# EVALUATION OF SEVERAL MUSCLE CHARACTERISTICS IN THREE MUSCLES OF TWENTY-FIVE DAY OLD BEEF CALVES OF TWO BREED-TYPES

By

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1978

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 1984

Thesis
1984
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CHARACTERISTICS IN THREE
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1184178

ACKNOWLEGMENTS

The author wishes to express sincere appreciation and thanks to her major adviser, Dr. John J. Guenther, for his advice and guidance through the course work and research phases of this project and especially for his patience and encouragement through the long manuscript writing period.

Thanks are also extended to the other members of the author's graduate committee: Dr. Donald G. Wagner and Dr. George V. O'Dell, for their helpful input in course work selection and in this thesis manuscript. Additional thanks to Dr. Robert D. Morrison, Linda Wilson and Dr. Richard Hintz for their assistance with the statistical analysis of the data.

The author gratefully acknowleges former graduate students Carolina Rodriguez and Carolynn Evans for their assistance with the chemical analysis; and a special thanks to Kris Novotny for not only assistance with chemical analysis, but also her friendship and guidance in teaching the author general laboratory skills.

The deepest graditude, appreciation and respect are extended to the author's parents for their reassurance and support throughout, and to the author's husband who through three long years has fought to see this manuscript off the author's mind and onto the printed page.

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## NOMENCLATURE

| | |
|---|---|
| LD | Longissimus dorsi |
| ST | Semitendinosus |
| TBL | Triceps brachii, lateral head |
| ATPase | Adenosine Triphosphatase |
| NADH-TR | Nicotinamide Adenine Dinucleotide-Tetrazolium Reductase |
| SDH | Succinic Dehydrogenase |
| PSE | Pale, Soft and Exudative |
| DNA | Deoxyribonucleic Acid |
| RNA | Ribonucleic Acid |

# CHAPTER I

## GENERAL INTRODUCTION

Muscle characteristics have long been a subject of intense study. Through the years various aspects of muscle have been examined, including: myofiber type, prenatal myofiber formation, prenatal myofiber innervation, postnatal myofiber development, total myofiber number, myofiber size, muscle concentration of DNA and RNA and muscle protein, moisture, fat and ash.

Past studies by many investigators have perfected techniques of measurement and shown characteristics of these various muscle parameters; however, most of the work has been done using laboratory animals. Without a basis of comparison it is difficult to tell whether the traits of these small animals relate to the much larger meat producing animals. Therefore it is necessary to study these animals themselves. Some recent work has been done using the ovine and the porcine, but comparatively little work has been done using the bovine as the experimental species.

For many years it has been the goal of meat producers to market their animals quickly and efficiently as a

product that the consumer desires. Selecting animals with fast growth rates and acceptable muscle quality, therefore is a primary concern of producers. If some muscle parameter could be easily examined and used as a predictor of growth without adversely affecting the animal, this could serve as a valuble selection tool to the producer.

With this in mind, this study was undertaken to study several muscle parameters in two breed-types of cattle at three different ages. It was beyond the scope of this thesis to report on all data collected, so presented here are data from 25 day old Angus and Charolais calves born in two different seasons. It was the goal of this research to determine the following:

1. Do differences exist between these two breed-types of cattle with respect to myofiber number; myofiber type; myofiber size; live body weight; carcass weight; muscle weight; total body lean, fat and bone; total lean and individual muscle protein, moisture, fat and ash; and muscle DNA and RNA concentrations.

2. If differences exist between the two breed-types for the above parameters, are they detectable at an early age.

3. If differences are detectable at an early age, can one or any combination of these parameters be used to predict ultimate muscle mass or animal size (and therefore be an index of growth potential) and be used as a selection tool.

# CHAPTER II

## REVIEW OF LITERATURE

### Myofiber Type Descriptions

For many years it has been known that muscles are made up of unique types of cells which are characterized by their elongated shape and multinucleation. According to Ogata (1958), differences in the contraction rates and general color of muscles have been known to exist since 1830 when Ranvier observed red and white muscles in rabbits. Later, it was discovered that these color and contraction differences were due to relative proportions of different types of individual muscle cells. In 1929, Denny-Brown described two fiber types as being either opaque or clear, and related these to muscle contraction rates.

Some early studies of muscle fiber types were conducted in the 1950's and 1960's, but it was not until the late 1960's that a real, in-depth study of the individual myofiber types was begun.

Many parameters have been used to classify myofibers into different types. The number of nomenclature classification schemes used for these fibers has been as numerous

as the scientists conducting the studies. Even today, a common system of nomenclature has not been established, nor have scientists agreed on the number of fiber types in existance.

Staining Procedures

A basic knowledge of some common staining reactions is necessary for understanding classification of myofibers into types. Some of the more common stains used for fiber type determination are: Adenosine Triphosphatase (ATPase), Nicotinamide Adenine Dinucleotide-Tetrazolium Reductase (NADH-TR) and Succinic Dehydrogenase (SDH). Some fiber parameters also used are fiber size and contraction speed. A brief description of the common staining reactions follows.

ATPase. The actomyosin ATPase reaction has been shown to be related to speed of contraction of muscle fibers (Guth and Samaha, 1969). These authors found two qualitatively different actomyosin ATPases in the muscle fibers of rats, rabbits and cats. Low activity fibers had actomyosin ATPase which was acid-stable and alkali-labile, while high activity fibers had actomyosin ATPase which was alkali-stable and acid-labile. When sections were subjected to fixation in formaldehyde prior to staining, a third fiber type of intermediate staining was observable.

Therefore, in muscle tissue sections stained with this procedure, generally three fiber types are distinguishable.

NADH-TR and SDH. These two stains work on the same general principle; they are both indicators of oxidative enzyme activity. The method of action is the reduction of Nitro-Blue Tetrazolium (NBT), a tetrazolium salt, which results in a blue colored pattern of diformazan granules in the mitochondria and sarcoplasmic reticulum of fibers. The oxidative enzyme acts on its normal substrate, however, NBT is the end point of the pathway and is reduced to the colored diformazan (Brooke and Engel, 1966). The intensity of color and/or the pattern of diformazan granule deposition determine the fiber type.

Myofiber Types

Ogata (1958) studied muscles of fish, frogs, birds and mammals using the SDH reaction. He found SDH activity to be high in red muscle fibers, low in white muscle fibers and intermediate in medium muscle fibers. He concluded that the observed variation in SDH reaction between fiber types was due to differences in number or activity of mitochondria in the fibers. Later, Ogata and Mori (1964) used, in addition to oxidative enzyme activity, fiber size and contraction speed to determine fiber type. Red fibers were small in size, displayed a high oxidative enzyme

activity and contracted slowly and tonically. White fibers were large in size, displayed a low oxidative enzyme activity and contracted quickly for phasic movement. Intermediate fibers were medium in size, displayed intermediate oxidative enzyme activity and their function was unclear.

Stein and Padykula (1962) described three fiber types in the rat as A, B or C based on SDH activity (both pattern of diformazan granule deposition and intensity of reaction). The type A fiber contained a network of small granules distributed throughout the fiber forming long streaks; the intensity was low. The type B fiber contained small diformazan granules arranged in small polygons. The type C fiber contained large diformazan particles, many of which were located beneath the sarcolemma. These authors suggested that the type A fiber was the "classical white fiber" while types B and C were both forms of red fibers.

Two types of myofibers were described by Dubowitz (1963). One had high oxidative enzyme activity and low phosphorylase activity while the other had low oxidative enzyme activity and high phosphorylase activity. (Phosphorylase activity is an indicator of glycolytic capacity; Cassens and Cooper, 1971.)

Dubowitz (1965) described red fibers as being generally adapted for sustained activity with a slow speed of

contraction, while white fibers were capable of short periods of activity but had fast contraction speeds. He called red fibers type I, white fibers type II and he also observed an intermediate fiber.

Small red, large white and intermediate were the names used by Gauthier and Padykula (1966) to describe the three fiber types which they identified in diaphragm muscle of several mammalian species.

Brooke and Engel (1966) used the nomenclature of type I and type II fibers and described type I fibers as high in oxidative enzyme activity while type II fibers were low in oxidative enzyme activity.

Brooke and Kaiser (1969) showed that the ATPase reaction was inhibited in type I fibers when sections were preincubated above pH 10.0. Type II fibers retained their normal staining pattern (strong positive reaction) during this preincubation.

Three fiber types, based on size and oxidative enzyme activity were detailed by Edgerton and Simpson (1969) in rats and guinea pigs. They maintained that red fibers were intermediate in diameter and had coarse diformazan crystals located primarily beneath the sarcolemma; white fibers had large diameters and low oxidative enzyme activity; intermediate fibers were small in diameter and had a moderate amount of fine diformazan crystals located throughout the cells. With the myosin ATPase reaction

they could only identify two types of fibers. Red and white fibers had high ATPase activity while the intermediate fiber had low myosin ATPase activity.

Yellin and Guth (1970) stated that most studies have shown there are three basic muscle fiber types. They concluded, based on studies of rat and cat muscle, that they could not use a single classification of fibers based on SDH and actomyosin ATPase (pH 10.4) to describe the fiber types in <u>both</u> rat and cat muscle.

According to Guth and Samaha (1970) the actomyosin ATPase reaction allowed muscle to be divided into three fiber types. $\alpha$ fibers had acid-labile, base-stable ATPase; $\beta$ fibers had base-labile, acid-stable ATPase; and $\alpha\beta$ fibers had intermediate stability in acid and base.

Cooper et al. (1970) used the NADH-TR stain to determine fiber type. They reported that red fibers had high NADH-TR activity located uniformly throughout the fiber; intermediate fibers had moderate NADH-TR activity located more beneath the sarcolemma; and white fibers had no NADH-TR activity.

