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I am submitting herewith a thesis written by Robert Edward Sutton, Jr., entitled "Histochemical Analysis of Bovine Longissimus as Related to Carcass Grades." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

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HISTOCHEMICAL ANALYSIS OF BOVINE LONGISSIMUS AS RELATED TO CARCASS GRADES

A Thesis

Presented for the

Master of Science

The University of Tennessee, Knoxville

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DEDICATION

This thesis is dedicated to the author's parents, Mrs. Catherine Sutton and Mr. Robert E. Sutton. Their undying love, support, and encouragement prevailed through the darkest hours. Without their help this paper would never have been a reality.

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ABSTRACT

Histological analysis was conducted on the longissimus dorsi muscle of sixty animals (one-half Hereford and one-half Angus). The study was conducted to ascertain the association between skeletal muscle fiber type, fiber size, fat cell size, and fat cell distribution with various carcass traits used to access quality and yield grade. Two reciprocal staining procedures, NADH-TR and ATP-ase, were used to study muscle fibers, where as an Oil Red O stain was employed to study fat cells. The diameter of both aerobic and anerobic fiber increased significantly ($P<.05$) between 56 and 84 days on feed. Also, all fiber size measurements were highly related ($P<.01$) to rib eye area. Fat cell diameter was found to be significantly correlated ($P<.01$) with carcass weight, rib eye area, percent kidney fat, fat thickness at 12th rib, U.S.D.A. yield grade, U.S.D.A. quality grade, maturity, marbling score, and fat by ether extract. Analysis of variance revealed a highly significant effect ($P<.01$) for days on feed as related to fat cell diameter. As days on feed increased, fat cell diameter also increased. It was noted that feeding the animals past 84-112 days did not increase fat cell diameter or any other histological trait. In an attempt to classify muscle samples into marbling degrees, a histological marbling score (HMS) was established after observation of stained fat cells. The HMS was found to be significantly correlated ($P<.05$) to actual marbling score and U.S.D.A. quality grade.

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CHAPTER I

INTRODUCTION

The era of the future will be one of efficiency. As the population increases, the food industry will be called upon to feed the increasing masses with as little loss in quality as economically feasible. The livestock industry as a whole, must constantly strive for technological advances to improve status quo procedures. One such area could be the use of an animal's histological skeletal muscle traits as a predictor of meat quality.

Histological evaluation of skeletal muscle tissue has proven very valuable in research applications in regard to fiber type (Melton et al., 1974), intramuscular fat (Jeremiah et al., 1970) capillary distribution (Melton et al., 1975; Cooper et al., 1969) and sarcomere length (Locker, 1960; Covington et al., 1970). However, practical application of these factors has yet to be demonstrated. Melton (1975) cited the need for further research to determine how histochemical and histological muscle traits are related to muscle quality and cutability. If these factors can be correlated then histological procedures could become an integral part of predicting muscle quality and cutability in the live animal. Therefore, the purpose of this study was to determine the association of percentage skeletal muscle fiber type, fiber size, intramuscular fat cell distribution, and relative fat cell size with the various carcass traits used to assess U.S.D.A. quality and yield grades. This was an attempt to classify bovine muscles into

quality grade groups using histochemically determined muscle and fat cell traits. If this relationship between carcass traits and histochemical determined traits can be established then perhaps it will be useful in breeding programs. Bulls could be sampled by an accepted biopsy procedure (Bennett and Harris, 1970; Everett, 1960; Link et al., 1970) and later tested histochemically to determine their value as future herd sires.

CHAPTER II

LITERATURE REVIEW

Webster's defines histology as a branch of anatomy that deals with the minute structure of animal and plant tissues as discernible with the microscope. This general definition appears simple by today's standards, yet it bewildered man in past centuries and fascinates him today.

It was first learned that normal muscle was heterogeneous and was composed of different fiber types (Ranvier, 1873; Denny-Brown, 1929). Since that time histological analysis has been employed to study different fiber types in muscle. George and Naik (1958) discerned two different distinct fibers in pigeon's pectoral muscles. One fiber, later named as white, was low in mitochondria content, low in myoglobin and of a smaller surface area than the other type. The second distinct fiber, later classified as red, was high in mitochondria content, high in stored fat, high in myoglobin and larger in overall area. However, another study conducted by Dubowitz and Pearse (1960a) discovered a reciprocal relationship in size between histologically determined red and white fibers. They found the mitochondria rich, red fiber to be of smaller size than the mitochondria poor, white fibers. Also, they found that the red fibers were rich in oxidative enzymes and poor in phosphorylase and that the opposite was true for the white fibers. However, the detailed analysis of individual fibers in serial cross sections of muscle

demonstrated at least three, rather than two, broad categories of muscle fibers (Guth and Samaha, 1969; Samaha et al., 1970).

Stein and Padykula (1962) were the first to employ the ATP-ase test to differentiate muscle fiber. They elucidated to the existence of at least three muscle fibers with their A fiber resembling the classical white fiber while B and C represented the classical red and the mysterious intermediate fiber respectively. (A comparison of Red, White and Intermediate fibers is represented in Table 1). Ogata and Mori (1964) studied, extensively, oxidative enzymes in vertebrates. The larger white fibers showed lower activity with the smaller red fibers showing higher incidence of oxidative enzymes activity. Also, they referred to a third fiber type as being intermediate in respect to oxidative enzyme activity.

Romanul (1964) postulated on the existence of as many as eight fiber types in the gastrocnemius muscle of the rat.

Many researchers tend to disregard this and retain the three fiber type theory. Even though the role of the intermediate fiber is not fully understood, it appears to have the ability of transformation (Ashmore and Doerr, 1971a; Ashmore and Doerr, 1971b).

