
| Distilled water | 90.00 ml |
| $Na_2HPO_4$ (Dibasic) | 0.65 g |
| $NaH_2PO_4$ (Monobasic) | 0.49 g |

### Physiological Saline

0.9% NaCl

Distilled water

### Staining Solutions

60:40, Formalin: Saline (neutral)

1%($CaCl_2$) Calcium chloride

2%($CaCl_2$) Calcium chloride

2%($CoCl_2$) Cobalt chloride

99%($CH_3CH_2OH$) Ethanol

Dilute $(NH_4)_2S$ Ammonium sulfide

### Clearing Solutions

30% Ethanol

60% Ethanol

99% Ethanol

50:50% Ethanol: Xylene

25:75% Ethanol: Xylene

100% Xylene

### Buffer Solutions

.1N (NaOH) Sodium hydroxide

.1N or 3%(HCl) Hydrochloric acid

# APPENDIX C

## Muscle Color

1. Black
2. Very dark red
3. Dark red
4. Moderately dark red
5. Slightly dark red
6. Cherry red
7. Very light cherry red

## Muscle Texture

1. Very coarse
2. Coarse
3. Slightly coarse
4. Slightly fine
5. Moderately fine
6. Fine
7. Very fine

## Muscle Firmness

1. Extremely soft
2. Very soft
3. Soft
4. Slightly firm
5. Moderately firm
6. Firm
7. Very firm

## Marbling Texture

1. Coarse
2. Medium
3. Fine

## Carcass Grade and Conformation Score

1.
2.
3.
4.
5. High Utility
6. Low Standard
7. Average Standard
8. High Standard
9. Low Good
10. Average Good
11. High Good
12. Low Choice
13. Average Choice
14. High Choice
15. Low Prime
16. Average Prime
17. High Prime

## Marbling Score

1. Devoid
2. Practically devoid
3. Traces
4. Slight
5. Small
6. Modest
7. Moderate
8. Slightly abundant
9. Moderately abundant
10. Abundant
11. Very abundant

## Maturity

1. -
2. A
3. +
4. -
5. B
6. +
7. -
8. C
9. +
10. -
11. D
12. +

Table 7 Coefficients of Correlation of Carcass Traits and Muscle Fiber Characteristics