Barnard et al. (1971) recommended nomenclature of fast-twitch red, fast-twitch white and slow-twitch red for muscle fibers of guinea pigs based on contractile activities and staining patterns. They found that red fibers stained dark with myosin ATPase and had many dark diformazan crystals located beneath the sarcolemma with NADH-

diaphorase; white fibers stained dark with myosin ATPase and had few diformazan crystals located beneath the sarcolemma with NADH-diaphorase; and intermediate fibers stained light with myosin ATPase and had small, uniformly dispersed diformazan crystals with NADH-diaphorase. However, the guinea pig and the rat are somewhat different from other species. Ashmore and Doerr (1971a, b) found that in chick, bovine and porcine muscle, light staining with ATPase did not correlate with intermediate SDH activity.

Ashmore and Doerr (1971a) stated that several factors such as, specific animal, muscle, age and experimental conditions, must be considered when determining muscle fiber types. Several authors also concluded that to adequately describe a myofiber type, one must show both ATPase activity and metabolic character (Ashmore and Doerr, 1971a; Edgerton and Simpson, 1969).

In their investigation of newly hatched and young chicks, Ashmore and Doerr (1971a) described two types of red fibers which could be distinguished by their ATPase activities. One of these red fiber types had the ability to convert to a third fiber type, a white fiber. From these observations, they developed a new nomenclature system for describing myofiber types. $\beta$ red fibers stained lightly for ATPase at pH 10.0 and had a high concentration of diformazan crystals with the SDH reaction. $\alpha$ red fibers stained darkly for ATPase and had a high concentration of diformazan crystals with the SDH reaction. $\alpha$

white fibers stained darkly for ATPase and had a low concentration of small diformazan crystals with the SDH reaction.

In a later investigation, Ashmore and Doerr (1971b) sought to apply their new nomenclature system to other species. They examined muscle tissue from chick, mouse, bovine and porcine and concluded that their dual nomenclature system was applicable to these other species.

Romanul (1964) described eight types of muscle fibers in rat gastrocnemius and plantaris muscles, however, he found only three of these types in rat soleus muscle.

Guth and Yellin (1971) stated that there were three major types of muscle fibers but when serial sections were stained for several different enzymes, more fiber types became visible.

Davies (1972) observed six types of muscle fibers in the pig. His classifications came from varying degrees of reactivity with myosin ATPase, SDH and glycogen phosphorylase. He admitted, however, that there were three <u>main</u> fiber types.

Burleigh (1974) described four fiber types. The first, called fast-phasic had high myosin ATPase activity, fast contraction speed and anaerobic metabolism. Another fiber, unnamed, had active myosin ATPase, frequent contraction, high mitochondrial content, aerobic metabolism and small size. The next fiber, called slow-phasic, had

low myosin ATPase activity, aerobic metabolism and was concerned with maintaining posture. The last fiber had low myosin ATPase and was moderately aerobic.

Engel (1974) recommended using the myofibrillar ATPase stain at pH 9.4 to determine fiber type as type I (light staining) or type II (dark staining). He then used a secondary procedure (ATPase at pH 4.35), which reverses the staining pattern, to confirm fiber type.

Beermann et al. (1977) chose to classify fibers into only two fiber types; type I and type II. They described type I fibers as being high in acid ATPase and NADH-TR activity and low in alkaline ATPase and phosphorylase activity while type II fibers were low in acid ATPase and NADH-TR activity and high in alkaline ATPase and phosphorylase activity. (With the alkaline ATPase stain they found that some type II fibers stained darkly while others stained with intermediate intensity; all were classified as type II fibers.)

Mature bovine muscles stained with ATPase and NADH-TR were examined by Hunt and Hedrick (1977a). With the ATPase stain they classified fibers as $\alpha$ red, $\beta$ red or $\alpha$ white. With the NADH-TR stain they classified fibers as only red or white. They found that the percentage of $\alpha$ red plus $\beta$ red fibers was positively correlated ($r=0.91$) with the percent red fibers as determined by the NADH-TR reaction.

Khan (1978) grouped muscle fibers of rats into three

major groups, but also found subtypes of these major groups. While Pool et al. (1979), reported that fiber typing based on visual determination of staining intensity might be biased, however, if large samples were used, the results seemed to be reproducible.

More recently, Suzuki and Cassens (1980) examined five porcine muscles from birth to 16 weeks of age using ATPase stain. They classified fibers as follows: type I acid-stable, alkali-labile; type II alkali-stable, acid-labile, subdivided into type IIA-unstained after preincubation at pH 4.4 to 4.5 and type IIB-stained weakly or moderately after preincubation at pH 4.4 to 4.5; and three intermediate fiber types, type SM-stained strongly for acid-stable ATPase and moderately or weakly after preincubation at pH 10.4 to 10.5, type SS-stained moderately or strongly after either acid or alkali preincubation, type MS-stained weakly or moderately after preincubation at pH 4.3 to 4.4 and strongly after alkaline preincubation.

From the above information, the diversity of the fiber-type classification schemes is readily apparent as is the classification differences between species. Against this background, myofiber type development will now be described.

## Prenatal Myofiber Development

### Prenatal Fiber Formation

Understanding prenatal myofiber development is essential to the study of postnatal fiber type development with age. According to Allen et al. (1979) and others, prenatal muscle growth is a period composed largely of cell hyperplasia. Muscle develops from the middle layer of embryonic cells, the mesoderm. From mesenchyme cells of the mesoderm arise myogenic cells which, after a period of proliferative mitosis, give rise to myoblasts. These myoblasts are mononucleated cells which eventually fuse to form myotubes, the multinucleated primative muscle fibers (Forrest et al., 1975).

### Prenatal Fiber Type Development

Currently, there exist two major theories of prenatal muscle fiber-type development. The first, called the stemline theory, was reported by Ashmore and Addis (1972). These authors maintained that prenatal development of myofibers is "biphasic". From studies of chick embryo (Ashmore et al., 1973), fetal lamb (Ashmore et al., 1972), fetal pig (Ashmore et al., 1973a) and fetal bovine muscle (Ashmore et al., 1974) the authors concluded that first there is formation of the presumptive $\beta$ fiber which has at least one large vaccuole in the center and myofibrillar

material around the periphery. From these presumptive fibers arise a secondary fiber type, smaller than, and many in direct contact with, the original fibers. These secondary fibers are destined to become the $\alpha$ fibers. These morphological differences in the fibers were observed before the fibers could be distinguished histologically and have also been described by Beermann et al. (1978) and Swatland (1973).

Ashmore and Addis (1972) regarded the presumptive $\beta$ fibers as serving as the framework upon which further myoblast fusion and proliferation occurred, ultimately resulting in the secondary myotube (or $\alpha$ fiber) formation. Swatland and Cassens (1973) believed that secondary myofibers were formed from myoblasts on the surface of primary myotubes brought into contact with one another through contraction of the primary fibers. The newly formed secondary myotubes were then released and pushed toward the periphery of developing muscle bundles by more new $\alpha$ fibers.

Ashmore and Addis (1972) reported that $\beta$ fiber generation was completed relatively quickly prenatally while $\alpha$ fibers continued to proliferate from the surface of the presumptive $\beta$ fibers for extended periods. Indeed, in an earlier paper (Ashmore and Doerr, 1971a), it was suggested from observations of young chicks that the relative uniformity of size of the $\beta$ fibers indicated they

were formed at the same time during development; while $\alpha$ fibers, being among the smallest and the largest fibers, were formed at different times during development.

Ashmore et al. (1973a) suggested that muscles or portions of muscles with a high proportion of $\beta$ fibers developed earlier in prenatal life than those with no $\beta$ fibers. In muscles with a large proportion of $\beta$ fibers on which the $\alpha$ fibers could form, formation of the secondary ($\alpha$) fibers proceeded quickly. On the other hand, in muscles with no $\beta$ fibers, the initial appearance of $\alpha$ fibers was delayed and $\alpha$ fibers probably formed on other $\alpha$ fibers. They therefore concluded that muscle development might be accelerated by the presence of $\beta$ fibers.

The second theory, described by Beermann et al. (1978) using fetal pig muscle agrees in part with the stemline theory: that primary myofibers are the first to form; they tend to be located in the center of developing faciculi and secondary myofibers are formed from fusion of myoblasts upon these primary myofibers and tend to be located around the periphery of developing muscle bundles.

The two theories part here. Ashmore and Addis (1972) maintain that all primary fibers (or presumptive $\beta$ fibers) will become $\beta$ muscle fibers and all secondary fibers will become $\alpha$ muscle fibers. Beermann et al. (1978) observed in fetal pig muscle that primary fibers could become either type I ($\beta$) or type II ($\alpha$) fibers and that

secondary fibers could remain type II ($\alpha$) or change to type I ($\beta$) fibers. Furthermore, Ashmore and Addis (1972) stated that, initially, all fibers were aerobic or "red" (either $\alpha$ red or $\beta$ red) fibers and near birth some $\alpha$ fibers changed from aerobic to anaerobic metabolism (becoming $\alpha$ white) while Beermann et al. (1978) observed conversion of secondary fibers from type II ($\alpha$) to type I ($\beta$), prenatally.

Swatland (1973) also studied fetal pig muscle and found a reduction or disappearance of primary fetal myofibers, which he attributed to a morphological change in the primary fiber. As fetal development proceeded, the central vaccuole of primary myofibers filled with myofibrils and disappeared (Ashmore and Addis, 1972; Swatland, 1973). Therefore, Swatland (1973) included the changed primary myofibers in his count of secondary myofibers. He concluded that two distinct populations of myofibers were formed during fetal porcine development and that primary myofibers underwent a metamorphosis to be included as secondary myofibers.

Role of Prenatal Muscle Fiber Innervation

It has been stated that oxidative enzyme activity in muscle does not occur sufficiently to distinguish fiber types prenatally until late gestation (Ashmore and Addis, 1972) or during early post-natal development (Ashmore et

al., 1973a; Beermann et al., 1978; Cooper et al., 1970). Burleigh (1974) suggested that this time gap might be required for complete innervation to be accomplished and that innervation might be a factor in determining muscle fiber type. This view was also shared by Beermann et al. (1977); Beermann et al. (1978); and others. Many authors maintain that since myofiber type ratios can be changed by altering innervation to muscles, the motor neuron innervating a particular myofiber must influence its fiber type (Beermann et al., 1977; Beermann et al., 1978; Cassens and Beermann, 1977; Cooper et al., 1970; Davies, 1972; Guth et al., 1970).