The number of muscle fibers in a particular muscle of an animal is relatively fixed at birth (Rowe and Goldspink, 1969). Since white fibers are initially red at birth, the accumulation of white fibers after birth must result from transformation. Transformation of the white fiber is theorized as being concerned with energy producing enzymes. As the percentage of mitochondria decreases, glycogen

TABLE I

CHARACTERISTICS OF RED, WHITE AND INTERMEDIATE MUSCLE FIBERS

Characteristic	Red Fiber	Intermediate Fiber	White Fiber
Color	Red	Red	White
Myoglobin Content	High	High	Low
Fiber Diameter	Small	Small-Im[a]	Large
Blood Supply	High	Im	Low
Contraction Speed	Slow	Fast	Fast
Contractile Action	Tonic	Tonic	Phasic
No. of Mitochondria	High	Im	Low
Mitochondrial Size	Large	Im	Small
Capillary Density	High	Im	Low
Metabolism	Aerobic	Aerobic and Anaerobic	Anaerobic
Oxidative Metabolism	High	Im	Low
Glycolytic Metabolism	Low	Im	High
Phosphorylase	Low	Im	High
Lipid Content	High	Im	Low
Glycogen Content	Low	High	High
ATP-ase Stain pH 9.4	Light (-)[b]	(+)[c]	Dark (+)
DPNH-TR	Dark (+)	Light (-)	Light (-)

[a]Im means intermediate response in regard to red and white fiber meats.

[b](-) means a negative reaction for that particular test.

[c](+) means a positive reaction for that particular test.

phosphorylase increases as indigative of white fiber (Bass, et al., 1969). Also, it was reported that there was fewer incidence of red fibers in less active porcine, ovine and bovine (Ashmore and Doerr, 1970). Whether exercise directly controls fiber transformation is not fully known. Increased exercise results in the increase of mitochondria and oxidative enzymes in white muscle fibers of adult animals thus, indicating a possible change to red fibers (Kowalski et al., 1969). Also, mitochondria extracted from routinely exercised muscle exhibited a decrease in ability to oxidize a-glycerol phosphate and an increase in ability to oxidize palmitate (Hallosgy and Oscai, 1969). These previous conditions are known to be associated with red fiber mitochondria, giving way to a possible degree of white fiber reversile.

Muscles used by animals to maintain posture or those which are used most frequently have a higher portion of red fibers. Consequently, muscles which are inactive by comparison (i.e., longissimus dorsi) have a higher incidence of white fibers. The pectoralis muscle of the chicken is almost entirely composed of white muscle fibers, where as flying birds have a higher portion of red fibers (Ashmore and Doerr, 1971a). As mentioned earlier, white fibers are larger than red fibers primarily due to their mode of metabolism. Red fibers require a continuous source of oxygen and nutrients, and a continuous waste removal system to facilitate its role of repetitive contraction. This role of continual energy production is best accomplished by small fiber size and high capillary to fiber ratios. Decreased exercise means decreased blood flow to the fiber and increased fiber size, exhibiting characteristics of white muscle fibers.

Moody and Cassens (1968) used diphosphopyrdine nucleotide tetrazdium reductase (DPNH-TR) to study different fiber types. The red fibers show a positive reaction for the test thus, staining the darkest. They also reported the existence of numerous intermediate fibers in the longissimus dorsi of porcine. In 1969, Cooper et al., further reported a higher incidence of intermediate fibers in the longissimus dorsi muscle of stress-susceptible porcine. They suggested that this higher incidence of intermediate fibers may be linked to the condition known as PSE (pale, soft and exudative). Conversely, Dildey et al. (1970), and Merkel (1971) proposed that the PSE condition was associated with an increase in the proportion of white fibers. In a test of stress-susceptible hogs, Swatland and Cassens (1972) observed that there was either an increase in the proportion of intermediate fibers or else an increase in the proportion of white fiber.

Another study conducted at the University of Kentucky by Johnson et al. (1975), investigated the effect of breed and time on feed on the fiber diameter distribution and size of individual fiber types. Thirty-six Angus and 36 Charolais steers of similar ages, weights and nutritional regimes were used. Half of each breed were slaughtered after 153 days on feed (Group I) while the remainder were slaughtered after 233 days on feed (Group II). One of the main points noted in the paper was the significantly larger (14%) mean fiber diameter increase in Group II as compared to Group I. Also, the percentage of area occupied by red fibers was somewhat larger in Group II but not on a ($P<.05$) significant level. In general, the breed comparisons revealed that the Charolais

cattle had larger mean fiber diameters for all fiber types compared with the Angus. This increase was not surprising since the Charolais cattle gained faster and were more muscular than their Angus counterparts.

Lewis et al. (1977), at the University of Arkansas, investigated the relationship of tenderness with fiber diameter and sarcomere length. Seventy-six steers all sired by Hereford, Angus, Santa Gertrudes and Charolais bulls mated to Hereford and Angus cows were used as experimental animals. The steers were slaughtered at approximately 454 kg body weight. The information reported indicated that breed of sire and breed of dam will affect fiber diameter and sarcomere length of the offspring. However, breed of dam and breed of sire had little effect on the tenderness of these same muscles. Also, there was a low correlation between fiber diameter and panel tenderness scores. Lewis et al., also speculated that the selection of animals for larger fiber diameter (more muscling) may have small effect on tenderness of the muscle.

In another study at Kansas State University, May et al., (1977) compared muscle fibers of the longissimus muscle from different types of crossbred steers to carcass and meat palatability traits. Twelve Simmental X Angus crosses, 12 Hereford X Angus crosses and 12 Limousin X Angus crossbred steers finished on the same predominately corn silage ration, were slaughtered in three lots (4 animals of each per lot) at 42 days apart. They found that the white fibers tended to be larger in diameter than the red fibers as reported by Padykula and Gauthier (1970) and Johnston et al. (1975). However, low and nonsignificant ($P>.05$)

correlation coefficients were obtained between carcass composition traits and proportion of fiber types. The diameter of red fibers was positively ($P<.05$) correlated with percentage of carcass fat trim and yield grade. The diameter of white fibers was not significantly associated ($P>.05$) with carcass composition traits.

Correlation coefficients between histological characteristics and indices of meat quality and palatability were generally low and nonsignificant except for diameter of white fibers which was positively ($P<.05$) related to tenderometer values.

Another aspect which has received wide attention in recent years is the histological determination of fat. Moody and Cassens (1968b) studied fat cell size and its distribution in muscles. They used muscles with varying degrees of marbling: traces, small and moderate. They then divided the fat cell masses into the following categories: 5-10 cells per group, 11-20 cells per group, and 20 and over cells per group. They discovered that as a fat cell mass increased in number, the size of the fat cells themselves increased. Consequently, the fat cells of the higher marbled cattle were larger than those of the lower marbled group. Subcutaneous fat cells were larger than intermuscular fat cells and that intramuscular fat cells were smaller than the intermuscular fat cells. This agrees with earlier work by Waters (1909) that intramuscular fat cells were small by comparison to other fat cells in the body.