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Carcass weight | 1 00 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | Carcass fat thickness | -0 27 | 1 00 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 | Estimated kidney fat | -0 19 | 0 58** | 1 00 | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 | Loin length | 0 45** | -0 47 | -0 11 | 1 00 | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | Loin weight | 0 82** | -0 03 | -0 03 | 0 33 | 1 00 | | | | | | | | | | | | | | | | | | | | | | |
| 6 | Rib eye area | 0 57** | -0 31 | -0 18 | 0 05 | 0 58** | 1 00 | | | | | | | | | | | | | | | | | | | | | |
| 7 | Marbling | -0 06 | 0 56** | 0 51** | -0 32 | 0 16 | -0 18 | 1 00 | | | | | | | | | | | | | | | | | | | | |
| 8 | Carcass grade | -0 07 | 0 40* | 0 56** | -0 39* | 0 15 | -0 00 | 0 78** | 1 00 | | | | | | | | | | | | | | | | | | | |
| 9 | Warner-Bratzler shear | 0 18 | -0 14 | -0 26 | 0 07 | -0 39 | 0 04 | -0 18 | -0 19 | 1 00 | | | | | | | | | | | | | | | | | | |
| 10 | Tenderometer | -0 14 | -0 09 | -0 02 | -0 12 | -0 19 | 0 00 | 0 01 | 0 04 | 0 05 | 1 00 | | | | | | | | | | | | | | | | | |
| 11 | Dressing percent | 0 45** | 0 21 | 0 11 | -0 24 | 0 35* | 0 38* | 0 29 | 0 23 | 0 08 | 0 02 | 1 00 | | | | | | | | | | | | | | | | |
| 12 | Yield grade | -0 31 | 0 75** | 0 55** | -0 15 | -0 18 | -0 80** | 0 49** | 0 28 | -0 09 | -0 09 | -0 03 | 1 00 | | | | | | | | | | | | | | | |
| 13 | Muscle firmness | 0 06 | -0 11 | 0 25 | 0 10 | 0 24 | 0 07 | 0 05 | 0 24 | -0 25 | -0 24 | -0 11 | -0 04 | 1 00 | | | | | | | | | | | | | | |
| 14 | Muscle texture | 0 18 | 0 06 | 0 12 | 0 20 | 0 20 | -0 11 | -0 01 | 0 10 | -0 01 | -0 18 | -0 08 | 0 19 | 0 52** | 1 00 | | | | | | | | | | | | | |
| 15 | Marbling texture | 0 21 | -0 11 | -0 45 | 0 06 | 0 01 | 0 15 | -0 02 | 0 06 | 0 17 | -0 13 | 0 19 | -0 12 | 0 28* | 0 37* | 1 00 | | | | | | | | | | | | |
| 16 | Kidney fat | 0 34* | 0 14 | 0 36* | 0 07 | 0 38* | 0 08 | 0 34* | 0 33 | -0 15 | 0 03 | 0 32 | 0 18 | 0 08 | -0 16 | -0 04 | 1 00 | | | | | | | | | | | |
| 17 | Rib eye area/100 lb carcass wt | -0 31 | -0 10 | -0 07 | -0 42* | -0 19 | 0 60** | -0 18 | 0 05 | -0 11 | 0 09 | 0 01 | -0 62** | -0 00 | -0 29 | -0 02 | -0 31 | 1 00 | | | | | | | | | | |
| 18 | Rib eye area/day of age | 0 02 | 0 01 | 0 00 | -0 39* | 0 11 | 0 73** | -0 00 | 0 19 | -0 10 | -0 07 | 0 31 | -0 56** | 0 08 | -0 26 | 0 20 | -0 07 | 0 84** | 1 00 | | | | | | | | | |
| 19 | Carcass weight/day of age | 0 50** | 0 15 | 0 04 | -0 14 | 0 50** | 0 56** | 0 22 | 0 27 | -0 01 | -0 26 | 0 55** | -0 20 | 0 15 | -0 08 | 0 37* | 0 28 | 0 18 | 0 68** | 1 00 | | | | | | | | |
| 20 | No of red fibers | -0 16 | -0 20 | -0 16 | -0 16 | -0 26 | 0 20 | -0 18 | 0 05 | 0 14 | -0 13 | -0 16 | -0 33 | -0 31 | -0 21 | 0 08 | -0 34** | 0 44** | 0 28 | -0 06 | 1 00 | | | | | | | |
| 21 | No of white fibers | 0 16 | 0 20 | 0 16 | 0 16 | 0 26 | -0 20 | 0 18 | -0 05 | -0 14 | 0 13 | 0 16 | 0 33 | 0 31 | 0 21 | -0 08 | 0 34** | -0 44** | -0 28 | 0 06 | -1 00 | 1 00 | | | | | | |
| 22 | Mean red fiber area | 0 14 | -0 04 | -0 17 | -0 27 | 0 03 | 0 36* | -0 21 | -0 05 | 0 23 | -0 19 | 0 38* | -0 29 | -0 18 | -0 08 | 0 05 | 0 20 | 0 33** | 0 43** | 0 34* | 0 19 | -0 19 | 1 00 | | | | | |
| 23 | Mean white fiber area | 0 11 | -0 00 | -0 11 | -0 27 | -0 08 | 0 38* | -0 22 | 0 00 | 0 23 | -0 18 | 0 41* | -0 29 | -0 21 | 0 05 | 0 31 | -0 17 | 0 38* | 0 40* | 0 22 | 0 44** | -0 44** | 0 75** | 1 00 | | | | |
| 24 | Total red fiber area | -0 00 | -0 11 | -0 16 | -0 29 | -0 14 | 0 84* | -0 21 | 0 01 | 0 23 | -0 22 | 0 18 | -0 35* | 0 33 | -0 16 | 0 08 | -0 31 | 0 46** | 0 46** | 0 22 | 0 69** | -0 69** | 0 83** | 0 80** | 1 00 | | | |
| 25 | Total white fiber area | 0 19 | 0 07 | -0 08 | -0 24 | 0 02 | 0 34* | -0 20 | -0 04 | 0 20 | -0 14 | 0 52** | -0 18 | -0 06 | 0 16 | 0 32 | -0 05 | 0 22 | 0 31 | 0 26 | -0 00 | 0 00 | 0 74** | 0 89** | 0 53** | 1 00 | | |
| 26, | Percent red fiber area | -0 11 | -0 25 | -0 23 | -0 16 | -0 17 | 0 23 | -0 20 | -0 01 | 0 16 | -0 15 | -0 18 | -0 38* | -0 30 | -0 32 | -0 11 | -0 37* | 0 44** | 0 34* | 0 06 | 0 86** | -0 86** | 0 41** | 0 30 | 0 80** | -0 09 | 1 00 | |
| 27 | Percent white fiber area | 0 11 | 0 25 | 0 23 | 0 16 | 0 17 | -0 23 | 0 20 | 0 01 | -0 16 | 0 15 | 0 18 | 0 38 | 0 30 | 0 32 | 0 11 | 0 37* | -0 44** | -0 34* | -0 06 | 0 86** | 0 86** | -0 41** | -0 30 | -0 80** | 0 09 | -1 00 | 1 00 |