## Postnatal Muscle Development

### General Muscle Growth

It is important to understand the concepts of general muscle growth and individual muscle growth before attempting to investigate their component parts; the myofibers.

In the fetal pig at approximately mid-gestation, Swatland (1973) recorded a medium rate of growth in the triceps muscle, a high rate of growth in three hind limb muscles and a low rate of growth in the longissimus muscle. Shortly after birth, he recorded decreased growth rates in all muscles except the longissimus which showed a substantial increase in growth rate.

Johnson (1974) studied growth rates of several muscle groups in fetal calves. He found that from 150-210 days gestation, muscle groups of the fore and hind limbs grew at significantly faster rates than total muscle, while the muscles surrounding the spinal column (including the longissimus) grew at rates not significantly different from total muscle. From 210 days gestation to one day of age, the above mentioned muscles all grew at rates not significantly greater than total muscle. Over those same periods, Johnson (1974) found that total muscle had a high growth impetus compared to total carcass. He stated that this could be understood in view of the hypothesis that tissue development is influenced by functional priority at birth.

Dubowitz (1963) investigated several species to determine the extent of muscle differentiation at birth. He concluded that muscle differentiation at birth might be partly influenced by length of gestation and need for functional activity at birth.

Berg and Butterfield (1976) stated that in general, large muscles grow proportionately faster than small muscles. Small muscles had a higher proportion of connective tissue and fewer muscle fibers than large muscles and the growth potential for muscle fibers is higher than that of connective tissue. In general, the muscles most closely related to the skeleton are smallest and have the lowest growth impetus.

These authors also reported that immediately before birth muscle growth responds to genetic influences which insure that the animal is ready for its "usual" environmental challenges. Immediately after birth there are great changes in muscle related to actual functional demands.

Berg and Butterfield (1976) classified all muscles of the bovine for growth impetus relative to weight increase in total muscle. The triceps brachii (lateral head) was classified as a low impetus muscle. It grew slower than total muscle throughout life. The semitendinosus and longissmus dorsi were both classified as high-average impetus muscles. They grew more rapidly than total muscle in early life and about the same as total muscle in later life.

Bendall and Voyle (1967) concluded that overall growth rate was very similar for the longissimus dorsi and the semitendinosus muscles of calves from one to 24 months of age.

Joubert (1956) indicated that muscle growth rate (based on myofiber diameter increase in sheep), appeared to reach an early maximum in lower limb muscles which proceeded to a late maximum growth rate in the loin region.

Berg et al. (1978) divided muscles into groups and calculated growth coefficients based on total muscle growth for bulls. Their coefficients reflect "centripetal

group impetus from distal to proximal limb muscles. There is a progressive rise in growth impetus from rump to neck." They also found differences in muscle distribution between breeds, however, they stated that these variations were probably due to differences in maturity and minor functional influences.

Causes for Increased Muscularity

According to Burleigh (1974), larger animals have a tendency for longer gestation periods and thus an opportunity for a longer period of cell replication prenatally. Therefore, they have the potential for a greater number of muscle fibers at birth and thus larger ultimate body size.

Holmes and Ashmore (1972) indicated that double muscled calves had more muscle fibers than normal calves at one month of age. They noted that average fiber size was the same for the two phenotypes, but the double muscled calves had larger muscles. Holmes and Ashmore (1972) concluded, from a study of double muscled and normal cattle, that increased fiber number must be the cause of the increased muscularity in double muscled animals, since the greater muscle size in the double muscled cattle could not be accounted for by increase in fiber size alone.

Ashmore and Robinson (1969) suggested that an increase in the proportion of glycolytic-type fibers was the cause for the observed muscle hypertrophy in double muscled

cattle. Holmes and Ashmore (1972) found a larger proportion of "white" fibers (with the SDH reaction) present in muscle from double muscled cattle than from normal cattle at several ages. Holmes and Ashmore (1972) again observed double muscled and normal cattle and concluded that increasing muscularity was correlated with increasing conversion of $\alpha$ red to $\alpha$ white fibers.

Dildey et al. (1970) found a "distinct linear association between light fiber content and muscularity" in PSE pigs. Cassens and Beermann (1977) suggested that the PSE condition might be the result of increased muscularity caused by increased proportion of white fibers in the muscles of these animals.

In normal animals, however, Johnston et al. (1975), in their study of Angus and Charolais cattle, found that Charolais steers had the same percentage of $\alpha$ red and about 0.75 percent fewer $\alpha$ white fibers than Angus steers.

## Postnatal Myofiber Type Development

Alterations in Fiber Type. Many authors have observed changes in the proportions of fiber types with increasing age or weight in several species. Most believe these changes to be the result of fiber type conversions. But the cause of these transformations is still controversial and probably is not the result of a single factor.

Guth and Yellin (1971) maintained that fiber types

are continually being changed throughout life due to changes in functional demands placed on them.

Stickland (1979) reported that many authors believe that the function of a given muscle determines the fiber type make-up of that muscle.

Ashmore and Doerr (1971a) suggested that muscle use might prevent conversion of $\alpha$ red to $\alpha$ white fibers. They had observed the sartorius and adductor leg muscles in chicks had higher percentages of red fibers than the pectoralis muscle (which is not used in chicks).

Cassens (1977) stated that fiber type composition could be altered somewhat by exercise or training, but the alteration would not be significant.

Ashmore and Doerr (1971a) studied muscle fiber types in three muscles of normal and dystrophic chicks. At hatching, in the normal pectoralis muscle, the ATPase stain indicated that all fibers were $\alpha$, however, with the SDH stain, two fiber populations were apparent. As age was increased to three weeks, the observed mitochondria rich SDH fibers were rapidly changed to mitochondria poor fibers. In the sartorius muscle, Ashmore and Doerr (1971a) observed two fiber types ($\alpha$ and $\beta$) with the ATPase reaction at hatching. Both $\alpha$ and $\beta$ fibers were classified as red with the SDH stain. By four days of age, two populations of fibers could be distinguished with the SDH stain. These authors observed a conversion of some

mitochondria rich $\alpha$ fibers to mitochondria poor $\alpha$ fibers which appeared to be nearly completed by two weeks of age.

In addition, Ashmore and Doerr (1971a) noted that the fiber type distribution pattern in the sartorius was different for opposite sides of the muscle. At hatching, $\beta$ fibers constituted 20-40 percent of the fibers on one side of the muscle, while they were completely absent on the other side. The adductor muscle also had an uneven distribution of fiber types. The deep adductor was composed mostly of $\alpha$ fibers while the superficial adductor had mostly $\beta$ fibers.

Ashmore et al. (1972) stated that in porcine, ovine, bovine and chick muscle, $\alpha$ fibers were initially red but had the capacity to change to $\alpha$ white. They also found that this change began earlier in calves and lambs than in pigs and chicks. They concluded that this fiber type transformation could result in increased muscularity.

Cassens et al. (1968b) believed there was a transformation of intermediate to white fibers, but red fibers stayed constant in porcine muscle.

In studies of rat muscle, Dubowitz (1965) found no differentiation of fibers into types at birth. He stated that differentiation occurred gradually between one and 14 days of age.

Cooper et al. (1970) could find no fiber type differentiation in muscles of one-day old pigs with ATPase,

NADH-TR or phosphorylase. However, clear differentiation was observed by four weeks of age and fibers were classified as red, white or intermediate. Based on total myofiber area, Cooper et al. (1970) observed a decrease in the percent area composed of red or intermediate fibers and an increase in the percent area composed of white fibers as age increased. The authors suggested that this was due to a conversion of red and/or intermediate to white fibers, or to more rapid growth of the white fibers.

Suzuki and Cassens (1980) believed that type II fibers were converted to type I fibers and the intermediate fiber was a transitional state between them. Using acid and alkaline ATPase to classify fibers in developing porcine muscle, these authors observed that the proportion of type II fibers decreased and the proportion of type I fibers increased from birth to eight weeks of age in the longissimus, rectus femoris, masseter, trapezius and vastus intermedius muscles. The proportion of intermediate fibers in the longissimus, rectus femoris and masseter muscles decreased from birth to eight weeks while in the trapezius and vastus intermedius this proportion remained constant from birth to four weeks and decreased from four to eight weeks of age. Conversion rate was dependent upon the muscle studied.

Fiber Type Differences Between Muscles. Ashmore and Robinson (1969), Ashmore and Doerr (1971b), Holmes and

Ashmore (1972) all agreed that the bovine triceps brachii muscle contained a majority of red fibers. Moreover, Ashmore and Doerr (1971b) declared that the red fibers were almost evenly divided between $\alpha$ red and $\beta$ red fibers. In addition, Holmes and Ashmore (1972) stated that the fiber type composition of the triceps brachii did not vary much with age.

Ashmore and Robinson (1969) determined that, in one year old beef animals, the semitendinosus muscle contained a majority of white myofibers. Beermann et al. (1977) showed that both the inner and outer portions of porcine semitendinosus had a majority of type II (white) fibers. Holmes and Ashmore (1972) maintained that all bovine muscles became "whiter" with age, but this was especially pronounced in the semitendinosus muscle.

Melton et al. (1975) stated that the bovine longissimus muscle contained more white than red fibers and was therefore considered to be a white muscle. Nevertheless, Hunt and Hedrick (1977a), in their study of choice, A - maturity beef carcasses, found that the longissimus muscle contained 29.3 percent $\beta$ red fibers, 24.7 percent $\alpha$ red fibers and 46.0 percent $\alpha$ white fibers. Hunt and Hedrick (1977a) also studied the semitendinosus muscle of the above carcasses and found that the inner semitendinosus had 35.8 percent $\beta$ red fibers, 24.7 percent $\alpha$ red fibers and 39.2 percent $\alpha$ white fibers, while the outer

semitendinosus had 12.2 percent $\beta$ red fibers, 20.6 percent $\alpha$ red fibers and 67.2 percent $\alpha$ white fibers. Beecher et al. (1968) also reported that there were more white fibers in the superficial portion of the porcine semitendinosus muscle and more red fibers in the deep portion.