Melton et al. (1974, in a study at Kansas State conducted biopsies (according to procedures by Wilson et al., 1955; Brisler et al., 1960) on the longissimus dorsi muscle of 21 Hereford yearling bulls for

histological analysis. He found that percentage of intramuscular fat or marbling determined histologically was significantly ($P<.05$) related to U.S.D.A. quality grade. He agreed with Jeremiah et al. (1970), who found a similar relationship. Melton et al., also reported that although it approached a significant level ($P=0.41$), there was no significant correlation between percent intramuscular fat and marbling score. Also, the correlation coefficient between intramuscular fat and percent ether extractable fat was low ($P=0.18$). They also found no significant correlation between fiber diameter and carcass composition, meat palatability or muscle proximate analysis. Brady (1937) and Tuma et al. (1962), also observed a low relationship between fiber diameter and tenderness for animals of similar age. However, other workers reported that as fiber diameter increased, tenderness decreased (Carpenter et al., 1962; Herring et al., 1965a). Melton et al., also postulated that the small degree of variation between the animals used could explain the low correlations.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

Histological analysis was conducted on sixty animals (one-half Hereford and one-half Angus steer calves). These animals were obtained from Tennessee graded feeder calf sales and wintered at the Plateau Experiment Station near Crossville, Tennessee. The cattle were divided into six lots of ten animals each. The first sample lot obtained was strictly forage fed. However, the remaining five lots were finished on grain with a time period of 28 days between lots. Consequently, the sixth lot of cattle had the most time on grain of 140 days. The test animals were fed ad libitum on a ration of U.S. No. 2 corn supplemented with Tend-R-Lean and dicalcium phosphate (IRN 6-01-080). Their pasture consisted of (IRN 4-02-931) three different types of grasses: Kentucky 141 fescue, ladino clover, and orchard grass.

The varying nutritional levels of the cattle produced considerable variation in quality grades which ranged from standard to prime. This wide range of grades was important in studying the relationship between histological traits and those carcass traits which indicate quality and yield.

Experimental Procedure

Following slaughter of the animals, the carcasses were chilled 48 hours at 35° F.. Sample steaks were taken from the longissimus dorsi

muscle between the 12th and 13th rib. The muscle samples used for making histological slides were then removed from the steaks approximately two inches from the midline of the animals. Muscle samples taken from this area would simulate the biopsy operation in a living animal. Bennet and Harris (1970) and others have shown the biopsy procedure to be safe and practical. Liquid nitrogen can be used to anaesthetize the skin with little apparent discomfort to the animal.

The samples were then cut into small pieces roughly one-half inch long and one-fourth inch in diameter and frozen and stored in liquid nitrogen to await sectioning. A quick freeze was necessary to prevent rupture of the muscle fibers which would result in freezing damage. This was accomplished by powdering the unfrozen samples in talcum powder to reduce the vapor barrier, thus hastening freezing. Also an understanding of the orientation of the fibers was essential in obtaining a proper transverse section.

Prior to cutting the histological sections, the sample were removed from the liquid nitrogen tank and allowed to adjust at -20°C., for one hour. This was done to ensure better sections because the samples immediately out of the liquid storage tank (around -273°F.) are very brittle and tend to crack on impact with the knife.

After the samples were adjusted, they were mounted on a metal chuck using a gelatin imbedding matrix. This material freezes quickly at -20°C. (internal temperature of the cryostat chamber) and is shown to be easier to use than its forerunner histowax which is solid at room temperature. Samples were then cut on a Lipshaw model 1500 microtome

using a blade angle of 33 degrees. The samples were cut at a thickness of 10 μ with at least three randomly chosen samples cut to represent each animal. After serial sections were obtained and mounted, three staining procedures were used.

The first staining procedure employed was Padkula and Herman's ATPase test as revised by Brook and Engels (1966). (See Appendix.)

The sections were incubated in a buffered medium at pH 9.4 containing equine ATP and calcium ions. The orthophosphoric acid contained in the ATP was then precipitated by the calcium ions. The calcium phosphate precipitate was then transformed into a visible form by treatment with a colbalt chloride solution and the resulting cobalt phosphate visualized as black cobalt sulfide after treatment with ammonium sulfide.

The pH of the incubating medium was adjusted to 9.4 with 0.1 molar NaOH because this corresponds to the pH optimum of most alkaline phosphatases. Below pH 9.0 the intensity of the reaction decreases and because of the increased solubility of calcium phosphate localization is influenced. White fibers show a positive reaction for this test (they stain the darkest) because they have higher glycogen and phosphorlase levels than red fibers and because they use anaerobic metabolism.

The second test conducted was NADH-TR (Reduced diphosphopyridine nucleatide--tetrazolium reductase). (See Appendix.) In this stain the tetrazolium salt, nitroblue acts as a hydrogen acceptor. DPNH is generated by the specific dehydrogenase and is transformed to diaphorase. The tetrazolium salt reacts with the diaphorase to form formazon, and it will localize the diaphorase system which is connected to the specific

dehydrogenase. The activity will thus be revealed. The red fibers will show positive for this reaction due to their aerobic mode of metabolism corresponding to their high blood supply and low content of glycogen.

The two enzyme techniques, ATP-ase and NADH-TR, distinguished the fibers with the positive stain of one technique being the negative stain of the other. This is crucial in fiber typing as demonstrated by Ashmore and Addis (1972).

The final test conducted was an Oil Red O stain. This test was fairly simple and straightforward. The fat cells absorb the Oil Red O stain mixture and assume an orange color. However, to better determine the fat cells from the muscle cells, a counter stain was employed involving Delafields Hematoxylin. This solution stains all muscle fibers blue but does not effect the fat cell color as distinguished by the Oil Red O solution.

After proper sections were obtained, photographs were made using a Wild M20 stage microscope. These were used in determining fiber percentage, fiber size and fat cell size. The ATP-ase negatives were then processed and enlarged into eight inch by ten inch photographs. Instead of counting the fibers within a certain grid area on the picture, all fibers which were completely in the photographic area were counted. Approximately 125 fibers per sample were analyzed by the "best fit" method on the Zeiss Particle Size Analyzer. This method was used to estimate diameter and thus to calculate fiber area. Also, relative percentages of red and white fibers were obtained to be compared to fiber percentages obtained from the NADH-TR stain.