*Significant, P < 05 level

**Significant, P < 01 level

## APPENDIX E

Table 8. Multiple Regression Equations - Partial Regression Coefficients Predicting Dependent Variables (Red Fiber Characteristics and White Fiber Characteristics)[a]

| Dependent Variable | Red Fiber Characteristics[b] | | | | | |
|---|---|---|---|---|---|---|
| | Constant | NRED ($X_1$) | MRFA ($X_2$) | TRFA ($X_3$) | PRFA ($X_4$) | $R^2$ |
| Carcass Traits | | | | | | |
| Fat color | 0.61 | 0.06 | 1.40 | -0.03 | -0.02 | 0.10 |
| Muscle color | 0.28 | 0.21 | 5.69 | -0.14 | -0.13 | 0.22 |
| Carcass length | 84.56 | 0.44 | 22.15 | -0.61 | 0.41 | 0.11 |
| Leg length | 50.13 | 0.62 | 15.29 | -0.36 | -0.30 | 0.07 |
| Loin length | 61.00 | -0.07 | -1.96 | -0.07 | 0.07 | 0.09 |
| Loin/100 lb. carcass wt. | 17.58 | 0.27 | 13.44 | -0.34 | 0.05 | 0.06 |
| Percent loin | 17.04 | -0.12 | -2.20 | 0.04 | 0.10 | 0.06 |
| Percent loin shrinkage | 0.07 | -0.00 | -0.02 | 0.00 | 0.00 | 0.10 |

| Dependent Variable | White Fiber Characteristics[b] | | | | | |
|---|---|---|---|---|---|---|
| | Constant | NWHITE ($X_1$) | MWFA ($X_2$) | TWFA ($X_3$) | PWFA ($X_4$) | $R^2$ |
| Carcass Traits | | | | | | |
| Fat color | 3.93 | -0.02 | -0.24 | 0.01 | -0.01 | 0.11 |
| Muscle color | 5.84 | -0.12 | -3.47 | 0.06 | 0.08 | 0.20 |
| Carcass length | 123.30 | 0.35 | 0.40 | 0.00 | -0.48 | 0.07 |
| Leg length | 96.26 | -0.64 | -18.37 | 0.31 | 0.13 | 0.12 |
| Loin length | 47.10 | 0.11 | 3.59 | -0.08 | 0.08 | 0.09 |
| Loin/100 lb. carcass wt. | 19.79 | 0.32 | 3.65 | -0.04 | -0.16 | 0.05 |
| Percent loin | 13.66 | 0.08 | 1.28 | -0.03 | -0.04 | 0.07 |
| Percent loin shrinkage | 0.08 | 0.00 | 0.00 | -0.00 | -0.00 | 0.11 |

Table 8. (continued)

[a]Models were selected on the basis of the highest $R^2$ value and level of significance.

[b]NRED = number of red fibers; MRFA = mean red fiber area; TRFA = total red fiber area; PRFA = percent red fiber area; NWHITE = number of white fibers; MWFA = mean white fiber area; TWFA = total white fiber area; PWFA = percent white fiber area.

# VITA

Jimmy Ted Keeton was born December 12, 1946, in Decatur County, Tennessee, to Mr. and Mrs. Ovid A. Keeton. He entered Parsons Elementary School in September, 1952, and later became an active 4-H club member with crop and livestock projects. Having a farm background influenced his decision to participate in F.F.A. activities while continuing with 4-H projects during high school. Being a member of the winning state 4-H Livestock Judging Team and serving as secretary of the Parsons F.F.A. chapter were two high school achievements. Upon graduating from Parsons High School in May 1964, he enrolled and attended the University of Tennessee, Martin from September, 1964 to June 1966. He transferred to the Knoxville campus in September 1966 and received the Bachelor of Science degree in Agriculture on August 24, 1968 with emphasis in two major study areas, animal husbandry and agricultural education. As an undergraduate, he was a member of the Ag-Club (Martin), the Block and Bridle Club, Society of Agricultural Education Students and student representative for the Student-Faculty Council.

A $300 scholarship was provided for graduate study in Animal Science. The Master of Science degree was conferred upon Jimmy T. Keeton March 19, 1973.

He served in the Army Medical Corps from January, 1969, to January, 1971, as an electrocardiogram technician and was stationed in Washington, D. C.

He is married to the former Jo Harriet Norsworthy of Newbern, Tennessee.