Fiber Type Differences Between Breeds. Johnston et al. (1975) examined the effect of time on feed and breed on muscle fiber type. Breeds used were Angus and Charolais of similar ages, weights and nutritional regimes. They found, with increasing time on feed, a slight (but nonsignificant) increase in the percent $\beta$ red fibers. Nearly one-quarter (24.92 percent) of the fibers were $\beta$ red, one quarter (26.71 percent) were $\alpha$ red and one-half (48.84 percent) were $\alpha$ white.

Stull and Albert (1980) examined two muscles of several breeds of horses and determined that percentages of the three fiber types did not differ significantly between breeds, even though data tended to show that Thoroughbreds had more red fibers than other breeds.

Fiber Type Differences Between Species. Gauthier and Padykula (1966) reported a study of diaphragm muscle in several mammalian species. They used the albino rat as a standard and found that in the diaphragm, in which activity is inversely related to body size, animals of

intermediate size had a heterogeneous population of myofibers, small animals had a homogeneous population of small fibers with abundant mitochondria and very large animals had a homogeneous population of large fibers with a low mitochondrial content.

These authors then examined hind limb muscles (gracilis or semitendinosus) of seven mammals and found that the same trend was present. With increasing body size there was a trend toward greater differences in fiber type in the gracilis and semitendinosus muscles.

Fiber Type Differences With Age. Spindler et al. (1980) examined the biceps femoris muscle of Angus, Hereford and Holstein steers and heifers for fiber type differences due to age, breed and sex. Samples were taken by biopsy beginning at 28 days of age and subsequently at 56 day intervals until slaughter at 392 days of age. Percent white fibers tended to increase, percent red fibers decreased and percent intermediate fibers remained steady with increasing age.

Judge (1978) found no significant difference in the proportion of NADH-TR negative fibers in the cutaneous trunci muscle of pigs from 23 to 113 kilograms body weight.

## Myofiber Number

Many authors believe that myofiber number is fixed at or shortly after birth and that any increase in animal

size must come from an increase in size of existing myofibers (Ashmore, 1977; Stickland and Goldspink, 1973; Hegarty, 1971; Joubert, 1956; Swatland, 1976).

Gonyea (1980), however, reported an increase in fiber number in cats trained to lift weights for a food reward. He attributed this increase to muscle fiber splitting. Bendall and Voyle (1967) reported a decrease in fiber number in the longissimus and semitendinosus of cattle, accompanied by a period of rapid increase in fiber area between 12 months and two years of age.

Hegarty (1971) pointed out the difficulties which arise in determination of fiber number in large animals. In large animals, most authors predict total fiber number through use of a fiber number per unit area technique. Stickland and Goldspink (1973) counted number of fibers per unit area by superimposing a grid onto a projected image of a tissue section. Total myofiber number within the muscle was calculated by multiplying fibers per unit area by the transverse sectional area of the entire section. Ezekwe and Martin (1975) counted myofibers of mouse muscle within a micrometer grid and then used similar calculations to find total myofiber number in the muscle.

Problems arise due to differences in intramuscular fat and connective tissue along the length and breadth of the muscle and differences in extracellular space in different parts of the muscle. According to Hegarty (1971)

any of these factors could influence myofiber count. In addition, due to intrafacicularly terminating muscle fibers the plane of sectioning might not be representative of muscle fiber number in the whole muscle (Swatland, 1976).

Burleigh (1974) stated that larger mammalian species have greater numbers of cells than smaller species because although the cells probably divide more slowly in prenatal life in larger species, they do so for a longer period of time.

Variation in muscle fiber number occurs between breeds of the same species (Hegarty, 1971). Stickland and Goldspink (1978) compared Large White pigs with a breed of miniature pigs at similar ages and found that the Large White pigs had about 68 percent more fibers.

Swatland (1973) counted myofibers in the sartorius muscle of fetal and neonatal pigs. He found that myofiber number increased with age in the fetus and continued increasing until about seven days postnatally. He concluded, however, that this increase in myofiber number postnatally might be the result of elongation of existing myofibers into sections where previously they were not viewed.

Ontell and Dunn (1978) observed in rats that all myofibers were present at birth, but not all were observable with the light microscope. Those not observable were located as "satellite myotubes or myofibers" which shared a basement membrane with a large primary fiber. They

called these occurrances "clusters". Ontell and Dunn (1978) observed that these clusters rapidly broke up during early postnatal life and could account for most of an observed increase in fiber number in rats, postnatally.

## Myofiber Size

### Determination Methods in Use

Several methods have been used to determine myofiber size in various species. Common measurements are fiber diameter and/or fiber area, but also in use is measurement of sarcomere length. Joubert (1956) teased apart fibers from thinly sliced muscle sections, then measured fiber diameters of the free floating fiber cylinders with an ocular micrometer mounted in the eye-piece of a standard light microscope. Other authors (Hanrahan et al., 1973; Stickland and Goldspink, 1973; Melton et al., 1974; Ezekwe and Martin, 1975) have used different fiber separation techniques, but the same general measurement method for myofiber diameter determination.

Bendall and Voyle (1967) determined fiber area by counting millimeter squares within magnified myofibers from tissue sections. They then calculated fiber diameter (assuming a circular fiber) from their area measurements.

Cross sectional area of individual fibers has been obtained using photographs or tracings of fibers, then measuring the area with a Compensating Polar Planimeter

(Stein and Padykula, 1962; Swanson et al., 1965; Johnston et al., 1975).

Davies (1972) measured trans-sectional area of muscle fibers by use of a paper weighing method after tracing individual fiber types onto transparent paper.

Myofiber diameter (Dildey et al., 1970; Johnston et al., 1975) and myofiber area (Cooper at al., 1970; Hunt and Hedrick, 1977a) have both been determined by use of a Ziess particle size analyzer.

Problems in Fiber Size Determination

Joubert (1956) examined the effect of 10 percent formalin fixation on myofiber size in rabbit muscle. He concluded that a slight but nonsignificant decrease in fiber diameter occurred in prerigor samples stored overnight in 10 percent formalin fixative. Following this, he found no difference in fiber size with storage up to six months.

Hegarty and Naude (1970), however, determined that separation procedures used prior to fiber size measurement could cause fiber distortion and therefore, erroneous results. Furthermore, Hegarty (1971) suggested that the small sample size available from muscles of farm animals may not give results representative of the whole muscle, since fiber diameter had been shown to vary along the length of the longissimus dorsi muscle of pigs and cattle.

Swanson et al. (1965) found in the longissimus muscle of market weight cattle that fiber size did, indeed, vary with location along this muscle.

Ultimate Fiber Size

According to Burleigh (1974) the ultimate width of fibers of different mammalian species does not vary nearly as much as myofiber number or growth rate between species. He reported an adult fiber diameter range between 30 and 70 $\mu$. Bendall and Voyle (1967) reported an increase in mean fiber diameter from 15 $\mu$ at one month of age to 45 $\mu$ at 24 months of age, in cattle. Joubert (1956) found that mean fiber diameter in different species was rated pig, rabbit, cattle, sheep, from largest to smallest, at maturity.

Several authors have reported that myofiber size increased with increasing age (Hegarty, 1971 in cattle; Spindler, 1980 in cattle). Joubert (1956) observed that fiber diameter increased 41 percent from birth to maturity in cattle. Cooper et al. (1970), in a study of pigs from one day to 26 weeks of age showed that myofiber size increased with increasing maturity. Stickland and Goldspink (1973) found an increase in fiber diameter with increasing muscle weight in a variety of pigs.

Size of Individual Fiber Types

"Red" muscle fibers are generally considered as being small in comparison to white fibers (Ashmore et al., 1972a; Cassens and Cooper, 1971; Davies, 1972; Hunt and Hedrick, 1977a; Melton et al., 1974; Stein and Padykula, 1962). Some authors (Ashmore et al., 1972a; Cassens and Cooper, 1971; Davies, 1972) regard this smaller size (cross sectional area) of the red fibers as a factor which favors cellular exchange of nutrients and wastes and increased capacity for diffusion needed for function of these fibers. Burleigh (1974) stated that fibers richest in mitochondria had a tendency to be comparatively small. Cassens and Cooper (1971) maintained that red fibers had a higher lipid content than white fibers. They also stated that red fibers needed greater blood flow than white fibers since they depended on blood oxygen for energy generation while white fibers were capable of glycolytic metabolism.

Cooper et al (1970), in a study of porcine muscle from one day to 26 weeks of age, stated at four and five weeks of age all fiber types were approximately the same size. After this period, white fibers increased in size more rapidly than red or intermediate fibers. Ashmore and Doerr (1971a), however, observed differences in size of individual fiber types before birth.

Hunt and Hedrick (1977a) reported that although white fibers were usually the largest fiber type in

market beef animals, $\beta$ red fibers were not always the smallest fiber type.

Melton et al. (1974) found that the cross sectional areas of fibers biopsied from bulls were ranked red, intermediate and white, from smallest to largest. Stein and Padykula (1962) ranked fibers as C (corresponding to $\alpha$ red), B ($\beta$ red) and A ($\alpha$ white), from smallest to largest. Stickland (1979), in a study of several species of East African wild game animals, found that in the longissimus dorsi, fiber size was ranked red, intermediate and white, from smallest to largest (with the SDH reaction).

Factors Affecting Myofiber Size

Variation in Fiber Size. Stickland and Goldspink (1973) found considerable variation in fiber size within several breeds of pigs which they attributed to differences in the herds from which pigs of the same breed came.

Stickland et al. (1975) stated that "the variation in size of fibers increased as the mean fiber size increased". Bendall and Voyle (1967) observed the same phenomenon.

Breed Differences With Respect to Fiber Size. Stickland and Goldspink (1978) studied differences between Large White pigs and a breed of miniature pigs. They concluded that there was no difference in fiber diameter between the two breeds at six to seven months of age.

Johnston et al. (1975) found that Charolais cattle had larger mean fiber diameters and areas for all three fiber types than Angus cattle at both feeding stages examined.

According to Stickland and Goldspink (1973), Staun (1968) associated a large number of fibers with smaller fibers. Stickland and Goldspink (1973) found this to be true in their study of a large variety of straight and crossbred pigs.