The NADH-TR negatives were placed in slide mounts and projected on a screen to obtain fiber percentages. The same method, mentioned earlier in counting the ATP-ase fibers, was employed. However, only percentages of fiber types were obtained from the aerobic stain. All size measurements on muscle fibers were obtained from the ATP-ase stain.

The fat cells, as determined by the Oil Red O Stain, were measured using a Wild M20 microscope. Approximately 25 fat cells per sample (when detectable) were measured by the use of an eye piece micrometer, and the average recorded. In an attempt to classify the samples into marbling grades, the Oil Red O slides were coded and placed randomly into a slide box. Upon viewing at 40X, a trained observer was then asked to classify these samples into marbling degrees using the scale in the Appendix. Subjective observations were made of fat cell size, number of fat cells per loci and number of loci in assigning a marbling degree score.

These histological data were analyzed by analysis of variance to determine days on feed, breed, and type score effects as well as interactions among the main effects. Duncan's multiple range test was used to establish significance among means. Orthagonal polynomial analysis was also performed to determine if significant linear, cubic, or quadratic effects were present. Simple correlation coefficients were calculated to determine relationships among histological, carcass, and chemical traits. These analyses were performed using the Statistical Analysis System programs by Barr et al. (1976).

CHAPTER IV

RESULTS AND DISCUSSION

Means and standard deviations of histological traits studied are presented in Table II. As days on feed increased from zero to one-hundred forty days, the percent NADH-TR positive fibers tended to decrease while NADH-TR negative fibers increased. This agrees with earlier work by Rowe and Goldspink (1968) who suggested that as animals matured fiber transformation occurred due to energy producing enzymes. However, the reciprocal ATP-ase stained fibers had a different relationship to time on feed. The percent ATP-ase negative fibers increased in a linear fashion from zero to one hundred and twelve days. At the 112 day period it was observed that some of the cattle in this group were sick and showed decreased feed consumption. This may explain the break in trends for this particular variable as well as others which will be discussed. At 140 days on feed the percent ATP-ase negative fibers were slightly higher than at 84 but according to Duncan's multiple range test in Table III not at a significant level. The ATP-ase positive fibers showed an increase ($P<.05$) at 112 days (Table III) and then a decrease at 140 days.

The ATP-ase and NADH-TR stains are, supposedly, reciprocal tests of one another. However, differences can occur. The NADH-TR positive and the ATP-ase negative fibers represent those muscle fibers which primarily use an aerobic mode of metabolism where as NADH-TR negative and ATP-ase positive fibers represent those which primarily use an

TABLE II

MEANS AND STANDARD DEVIATIONS OF HISTOLOGICAL TRAITS AS INFLUENCED BY DAYS ON FEED

Histological Traits	Days on Feed (N=10)												Overall	
	0		28		56		84		112		140		Mean	C.V.[1]
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
NADH-TR(+)[2] Fibers (%)	32.3	5.3	30.6	4.5	34.3	6.2	35.2	6.4	24.8	2.7	24.8	5.0	30.4	21.3
NADH-TR (-)[3] Fibers (%)	67.7	5.3	69.4	4.5	65.7	6.2	63.8	5.9	75.2	2.7	75.2	5.0	69.4	9.4
ATP-ase (-) Fibers (%)	27.6	5.6	28.8	4.0	30.9	5.7	32.6	3.6	25.9	6.5	32.8	4.4	29.7	18.5
ATP-ase (+) Fibers (%)	72.4	5.6	71.2	4.0	69.1	5.8	67.4	3.6	74.0	6.5	67.6	4.2	70.3a	7.8
ATP-ase (-) Fiber Dia.[4]	49.9	6.6	42.6	3.2	39.6	5.4	48.2	5.6	53.3	7.9	50.3	6.9	47.3	15.9
ATP-ase (+) Fiber Dia.[4]	54.7	7.5	48.8	4.5	47.8	4.9	55.5	5.1	60.7	7.3	58.3	6.5	54.2	13.8
ATP-ase (-) Fiber Area[5]	1.9	0.5	1.4	0.2	1.3	0.4	1.8	0.4	2.3	0.7	2.0	0.7	1.8	32.4
ATP-ase (+) Fiber Area[5]	2.4	0.7	1.9	0.4	1.8	0.4	2.3	0.3	2.8	0.7	2.7	0.6	2.3	27.5
Histological Marbling Score[6]	10.9	2.1	9.3	3.02	15.1	4.4	15.6	4.9	13.8	5.3	16.2	5.0	13.4	35.8
Fat Cell Dia.[4]	40.1	4.2	40.9	6.3	59.1	15.1	77.8	14.8	77.5	9.1	80.3	13.5	62.3	32.7

1 = Coefficient of Variation
2 = (+) A positive reaction for that test
3 = (-) A negative reaction for that test
4 = Fiber and fat cell diameter expressed in microns.
5 = Actual area in mm^2 obtained by multiplying by 10^{-3}
6 = Histological Marbling Score = 30 unit scale; 30 = AB^+, 1 = D^-

TABLE III

DUNCAN'S MULTIPLE RANGE TEST FOR MEANS OF HISTOLOGICAL TRAITS
AS INFLUENCED BY DAYS ON FEED

Histological Traits	Days on Feed (N=10)					
	0	28	56	84	112	140
NADH (+)[1] Fibers (%)	32.3[a]	30.6a	34.3a	35.2a	24.8b	24.8b
NADH (-)[2] Fibers (%)	67.7[c]	69.4[b]	65.7[bc]	63.8[c]	75.2[a]	75.2[a]
ATP-ase (-) Fibers (%)	27.6[ab]	28.8[ab]	30.9[ab]	32.6[a]	25.9[b]	32.8[a]
ATP-ase (+) Fibers (%)	72.4[ab]	71.2[ab]	69.1[ab]	67.4[b]	74.0[a]	67.6[b]
ATP-ase (-) Fiber Dia.[3]	49.9[a]	42.6[b]	39.6[b]	42.8[a]	53.3[a]	50.3[a]
ATP-ase (+) Fiber Dia.[3]	54.7[b]	48.8[c]	47.8[c]	55.5[ab]	60.7[a]	58.3[ab]
ATP-ase (-) Fiber Area[4]	1.98[a]	1.4[bc]	1.3[c]	1.8[ab]	2.3[a]	2.0[a]
ATP-ase (+) Fiber Area[4]	2.4[a]	1.9[bc]	1.8[c]	2.3[ab]	2.8[a]	2.7[a]
Histological Marbling Score[5]		9.3[c]	15.1[a]	15.6[a]	13.8[ab]	16.2[a]
Fat Cell Diameter[3]	40.1[c]	40.9[c]	59.1[b]	77.8[a]	77.5[a]	80.3[a]

1 = (+) A positive reaction for that test
2 = (-) A negative reaction for that test
abc - Means in the same row followed by the same superscript letter are not different ($P<.05$)
3 = Fiber and fat cell diameter expressed in microns
4 = Actual area in mm^2 obtained by myltiplying by 10^{-3}
5 = Histological Marbling Score = 30 unit scale; 30 = AB^+, 1 = D^-

an anaerobic mode of metabolism. Differences could be due to intermediate fibers, or those which are intermediate in regard to their mode of metabolism, could cause a discrepancy by showing enough reaction to the NADH-TR test to be counted as aerobic fibers. This reaction would not be as strong in reverse in the ATP-ase test thus giving a different positive or negative count. These data are somewhat related to work by Bocek and Beatty (1966) who reported problems in these reciprocal staining procedures.

Even though differences occurred in the groups, the overall means of the two tests were similar. Also, related percentages of ATP-ase fibers agrees with earlier work by Romanohanaro (1968). He observed averages of 32 aerobic and 68 anaerobic fibers per 100 for the longissimus dorsi muscle.

Analysis of variance revealed a highly significant relationship ($P<.01$) between percent fiber types and days on feed. (Table IV). Also, there was a difference in type score among the days on feed groups. The type score number was a measure of maturity with respect to frame score. Animals were grouped into five different categories based on subjective and objective evaluation. Subjective evaluation included visual observation, where as objective evaluation included fat thickness and wither height. The type score number was assigned to each animal before the start of the test. Both ATP-ase fiber types showed a highly significant effect ($P<.01$) for type score, where as percent NADH-TR positive and negative fibers showed no significant type score effect.

TABLE IV

ANALYSIS OF VARIANCE OF HISTOLOGICAL TRAITS AS EFFECTED DAYS ON FEED BREED ON TYPE SCORE

		Mean Squares									
Source	Degrees of Freedom (DF)	NADH-TR (+)[e] Fibers %	NADH-TR (-)[f] Fibers %	ATP-ase (-) Fibers %	ATP-ase (+) Fibers %	Fiber Dia. (-) ATP-ase[a]	Fiber Dia. (+) ATP-ase[a]	Fiber Area (-) ATP-ase[b]	Fiber Area (+) ASTP-ase[b]	Fat Cell Dia.[a]	Histo. Marbl. Score[c]
Days on Feed	5	199.6**	218.1**	75.9**	72.7**	268.4**	258.3**	1.49**	1.7**	3623.9**	77.0**
Breed	1	--[g]	--	--	--	188.7*	138.34*	1.03*	--	628.2*	--
Type Score[h]	1	--	--	186.6**	173.6**	148.7*	334.4**	--	1.82**	--	--
Error	52	26.6	25.9	22.43	22.5	31.7	28.9	0.2	0.24	126.2	17.3

a = Fiber and fat cell diameter expressed in microns
b = Actual area in mm^2 obtained by multiplying by 10^{-3}
c = Histological Marbling Score = 30 unit scale; 30 = AB^+, 1 = D^-
e = (+) A positive reaction for that test
f = (-) A negative reaction for that test
g = (--) Nonsignificant at the .05 level (P>.05)
h = This score was assigned after a visual observation of each calf before the start of the test

* = Significant at the .05 level (P<.05)

** = Significant at the .01 level (P<.01)

Simple correlation coefficients are presented in Table V. The most striking observation is the variation between the correlation coefficients of the two reciprocal stains. The percent NADH-TR fibers showed highly significant correlation coefficient ($P<.01$) for carcass weight, rib eye area (REA), fat thickness at 12th rib, yield grade, maturity, and lean texture. However, the percent ATP-ase fibers only showed a significant relationship ($P<.05$) for yield grade. This disagrees with work by May et al. (1977), who found no association between percent fiber types and carcass traits.

The percent ATP-ase positive fibers showed a significant negative ($P<.05$) correlation with respect to yield grade number where as its counterpart (NADH-TR negative) in the NADH-TR stain showed a highly significant positive ($P<.01$) correlation with respect to yield grade number. Again, intermediate fiber interference could explain this difference in percent fiber types for the two staining procedures, but the statistical model employed could also account for some of the differences in correlation coefficients. The correlation procedure used measured correlation coefficients across days on feed groups. Since the groups were slaughtered over a 140 day period at different feeding levels, the groups were not as homogenous as they would be if they had been slaughtered at the same time.

Means and standard deviation for fiber sizes are also presented in Table II. Aerobic fibers, ATP-ase negative, tended to decrease in size as days on feed increased. However, there was a significant increase in diameter (Table III) between 56 and 84 days for both ATP-ase

TABLE V

SIMPLE CORRELATION COEFFICIENTS

	NADH (+)[1] Fibers %	NADH (-)[2] Fibers %	ATP-ase (-) Fibers %	ATP-ase (+) Fibers %	Fiber Dia. (-) ATP-ase	Fiber Dia. (+) ATP-ase	Fiber Area (-) ATP-ase	Fiber Area (+) ATP-ase	Fat Cell Dia.	Histo. Marbl. Score
Carcass weight (48 hour chill)	-.41**	0.42**	0.06	-.05	0.28*	0.41**	0.28*	0.37**	0.61**	0.26*
Rib eye area	-.44**	0.44**	-.07	0.08	0.36**	0.46**	0.36**	0.40**	0.46**	0.15
% Kidney fat	-.32*	0.34**	0.15	-.14	0.14	0.16	0.14	0.12	0.51**	0.20
Fat Thickness at 12th Rib	-.35**	0.33**	0.17	-.16	0.14	0.29*	0.12	0.30*	0.69**	0.39**
Yield Grade	-.33**	0.33**	0.26*	-.25*	0.03	0.15	0.03	0.18	0.66**	0.37**
Quality Grade	-.27*	0.28*	0.07	-.05	0.16	0.24	0.16	0.19	0.51**	0.26*
Maturity	-.35**	0.35**	0.15	-.13	0.20	0.27*	0.19	0.26*	0.49**	0.29*
Marbling Score	-.06	0.001	0.06	-.06	0.21	0.19	0.20	0.15	0.33**	0.30*
Lean Color	-.24	0.27	-.14	0.15	0.13	0.08	0.13	0.09	0.32*	0.15
Lean Texture	-.49**	0.27	-.17	0.18	0.10	0.005	0.13	0.04	0.11	-.05
Fat on Ether Extract	-.30*	0.32*	0.13	-.11	0.21	0.28*	0.18	0.27*	0.34**	0.10
Moisture	0.04	-.06	0.11	-.12	-.22	-.17	-.19	-.15	-.03	-.18