Hegarty (1971) reported that breed differences in fiber size have been shown in many species. Joubert (1956) found marked interbreed differences in fiber diameter between Dairy and Friesian cattle. Bendall and Voyle (1967) found that Friesians had larger fibers than Herefords in both the longissimus and the semitendinosus at any stage of growth.

Age Differences With Respect to Fiber Size. Cornforth et al. (1980) reported that at a given body weight, late maturing cattle would have smaller muscle fibers and less extensive fat cell development than early maturing cattle. However, if muscles were examined from calves of equivalent maturity and differing body weights, these differences no longer appeared.

Muscle Differences With Respect to Fiber Size. Stickland and Goldspink (1973) reported that differences

in fiber size between muscles was quite common and that the difference was probably due to variation of work loads on the particular muscles.

Joubert (1956) associated increase in muscle fiber size in a particular muscle with rate of growth of that muscle. Therefore, at different stages of development, individual muscles have fibers of varying sizes.

Bendall and Voyle (1967) reported that mean fiber diameter of longissimus and semitendinosus muscles had about the same growth rate at all stages.

Plane of Nutrition With Respect to Fiber Size. Many authors have found that nutritional state can alter myofiber size in different species. Stickland et al. (1975) found, in young nutritionally deprived pigs, that myofiber number was unchanged, but the ability of fibers to increase in size was impaired.

Hegarty (1971) stated that alteration of the plane of nutrition would have a more noticeable affect during the period of rapid growth and that a low plane of nutrition generally decreased fiber diameter, while return to a high plane would restore fibers to their normal diameters.

Joubert (1956) found that lambs of ewes on different nutritional levels during pregnancy showed no significant difference in fiber diameter at birth, but were significantly different at 60 and 290 days postnatally.

<u>Exercise With Respect to Fiber Size</u>. According to Hegarty (1971), exercise can increase fiber diameter and some authors believe duration is more important than the intensity of the exercise.

## Muscle DNA and RNA

The monitoring of changes in DNA and RNA concentrations and their relationships with other chemical components in the body are yet another method of observing differences in growth of muscle between species, breeds and even individual muscles.

### Prenatal Muscle DNA Concentration

In prenatal rat muscle, Winick and Noble (1965) observed a rapid increase in total organ DNA which continued through early postnatal life, then leveled off due to a decrease in the rate of DNA synthesis.

Burleigh (1974) observed that muscle DNA continued to increase in mammalian species postnatally, but at a slower rate than in prenatal development.

According to Allen et al. (1979) the muscle cell cycle (prenatally) has four general phases. First, the S phase (also called the DNA synthetic phase) in which cells with a 2N compliment of DNA replicate their DNA. Second, the $G_2$ phase (or post synthetic gap) in which cells with a 4N compliment of DNA (from the S phase) prepare for

mitosis and cytokinesis. Third, the M phase (mitosis) in which two, 2N cells are produced for the last, or $G_1$ phase (the presynthetic gap). From $G_1$, cells either start the cycle over again or remain without replicating.

It appears that presumptive myoblasts are the last prenatal muscle cell precursors capable of DNA synthesis and cell division. After differentiating to the myoblast stage, muscle cells remain in the $G_1$ phase and further DNA synthesis is thought to occur via satellite cell incorporation (Allen et al., 1979 and others).

Satellite Cell Incorporation

According to Cheek et al. (1971), Burleigh (1974) and Allen et al. (1979), the DNA concentration in muscles of mammals increases during postnatal life. Burleigh (1974) concluded that some nuclei must be replicating their DNA during this period. He also stated that nuclear number increased postnatally due to satellite cell incorporation.

Satellite cells are mononucleated cells located beneath the plasma membrane of muscle cells (Cardasis and Cooper, 1975). Satellite cells have the capacity to replicate their DNA and fuse into existing myofibers. They have also been shown to decrease both in absolute number and as a percentage of total muscle nuclei with increasing age (Cardasis and Cooper, 1975; Allen et al., 1979).

Muscle RNA Concentration

Total amount of RNA has been observed to increase with age (Burleigh, 1974; Winick and Noble, 1965) in rats and mice.

According to Goldspink (1977), 80 percent of the RNA in muscle is ribosomal RNA. Garlick et al. (1976) noted a proposal by Munro (1969) that the RNA concentration in a tissue was related to that tissue's capacity for protein synthesis. Winick and Noble (1965) stated that the tissues which actively synthesize protein were high in RNA content. Cassens and Cooper (1971) found that "red" muscles synthesized more protein and had higher RNA concentrations than "white" muscles.

Burleigh (1974) stated that during growth, accumulating protein diluted the RNA concentration in muscle and in individual myofibrils. He commented that Munro and Gray (1969) found a decrease in concentration of RNA as body size increased from mice to horses, but attributed the decrease to increased tissue per unit RNA in the larger species.

DNA Concentration per Nucleus

Many authors maintain that the amount of DNA per diploid nucleus in mammalian species is constant for individual species (Enesco and LeBlond, 1962; Ashmore and Robinson, 1969; Moss, 1969; Cheek, 1968; Robinson and

Lambourne, 1970a; Robinson, 1971; Cheek et al., 1971; LaFlamme et al., 1973). Ashmore and Robinson (1969) and Moss (1969) used a value of $2.5 \times 10^{-9}$ milligrams DNA per diploid nucleus which Moss (1964) established in chickens; while several other authors (Enesco and LeBlond, 1962; Cheek, 1968; Robinson, 1971; Cheek et al., 1971) used a value of $6.2 \times 10^{-12}$ grams per nucleus to calculate nuclear number.

## Nuclear Number

The established constancy of DNA concentration per nucleus allows estimation of nuclear number in muscle tissue (Enesco and LeBlond, 1962; Moss, 1969; Cheek et al., 1971; Robinson, 1971). Enesco and LeBlond (1962) offered the following equation for the calculation of number of diploid nuclei:

$$\text{nuclear number (millions)} = \frac{\text{mg DNA in whole organ or tissue} \times 10^{3}}{6.2 \times 10^{-12}\ \text{g}}$$

Enesco and Puddy (1964) found that total nuclei in muscle tissue of rats consisted of approximately 65 percent muscle fiber nuclei, 25 percent endomysial nuclei and 10 percent perimysial nuclei.

Cheek et al. (1971) stated that in addition to muscle cell nuclei, histocytes, fibroblasts, neuronal cells and adipocytes contributed DNA (from their nuclei) to muscle tissue. They further suggested that one-quarter of the

DNA in a muscle sample was located outside the muscle fiber.

Enesco and LeBlond (1962), after studying many different tissues, found that the number of nuclei in muscle tissue increased with age and that muscle fiber size increased more rapidly than nuclear number as skeletal muscles gained weight.

Enesco and Puddy (1964) reported that the number of muscle fiber nuclei in rats increased between suckling and young adult stages, but the rate of increase was different for different muscles.

Cardasis and Cooper (1975) observed muscle of mice from the 19th day of gestation until 63 days of age. They found that the number of nuclei per fiber increased from 84 to 354 during that period. Furthermore, it appeared that the greatest increase in nuclei per fiber occurred just prior to birth and the next greatest increase, just after birth. They concluded that the observed increase in nuclear number could be accounted for by the satellite cells associated with each fiber.

Moss (1968) found that in the muscles of most chickens the number of nuclei increased in proportion to the 2/3's power of the weight of the muscle.

Harbison et al. (1976) found that the number of nuclei in the longissimus dorsi muscle increased more rapidly in a muscular line of pigs than in an obese line from

68 to 118 kilograms live weight.

In a study of double muscled versus normal cattle, Ashmore and Robinson (1969) found that the double muscled animals had more nuclei and cytoplasm than the normal animals.

Trenkle et al. (1978) found an increase in number of nuclei and in cell size between 110 and 360 kilograms body weight in steers sired by either an Angus or a Charolais bull. After 360 kilograms, cell size continued to increase without further increase in nuclear number. The steers sired by the Charolais bull reached slaughter weight sooner than those sired by the Angus bull. Trenkle et al. (1978) attributed this to a more rapid accretion of DNA causing a faster muscle growth.

Weight per Nucleus

Cytoplasm (or weight) per nucleus is equal to the wet weight of muscle divided by total DNA content (Enesco and LeBlond, 1962; Moss, 1968; Ashmore and Robinson, 1969; Moss, 1969; Robinson, 1971). Enesco and Leblond (1962) gave this equation:

$$\text{muscle weight per nucleus} = \frac{\text{weight of fresh tissue or organ (g)} \times 10^3}{\text{number of diploid nuclei (millions)}}$$

Enesco and LeBlond (1962) stated that there was a steady rise in muscle weight per nucleus with increasing age.

Cheek et al. (1971) reported on a study conducted by Enesco and Puddy (1964) on rat muscle. They noted that the muscle weight per nucleus was fairly uniform in three of the four muscles studied from 16 to 86 days of age.

Munro and Gray (1969) found that weight per nucleus varied by two-fold or less between mammalian species ranging in size from mice to horses.

According to some authors, muscle tissue reaches an ultimate maximum size because each nucleus can support only a given amount of tissue (Robinson, 1971; Cheek et al., 1971; Allen et al., 1979). Burleigh (1974) and Harbison et al. (1976) stated that the number of nuclei determine total muscle mass. Increase in muscle mass per unit DNA is responsible for some of the growth of skeletal muscle postnatally (LaFlamme et al., 1973).

LaFlamme et al. (1973) found that DNA concentration decreased with increasing body weight and muscle size in cattle of dairy and beef breeding.

Harbison et al. (1976) stated, "Increase in total muscle DNA (or a higher amount of total muscle DNA) has been shown to be related to increased (or a greater amount of) muscling in pigs, mice and cattle."

Goldspink (1977) found in diaphragm of normal hampsters that DNA per gram of tissue decreased with increasing age.

According to Trenkle et al. (1978), if nuclear

number in muscle continued to increase for a longer period of time, then skeletal muscle would have an increased impetus for growth.