1 = (+) A positive reaction for that test
2 = (-) A negative reaction for that test

* = Significant at the .05 level (P<.05)

** = Significant at the .01 level (P<.01)

negative and ATP-ase positive fibers. Aerobic fibers do tend to decrease in size as an animal gets older, especially in inactive animals (Ashmore and Doerr, 1970). However, the results of the present study agree somewhat with a study by Johnson et al. (1975), who found that aerobic fibers occupied a larger percentage of area as days on feed progressed but not significantly. Also, the area of the different fibers varied similarly to the diameter of the fibers. This is understandable since the area was mathematically derived from the measured diameter.

The areas of aerobic and anaerobic fibers measured $1.8 \times 10^{-3} mm^2$ and $2.3 \times 10^{-3} mm^2$, respectively (Table II, page 17). These results were smaller than those determined by Melton et al. (1974). The reason might be explained since they used a compensating polar planimeter to measure the outside border of the fibers where as in this study a particle size analyzer was used. Their area would be larger because they traced the entire area of the fiber. The particle size analyzer utilizes the "best fit" method which would tend to reduce fiber area. Also, Melton et al. (1974), used 21 Hereford bulls slaughtered at the same age and time where in this study animals were slaughtered during a 140 day period at various feeding levels and were younger at slaughter.

Analysis of variance revealed significant ($P<.05$) breed effects for both ATP-ase fiber type diameters (Table IV). Both anaerobic and aerobic fiber diameters and areas were highly significant ($P<.01$) as respect to days on feed. Aerobic fiber area revealed a significant ($P<.05$) effect for type score but no significant effect for breed. Since the type score was a measure of maturity, and frame size differences could

be explained by earlier maturity among the Angus steers as compared to the Hereford steers (Brown et al., 1972). Lewis et al. (1977), found a similar relationship among Angus and Hereford steers.

In Table V, anaerobic fiber diameter and area were highly correlated with carcass weight ($P<.01$). Aerobic fiber diameter and area were also significantly related to carcass weight but only at the ($P<.05$) level. However, all fiber size measurements were highly correlated to rib eye area ($P<.01$). Anaerobic fiber diameter and area were significantly correlated ($P<.05$) to maturity score where as aerobic fiber size measurements were not related. This agrees with earlier work by Bendall and Voyle (1967) who reported increases in anaerobic fiber diameter with advanced age in cattle.

Means and standard deviations of fat cell diameter are presented in Table II, page 17. Fat cell diameter increased substantially between the second and third lot (28 and 56 days respectively, Table III, page 18) with another significant increase ($P<.05$) between the third and fourth lots (56 and 84 days respectively, Table III, page 18). There was no change between lots four and five and no significant increase in lot six. It is interesting to note that fiber diameter was larger than fat cell diameter at the onset of the study, 49.9μ and 40.1μ respectively. However, as days on feed progressed, fat cell diameter increased considerably more than fiber diameter (Table II, page 17). Analysis of variance (Table IV) revealed a highly significant relationship ($P<.01$) between fat cell diameter and days on feed. Also, there was a significant relationship ($P<.05$) for breed effect as related to fat

cell diameter. The Angus cattle exhibited large fat cell diameter as compared to Hereford. This could be accounted for by earlier maturity in the Angus.

Simple correlation coefficients listed in Table V showed a positive relationship for fat cell diameter with carcass traits. Fat cell diameter was highly significant ($P<.01$) as related to carcass weight, rib eye area, percent kidney fat, fat thickness at 12th rib, U.S.D.A. yield grade, U.S.D.A. quality grade, maturity, marbling score, and fat by ether extract basis. Melton et al. (1974), observed a low correlation between percent ether extractable fat and intramuscular fat. Fat cell diameter was also significantly correlated ($P<.05$) with lean color. This indicates that as fat cell diameter increased, the lean color became brighter red in color.

Means and standard deviations for the histological marbling score (HMS) are listed in Table II, page 17. This score as mentioned earlier, was assigned by a trained observer as described in the Appendix. It is interesting to note that the HMS increased significantly ($P<.05$, Table III, page 18) between the second and third lots. However, there was no significant change for lots three, four, five, or six. Again, a slight break in trend occurred at the 112 day lot but not significantly.

Table V lists the correlation coefficient of the HMS with selected carcass traits. The HMS was highly correlated ($P<.01$) with yield grade and fat thickness at the 12th rib. It was also correlated ($P<.05$) with carcass weight, U.S.D.A. quality grade, maturity and marbling score. The most important aspect was the HMS was related significantly to U.S.D.A.

quality grade and the factors used in determining quality grade. This relates somewhat to Melton et al. (1974), who found a significant relationship ($P<.05$) between percent intramuscular fat and U.S.D.A. quality score.