RNA:DNA Ratio

Increase in the RNA:DNA ratio in muscle is an indication of increased protein synthesis (Topel, 1971; Ezekwe and Martin, 1975; Harbison et al., 1976).

Munro and Gray (1969) stated that the RNA:DNA ratio was the same in horses as it was in mice.

In a comparison of genetically muscular or fat pigs, Topel (1971) found that the muscular strain of pigs had consistantly higher RNA:DNA ratios in the longissimus muscle than the fat strain between 22.7 and 137 kilograms body weight.

Ezekwe and Martin (1975) compared Yorkshire pigs to the feral obese pig (Ossabaw) and found that the Yorkshire pigs had a greater RNA:DNA ratio than the obese pigs. However, when they compared mice selected for increased body weight with control mice, they found that the controls had greater RNA:DNA ratios.

In comparison of a muscular line and an obese line of pigs, Harbison et al. (1976) found that in both lines the RNA:DNA ratio increased up to 104 kilograms live weight. However, between 23 and 91 kilograms live weight, the muscular line had a higher RNA:DNA ratio than the

obese line.

Protein:DNA Ratio

Increased protein:DNA ratio indicates larger muscle cells, since the ratio of cell protein to cell water is constant (Cheek, 1968; Robinson, 1971; Cheek et al., 1971; Ezekwe and Martin, 1975). Robinson and Lambourne (1970a) used the ratio of cellular nitrogen or protein to DNA to indicate cellular hypertrophy.

Winick and Noble (1965) stated, in rats, that during early prenatal growth the increse in protein is proportional to the increase in DNA. After this time, protein increases more rapidly than DNA until the animal is full grown. These authors observed an increase in the protein: DNA ratio near maturity in rats which they attributed to a slowing of DNA synthesis, not an increase in protein.

Cheek et al. (1971) stated that protein:DNA ratio changes during growth were due mainly to changes in DNA, not protein. They also found that the protein:DNA ratio appeared to be consistant between muscles of an individual rat. In addition, there appeared to be agreement between muscle groups for the protein:DNA ratio in 2.5 year old monkeys.

According to Cheek et al. (1971), Munro and Gray (1969) found that the horse had twice the amount of protein:DNA as the mouse. Ezekwe and Martin (1975) found

that Yorkshire pigs had greater protein:DNA ratios than feral obese pigs.

## Breed Differences in DNA

A muscular line of pigs was compared to an obese line by Harbison et al. (1976). They found that between live weights of 23 and 68 kilograms, DNA and RNA concentrations were not significantly different between genetic lines. However, between 68 and 118 kilograms, the muscular line had a significantly ($P < .05$) greater concentration of both DNA and RNA than the obese line.

LaFlamme et al. (1973) found that breed-type (dairy or beef) in cattle had no significant effect on DNA concentrations at several live weight. (However, the breed-types were compared at a constant live weight, not at a constant age.)

## Nutritional Effects

Robinson (1971) found that restricted nutrition in pregnant sows and later in their neonatal pigs, caused a permanent decrease in accumulation of RNA, DNA and protein in muscle tissue.

## Exercise Effects

Burleigh (1974) stated that satellite cells increased in number during work induced hypertrophy. However,

Cheek et al. (1971) reported that certain types of exercise could cause an increase in muscle growth, but the protein:DNA ratio did not change.

## Carcass Composition

At birth, carcass composition of the calf consists of about two parts muscle to one part bone. Bone is essential for function at birth and therefore develops early during prenatal development. Muscle, on the other hand, is needed at birth but does not reach peak usage until the young adult stage and therefore has an intermediate development rate between fat and bone. Fatty tissues are the least essential and thus develop latest. At birth the bovine carcass contains very little fat (Berg and Butterfield, 1976).

Berg and Butterfield (1976) maintain that with increasing age, the ratio of muscle to bone in the bovine increases because muscle grows more rapidly than bone postnatally. Fat increases slowly during growth until the fattening phase sets in. During this phase, with adequate nutrition, fat deposition increases. As a percentage of carcass, with increasing weight after birth, bone decreases slowly and continuously, muscle increases slightly and then begins to decrease as the fattening period begins.

Berg and Butterfield (1976) stated that breed differences in the onset of fattening and in muscling occur

in the bovine. They, as well as Lawrie (1961), found that early maturing cattle have a smaller mature size and generally enter the fattening phase at lighter weights than later maturing cattle. In addition, heavier muscled animals generally have higher muscle to bone ratios throughout life.

## Chemical Composition

Berg and Butterfield (1976) stated that the major chemical components of the body are water, protein, fat and ash. Cassens (1977) described muscle composition as 75 percent water, 20 percent protein, less than one percent carbohydrate and the rest fat and organic and inorganic substances.

Berg and Butterfield (1976) found that the bodies of calves are high in water and low in fat. With increasing age there is a decrease in percent protein, ash and water and an increase in percent fat. The muscle composition of the steer at more than one year of age consisted of 74 percent water, 21 percent protein, 4 percent fat and 1 percent ash. They also stated that the percent fat varied with the muscle examined. According to Hunt and Hedrick (1977a), "red" muscle generally contains more lipid than "white" muscle.

Lawrie (1961) described a decrease in the moisture content of the bovine longissimus muscle with increasing

age. He said that it was accompanied by an increase in fat content.

Hunt and Hedrick (1977a) observed, in five muscles of the mature bovine, that percent moisture was inversely related to percent ether extractable material.

Link et al. (1970a) noted an increase in intramuscular lipid with increasing age in bovine steers and heifers. Under the same conditions, Link et al. (1970b) observed a decrease in the relative amount of muscle protein and moisture.

Dickerson and Widdowson (1960) described a decrease in percent water and an increase in percent protein in skeletal muscle during development.

In pigs selected for increased body weight, Ezekwe and Martin (1975) observed greater total protein in the muscular line of pigs than in the obese line of pigs.

Winick and Noble (1965) found in rats that total organ protein increased until maturity. They stated that protein increases linearly with maturity.

Cheek et al. (1971) stated that early in postnatal life, protein per unit muscle reaches a stable level.

Trenkle et al. (1978) examined steers from Hereford x Angus dams which were sired by either an Angus or a Charolais bull. They found that overall growth rate was 15 percent faster for steers sired by the Charolais bull. Futhermore, at four different slaughter weights, the

steers sired by the Angus bull had greater muscle lipid in the longissimus dorsi muscle and total muscle protein increased with each larger body weight examined.

# CHAPTER III

## GENERAL MATERIALS AND METHODS

### Animals

This project was designed to study several muscle parameters in two breed-types of cattle at three stages of development. The breeds selected were Angus, representing a smaller framed, "early maturing type" and Charolais, representing a larger framed, "late maturing type". Cattle used in this study were obtained from two purebred herds (one Angus and one Charolais) within the state of Oklahoma.

The initial group of calves sampled were to be 25 days old, however, exact birth dates were not available for some calves and thus ages were approximated. The first slaughter group consisted of three Angus and three Charolais calves, all born in the spring of 1978 (spring-baby calves). The second slaughter group consisted of three Angus and three Charolais calves, all born in the fall of 1978 (fall-baby calves). Thus, a total of 12 approximately 25 day old calves were used in the study. (See table I, appendix C.)

These baby calves, all intact males, were brought to the O.S.U. meat laboratory directly from their respective

farms. They were held overnight, weighed and slaughtered the next morning.

The second age group of calves were to be 240 days old (or weanling age). Again exact birth dates were not available for all calves and thus some ages were approximated. The first calves slaughtered from this group were born in the spring of 1978 (same as the spring-baby calves) and consisted of six Angus and six Charolais (spring-weanling calves). The second set of calves for this group consisted of six Angus and six Charolais calves born at the same time as the fall-baby calves (fall-weanling calves). Thus, a total of 24 weanling calves were sampled. (Table I, appendix C.)

The weanling calves had been weaned at their respective farms and then transferred to the Experiment Station at El Reno, Oklahoma. From there, calves were transported to the O.S.U. meat laboratory in Stillwater in their respective groups at the appropriate ages. All calves at this stage were castrated males. Again, they were held overnight, then weighed and slaughtered the next morning.

The third age group of calves were approximately 650 days old. Once more, exact birth dates were not available for all calves in this group, as reflected in table I, appendix C. In addition, due to the larger animal size and thus greater work load, all calves in a slaughter group were not necessary killed on the same day (as they

were in the baby and weanling groups). Calf numbers per group differed in this slaughter group, because some animals died before reaching market weight; therefore, there were six spring-born Angus, five spring-born Charolais, four fall-born Angus and five fall-born Charolais calves. Thus, a total of 20 market weight calves were sampled.

The market weight calves had been weaned and transfered to the El Reno facility at the same time as the weanling calves. Transport and pre-slaughter procedures were the same as for the weanling calves.