CHAPTER V

CONCLUSION

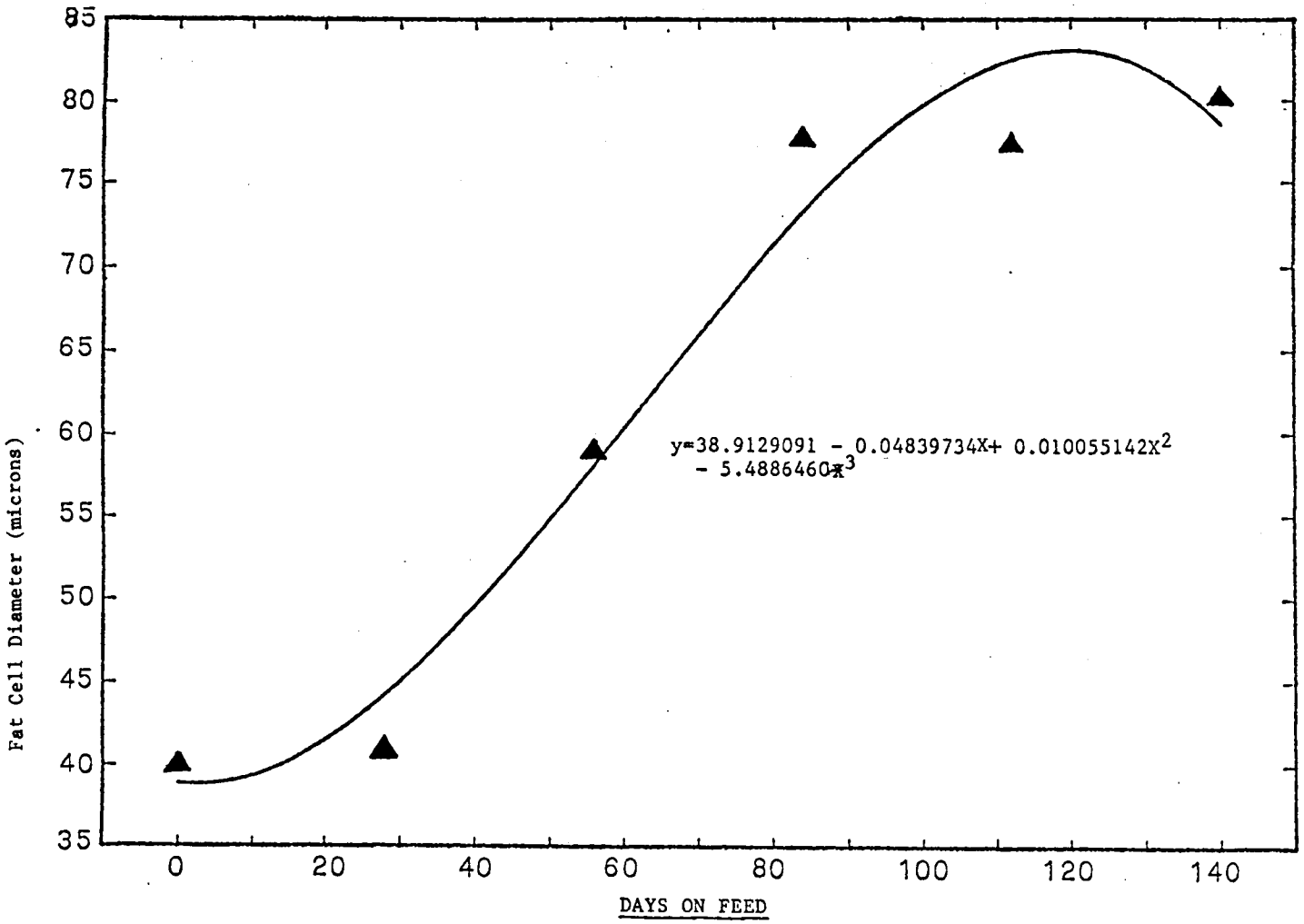
Percent anaerobic fibers increased significantly ($P<.05$) in the NADH-TR stain but not in the ATP-ase stain as days on feed progressed (Table III, page 18). Aerobic fibers decreased significantly ($P<.05$) in the NADH-TR stain but not in the ATP-ase stain as days progressed. The contradiction between the two reciprocal stains, as discussed earlier, makes it extremely difficult to note trends with respect to fiber percentage. However, even with some significant differences between individual days on feed lots and reversed tendencies of the two stains, the data indicated little change in overall fiber percentage.

In relationship to fiber size, several trends were noted. Overall fiber diameter did not increase significantly for the 140 day feeding period. However, a significant increase did occur between the 56 and 84 day periods. Joubert (1956) stated that from birth to maturity fiber diameter of bovine increase 41%. The present study, however, only encompassed a 140 day period not the entire growth process of the animal. The above agrees somewhat with Berry (1972) who discovered little fiber size change in cattle of different maturities. However, Bendall and Voyle (1967) and Roman et al. (1965), reported increases in fiber diameter in relationship to age. Anaerobic and aerobic fiber diameter were highly significant ($P<.01$) as respect to days on feed. All fiber size measurements were highly correlated ($P<.01$) to rib eye area. Also,

anerobic fiber sizes were significantly correlated ($P<.05$) to the maturity score while aerobic fibers were not.

The major aspect of this study was the determination of histological fat and its usefulness in predicting meat quality. It was noted that fat cell diameter was highly related ($P<.01$) to days on feed (Figure 1). Fat cell diameter or other histological traits did not change significantly after 112 days. In correlation procedures fat cell diameter was highly significant with respect to carcass weight, REA, percent kidney fat, fat thickness at 12th rib, U.S.D.A. yield grade, U.S.D.A. quality grade, maturity score, marbling score and ether extract.

One of the major purposes of this study was to classify histologically determined fat into marbling degrees. This could then be translated into quality grades. This was accomplished by the establishment of a histological marbling score (HMS) number. The HMS was found to be significantly correlated ($P<.05$) to marbling score and U.S.D.A. quality grade. It appears from the study that both quality and yield of an animal's carcass can be estimated by using certain histological traits such as fat cell diameter and histological marbling score.



▲ - Actual means compared to predicted curve calculated from orthaganol polynomial components.

Figure I. Effect of Days on Feed with Fat Cell Diameter.

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APPENDIX

I. ATP ase (Staining Procedure)

A. Prepare the staining solution just prior to staining:

0.1 M sodium barbitol	2.0 ml
0.18 M calcium chloride	2.0 ml
Distilled H_2O	6.0 ml
Adenosine triphosphate	25.0 mg

Adjust the pH of the solution to 9.4 using 0.1 M NaOH. Filter solution through Whatman No. 41 filter paper. Remember: this solution must be prepared fresh.

0.1 M sodium barbitol is prepared by dissolving 2.06 g in 100 ml distilled H_2O.

0.18 M calcium chloride is prepared by dissolving 1.998 g in 100 ml distilled H_2O.

B. After the sections of muscle have dried on the converslips:

1. Preincubate in 1% Ca Cl_2 for 7-10 minutes at 36°C
2. Rinse in distilled H_2O
3. Incubate for 10-15 minutes in the ATP ase staining solution at 36°C
4. Wash in 1% Ca Cl_2 three times (using fresh Ca Cl_2 each time) for a total of 10 minutes
5. Rinse in distilled H_2O
6. Transfer to 2% cobaltous chloride for 3 minutes
7. Rinse in distilled H_2O
8. Immerse in yellow ammonium sulfide for 15-30 seconds
9. Rinse in distilled H_2O
10. Mount in glycerogel

II. NADH-TR (Staining Procedure)

A. Prepare TRIS buffer:

0.2 M tris	100 ml
0.1 M HCL	168 ml
Distilled H_2O	232 ml
	= 500 ml buffer

Adjust to pH 7.4 with .01 M NaOH

B. Prepare staining solution:

0.2 M tris buffer (pH = 7.4)	10 ml
Nitro blue tetrazolium	10 ml
Reduced diphosphopyridine nucleotide	8 mg

C. After the selection of muscle have been dried on the coverslips:

1. Incubate in the staining solution for 30 minutes at 36°C
2. Rinse in acetone: water mixtures of the following strengths <u>in order</u>: 30:70, 60:40, 90:10, 60:40 and 30:70
3. Rinse in distilled H_2O
4. Mount in glycerogel

III. Oil Red O (Staining Procedure)

A. Prepare stock solution (oil red O is saturated in 99% alcohol):

Oil Red O dye	300 mg
Distilled ethyl alcohol	100 mg

B. Place coverslips on which sample sections are adhered into a staining jar; set aside.

C. Dilute stock solution:

Stock solution	6 ml
Distilled H_2O	

D. Immediately pour diluted solution into the staining jars in which the sections were placed. Let stand for 3 1/2 minutes (3 minutes 30 seconds).

E. Wash with distilled H_2).

NOTE: Fat will be stained red to orange.

Follow the Oil Red O procedure with Delafield's Hematoxylin Stain.

Carcass Characteristics Evaluated:

Carcass weight (48 hours)
Marbling degree
Muscling score
Firmness
Maturity
Quality grade
Color
Yield grade
Loin eye area
Percent Kidney fat
Fat thickness at 12th rib

Chemical Characteristics Evaluated:

Ether extract
Percent moisture

Histological Characteristics Evaluated:

Number fat cells/loci
Size of fat cells (diameter)
Diameter of ATP-ase negative fibers
Diameter of ATP-ase positive fibers
Area of ATP-ase negative fibers
Area of ATP-ase positive fibers
Percent ATP-ase negative and NADH-TR positive fibers
Percent ATP-ase positive and NADH-TR negative fibers

Histological Marbling Groups

30 29 28	abundant
27 26 25	moderately abundant
24 23 22	slightly abundant
21 20 19	moderate
18 17 16	modest
15 14 13	small
12 11 10	slight
9 8 7	traces
6 5 4	practically devoid
3 2 1	devoid

TABLE VI

MEANS AND STANDARD DEVIATIONS OF CARCASS AND CHEMICAL TRAITS
AS INFLUENCED BY DAYS ON FEED

	Days on Feed (N=10)												Overall	
	0		28		56		84		112		140		Mean	C.V.[1]
Carcass Traits	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Marbling Score[2]	15.40	1.17	15.4	0.97	15.7	1.57	17.0	2.05	16.2	2.04	16.6	1.26	16.05	10.1
Lean Color[3]	4.5	1.27	4.5	1.58	5.5	0.97	4.5	1.27	5.6	1.5	5.8	1.03	5.06	26.9
Lean Texture[4]	4.0	1.15	5.0	1.69	4.3	1.49	3.5	0.97	5.5	1.78	5.2	1.23	4.58	33.5
Maturity[5]	7.7	1.77	10.5	2.5	10.7	1.06	11.0	2.58	13.2	3.64	16.2	2.82	11.6	31.1
Quality Grade[6]	7.7	1.34	9.6	1.84	10.2	1.13	9.9	1.97	11.1	1.59	12.7	0.95	10.2	20.6
Fat Thickness at 12th Rib[7]	4.2	1.75	7.3	2.94	10.7	4.22	11.6	2.32	12.7	4.57	18.1	5.3	10.6	52.6
% Kidney Fat	0.95	0.28	1.6	0.32	1.85	0.47	1.65	0.53	1.95	0.28	2.45	0.49	1.74	34.5
Rib Eye Area at 12th Rib[8]	8.43	0.87	8.87	1.13	9.21	0.69	9.6	1.23	11.5	2.38	11.8	1.01	9.91	18.5
Yield Grade number[9]	1.91	0.25	2.36	0.48	3.03	0.52	2.9	0.48	3.01	0.72	3.79	0.40	2.83	26.7
Carcass Weight (after 48 hour chill)	407.7	56.5	480.6	48.7	541.5	48.4	553.0	83.6	657.8	107.2	710.6	47.6	558.5	21.8
Moisture	73.1	1.92	72.5	1.46	72.5	2.33	73.0	1.08	72.3	1.36	72.6	1.11	72.71	2.16
Fat from Ether Extract	11.8	4.34	13.0	3.49	12.6	3.97	13.0	4.79	14.3	4.79	19.9	3.93	13.9	33.8

1 = Coefficient of Variation
2 = Marbling score = 30 unit scale; 30 = AB^{+}, 1 = D^{-}
3 = Lean color = 8 unit scale; 8 = light grayish red (pink), 1 = black
4 = Lean texture = 8 unit scale; 8 = very fine, 1 = very course
5 = Maturity = A^{00} = 10, A^{50} = 15, A^{100} = 20
6 = Quality grade = 17 unit scale; 6 = STD^{-}, 17 = P^{+}
7 = Fat thickness in centimeters
8 = Rib Eye Area in square inches
9 = Yield Grade number = 5 point scale; 5 = highest yield, 1 = lowest yield

VITA

The author was born in Nashville, Tennessee, September 12, 1954, the only child of Mr. and Mrs. Robert E. Sutton.

He graduated from Battle Ground Academy in 1972. He then entered The University of Tennessee, Knoxville, where he was president of Pi Kappa Phi social fraternity. Also, he served on I.F.C. Council and was a member of the Block and Bridle Club. A B.S. degree in Animal Science was achieved in 1977 where upon graduation the author started upon his M.S. degree program in Food Technology and Science. The author is also a member of the Institute of Food Technologists.

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