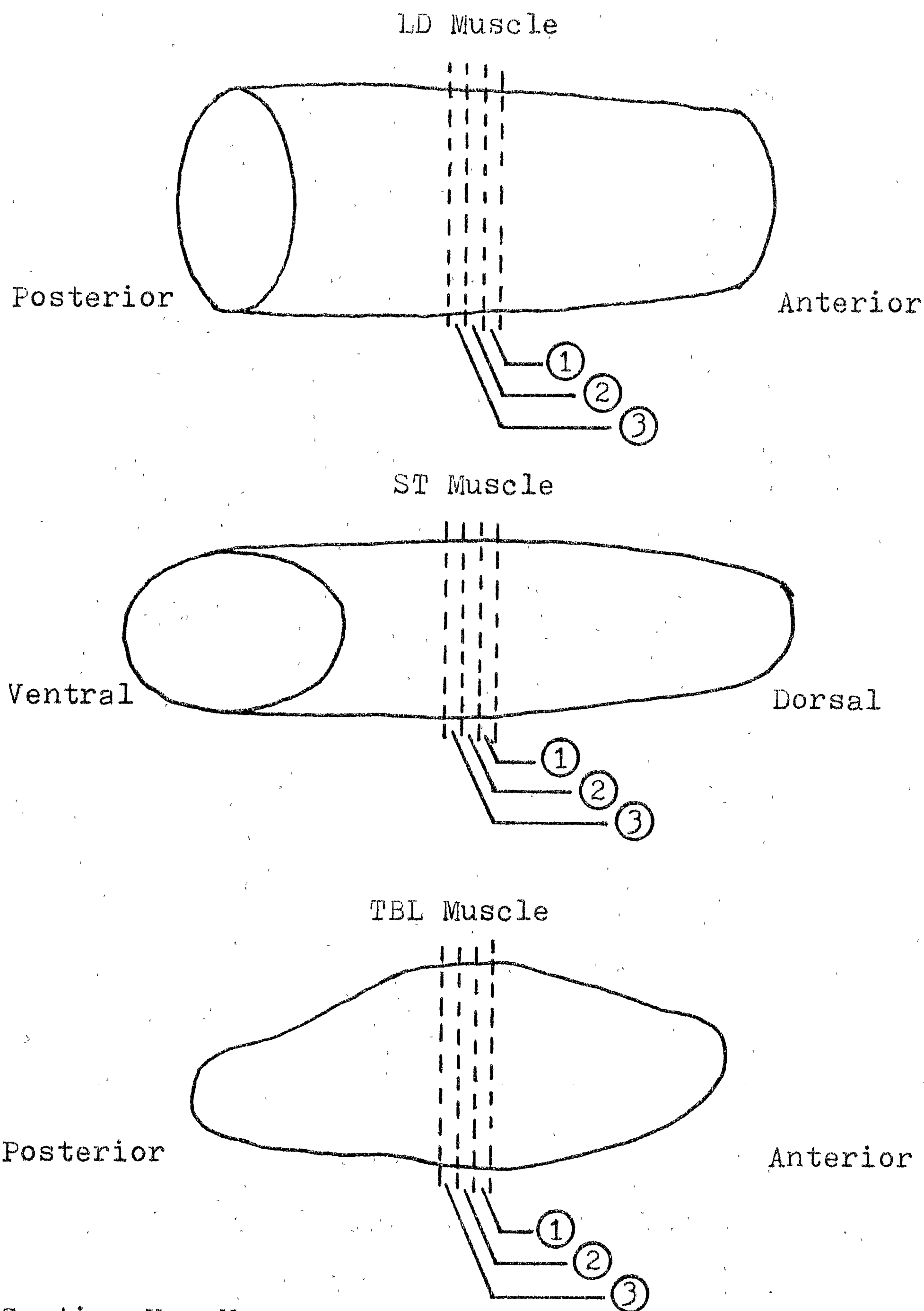
## Sampling Procedures

Within 30 minutes postmortem, carcasses were weighed (by side) and the Longissimus dorsi (LD) (a section from the twelfth rib to the fourth lumbar vertebrae), the Semitendinosus (ST) and the Triceps brachii-lateral head (TBL) were removed from each side. Following removal from the side, each muscle was trimmed of visible fat, weighed and measured for length. Additionally, a water weight was determined in 2°C water for later calculation of muscle specific gravity. Muscles were then wrapped in aluminum foil, frozen by immersion in liquid nitrogen and placed in storage at -20°C until analyses could be performed.

## Determination of Total Myofiber Number and Myofiber Type

In preparation for cryostat sectioning each muscle was removed from freezer storage and cross-sectioned at 50 percent of its length in the frozen state. (For the LD and TBL muscles this was the anterior-posterior orientation and for the ST muscle it was the dorsal-ventral orientation - figure 1.) A one centemeter wide section was removed from the posterior (ventral for ST) surface and its circumference traced onto paper for muscle cross sectional area determination. Cross sectional area was later measured from the paper tracings using a Compensating Polar Planimeter.

The frozen muscle sections were then placed posterior (or ventral for ST) side down on a board and visually divided into quadrants. A 6.35 millimeter diameter core was taken from each quadrant using a drill and a specially made coring device (see figure 2). Cores (still in the frozen state) were then placed in separate beakers of physiological saline solution and allowed to thaw. When thawing was completed, cores were oriented longitudinally, affixed onto microtome chucks using O.C.T. compound and quick-frozen with Cryokwik. They were then placed in a Slee Cryostat at -15 to -20°C for acclimation to temperature. Throughout this procedure the orientation of the cores was maintained.




Section Use Key

(1) Section used for nucleic acid analysis
(2) Section used for fiber type and proximate analysis
(3) Section used for myofiber width determination

*Note: All sections one centimeter wide, section two taken at 50 percent of transverse length.

Figure 1. Muscle Orientation for Sampling

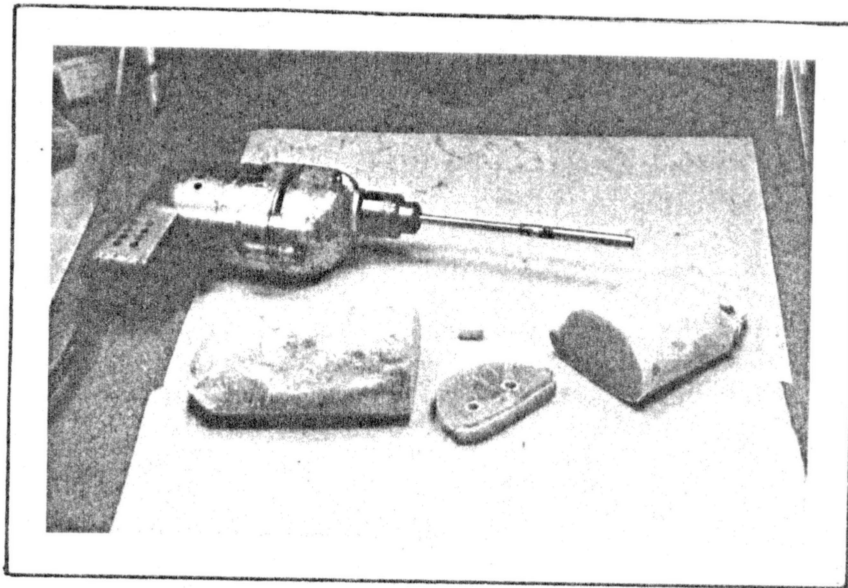


Figure 2. Coring Device and Procedure for Taking Cores for Myofiber Type Determination

Four, 12 $\mu$ transverse serial sections were made from each core and mounted on room temperature microscope slides. The first and third sections were stained using a NADH-TR method (modification of the method of Engel and Brooke, 1966) (see appendix A) and the second and fourth sections were stained using an alkaline ATPase method (modification of the method of Guth and Samaha, 1969) (see appendix A). Slides were then examined for myofiber type and number.

Myofiber type was determined in one random field from each slide. Therefore, two fields per core were counted for each stain; or a total of eight fields per muscle for each stain.

Slides were placed under the microscope and a random field selected. (The same microscope was not available for all fiber type determinations, therefore, the slides weren't all examined at the same magnification. Sections from spring-baby calves were examined at 20x with an American Optics microscope; sections from fall-baby calves were examined at 10x with the same A/O microscope; and sections from both spring and fall-weanling calves were examined at 16x with a Ziess microscope. All data, however, were corrected to a standard basis for statistical analysis.) The image of this field was projected onto a Viso-pan screen which contained a grid of known area. Fiber type was determined for each fiber within the grid

as follows. NADH-TR stain: darkly stained, high activity fibers - $\beta$ red; medium stained fibers - $\alpha$ red; light or no NADH-TR activity - $\alpha$ white (Cooper et al., 1970; Ashmore and Doerr, 1971a; Beermann et al., 1977). ATPase (alkaline) stain: darkly stained, high activity fibers - $\alpha$ white; medium activity fibers - $\alpha$ red; no activity fibers - $\beta$ red (Guth and Samaha, 1970; Ashmore and Doerr, 1971a; Beermann et al., 1977). Fibers located partially within the grid were counted as in the grid area if they touched the top or right-hand border of the grid and out if they touched the bottom or left-hand border. Total myofiber number was expressed as the sum of the three fiber types for each field examined.

## Myofiber Width Determination

For myofiber width determination a second transverse muscle section was taken adjacent and posterior (or ventral for ST) to that used for myofiber type (see figure 1). A 6.35 millimeter diameter core was removed from each of two randomly selected quadrants of the muscle section. Cores were identified, placed in individual containers of 10 percent buffered formalin solution and stored for 48 hours at 2.8°C. After this time, individual cores were placed in fresh, cold 10 percent buffered formalin in a Waring blender equipped with inverted blades. Individual myofibers were dispersed from the cores by blending for

two minutes. The containers of dispersed fibers were stored at 2.8$^{o}$C until fiber width measurements could be made.

In preparation for measurement, fibers in solution were pipetted from their containers into a watch glass. The watch glass was placed under the 10x objective of an American Optics light microscope and fiber widths were measured using an occular micrometer (Joubert, 1956; Hanrahan et al., 1973; Stickland and Goldspink, 1973; Melton et al., 1974; Ezekwe and Martin, 1975). Fibers measured were selected at random and measurements were made within the body of each fiber (not near the ends). Care was taken to avoid measuring any obviously damaged or broken fibers. Twenty-five fibers were measured per core or a total of fifty fibers per muscle.

## Nucleic Acid Analysis

For nucleic acid analysis a third transverse section was taken from each muscle. This section was located adjacent and anterior (or dorsal for the ST) to the section used for fiber type determination (see figure 1). Four random cores were taken from the muscle section to insure enough tissue for duplicate extractions. Nucleic acids were extracted and DNA and RNA concentrations were determined according to procedures described by Escoubas (1977) (appendix B).

## Proximate Analysis

### Muscle Composition

For muscle composition, the remaining portion of the transverse muscle section previously used for fiber type determination was finely minced and then Omni-mixed. The Omni-mixed muscle was used for determination of moisture, ash and ether extract by standard A. O. A. C. methods and protein by the Kjeldahl method.

### Carcass Composition

For carcass composition analysis only the right side of each carcass was used. Sides were chilled at least 24 hours and not longer than seven days before carcass analysis was begun. The chilled right sides were weighed then separated into bone, soft tissue (muscle and fat), kidney fat and kidney components. Each portion separated was weighed.

Following separation, the soft tissue was ground using a commercial meat grinder equipped with a 3/8's inch plate. The ground side was then hand mixed and ground again through a 1/8 inch plate. During the second grinding, periodical grab samples were taken so that upon completion there were two, approximately one pound samples. These samples were each hand mixed and a smaller grab sample removed for Omni-mixing. Duplicate aliquots were

then taken from each Omni-mixed carcass sample for determination of moisture, ash, ether extract and protein in the carcass by the methods described previously for muscle composition.

## Total Carcass Lean, Fat and Bone

Calculation of total carcass lean, fat and bone was not readily available since the LD, ST and TBL muscles were removed from the carcass prior to grinding for carcass composition estimates. To build back to the total carcass, the pounds of protein, moisture, ash and ether extractable components were calculated using the proximate analysis data and air weights of the muscles removed from the right side of the carcass. Then, the pounds of protein, moisture, ash and ether extractable components were calculated using the proximate analysis from the ground right side (minus the removed muscles) and the soft tissue weight as boned from the carcass.

Right side lean weight was determined by adding together the protein, moisture and ash components of the individual muscles and the ground side. Right side fat weight was determined by adding together the ether extractable components from the individual muscles and from the ground side and the kidney and pelvic fat. Right side bone weight was measured directly from the boned carcass.

To arrive at respective total carcass lean, fat and

bone percentages, the above weights were divided by total right side weight. (It was assumed that the right side lean, fat and bone were representitive of the total carcass lean, fat and bone, since time and personnel allowed only for boning and analysis of one side of the carcass.)

## Statistical Analysis

This experiment was designed as a Completely Randomized Design with multiple factorial arrangement of treatments. Analysis of variance were provided by the SAS 72 system. Comparisons between muscles for statistical significance were done using Duncan's Multiple Range Test.

CHAPTER IV

# TOTAL MYOFIBER NUMBER AND MYOFIBER TYPE PER MUSCLE IN TWENTY-FIVE DAY OLD BEEF CALVES

## Introduction

It is believed that myofiber number is essentially fixed at birth and that increase in muscle mass after birth is primarily due to muscle cell hypertrophy (Joubert, 1956; Hegarty, 1971; Stickland and Goldspink, 1973; Swatland, 1976; Ashmore, 1977). It has been reported that individual species, as well as individual breeds or animals within species vary with regard to total myofiber number (Hegarty, 1971; Stickland and Goldspink, 1978). These observed variations in total myofiber number can influence ultimate muscle mass. Additionally, it has been shown that there are several different types of muscle fibers. These myofiber types differ not only in certain histochemical and biological reactions, but also in size and relative proportions in individual muscles, breeds and species. All of these factors can influence the ultimate muscle mass of the animal.

This study was undertaken to determine if differences

exist with regard to total myofiber number, myofiber type and myofiber size between the two breed-types of cattle examined. Furthermore, it was desired to learn if examination of these parameters at an early age could be used to predict ultimate muscle mass.

## Materials and Methods

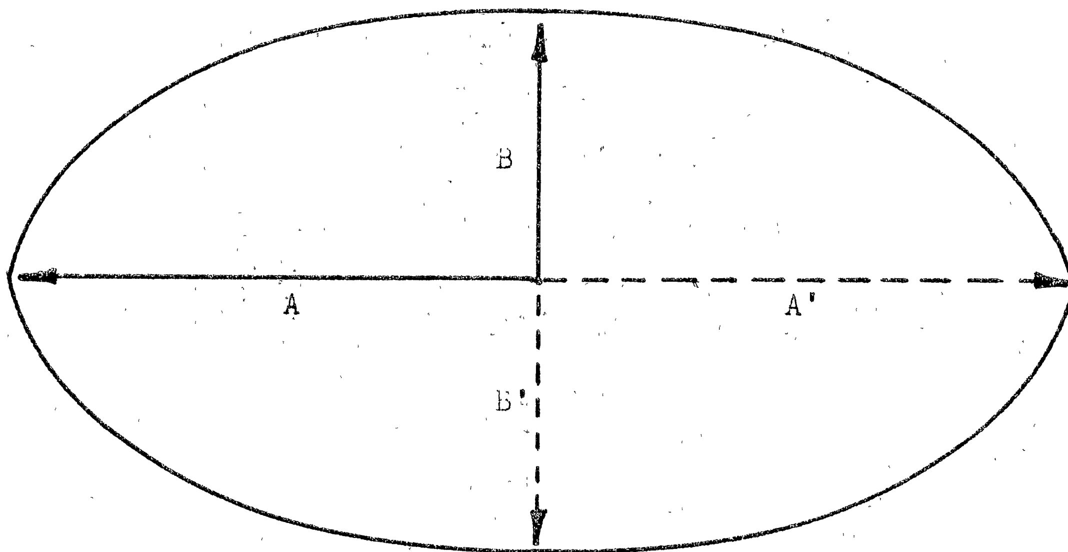
Muscle sections were obtained (transversely) from the longissimus dorsi (LD), the semitendinosus (ST) and the triceps brachii-lateral head (TBL) of 25 day old Angus and Charolais calves as previously noted. A 6.35 millimeter diameter core was taken from each of four quadrants of each muscle section. The cores were allowed to thaw in physiological saline solution, then mounted on microtome chucks and quick-frozen. After acclimation at -15 to -20°C in a Slee Cryostat, the cores were sectioned. Four, 12 μ transverse serial sections were made from each core and mounted (at room temperature) on glass microscope slides. Sections were then stained using either an Alkaline ATPase or a NADH-TR method (Chapter III).

The stained sections were microscopically examined and total myofiber number and myofiber number by type were determined. Myofiber type was determined in one random field which was projected onto a Viso-pan screen containing a grid of known area. Myofibers were enumerated within the grid as follows: Alkaline ATPase Stain (a) darkly

stained, high activity fibers = $\alpha$ white; (b) medium activity fibers = $\alpha$ red; (c) no activity fibers = $\beta$ red (Guth and Samaha, 1970; Ashmore and Doerr, 1971a; Beermann et al., 1977). NADH-TR Stain (a) darkly stained, high activity fibers = $\beta$ red; (b) medium stained fibers = $\alpha$ red; (c) light or no NADH-TR activity = $\alpha$ white (Cooper et al., 1970; Ashmore and Doerr, 1971b; Beermann et al., 1977). Total myofiber number was expressed as the sum of the three myofiber types for each field examined.

Myofiber type count per muscle cross sectional area was determined by: (1) counting the fibers of each fiber type in the projected grid and correcting this count for differences in magnification; (2) multiplying the myofiber count per grid by the area of the tissue slice on the microscope slide (the area of the tissue slice was calculated by assuming an ellipse and measuring the long [A + A'] and short [B + B'] axises of the tissue slice and then applying the formula $\frac{\pi[A + A'][B + B']}{4}$ to determine the area of the tissue slice [figure 3]); (3) the resulting myofiber count per thawed tissue slice was multiplied by the muscle cross sectional area (Chapter III) and divided by the area of the standard frozen core (see below).

$$\frac{\text{myofiber count per grid}}{\text{correction for magnification}} \times \text{area of tissue slice} = \text{myofiber count per thawed tissue slice.}$$




Ellipse

Area = $\pi AB$

A = $\frac{1}{2}$ long axis

B = $\frac{1}{2}$ short axis
(90$^{o}$ from long axis)

Figure 3. Area Determination of Tissue Slice

$$\frac{\frac{\text{myofiber count per}}{\text{thawed tissue slice}} \times \text{muscle cross sectional area}}{\text{area of frozen core (constant = 31.669 mm}^2\text{)}} =$$

myofiber count per muscle cross sectional area.

These calculations were necessary to adjust the thawed tissue slice area back to the original muscle cross sectional area. The thawed tissue slice was measured in the post rigor thawed state because the muscle cores were allowed to thaw prior to mounting on microtome chucks for sectioning (Chapter III) and measurements of muscle cross sectional area were taken with the muscle in the prerigor frozen state.

As previously explained, two stains were used in this study for the determination of myofiber type. Several authors have noted the necessity for use of more than one staining procedure for myofiber typing (Edgerton and Simpson, 1969; Ashmore and Doerr, 1971a) and have reported methods for simultaneous determination of myofiber type with more than one stain. Due to lack of appropriate equipment, the present study was not prepared for simultaneous determination of myofiber type with both the ATPase and NADH-TR stains, therefore, myofiber type was determined separately with each stain. This procedure left discrepancies with regard to enumeration of myofiber type between stains. These discrepancies caused a significant difference in the enumeration of myofibers between stains.

It was therefore decided to analyze data from the two stains separately.

As noted in the general materials and methods, half the calves for this study were born in the spring of the year and half were born in the fall. When data for both seasons were pooled it was readily apparent that significant differences existed between the two calving seasons for individual myofiber types and for total myofiber number in the 25 day old calves. It was therefore decided to examine the two seasons separately.

## Results and Discussion

### Total Myofiber Number - ATPase Stain

Season I results show that the Charolais calves had a greater number of total myofibers in each test muscle than the Angus calves at 25 days of age. These differences, however, were not large enough to be statistically significant. Season II results show the same trend as season I, except that the total number of myofibers in the LD muscle was significantly greater ($P < .05$) in the Charolais calves than in the Angus calves (tables II, III and IV, appendix C).

### Total Myofiber Number - NADH-TR Stain

In each muscle of season I the Charolais calves had a greater total number of myofibers than the Angus calves.

In the LD muscle the total number of myofibers was significantly greater ($P < .05$) for the Charolais calves. Similar results were apparent in season II. Total myofiber number in the LD muscle was significantly greater ($P < .05$) for the Charolais calves (tables II, III and IV, appendix C).

Discussion

It was readily apparent from table V, appendix C, that the Charolais calves were larger in both seasons than the Angus calves. Burleigh (1974) hypothesized that larger animals may have the potential for a greater number of muscle fibers at birth due to a tendency for longer gestation periods and thus increased time for prenatal muscle cell replication. Though statistical significance was not obtained in all comparisons, the results shown in tables II, III and IV, appendix C, certainly would support Burleigh's hypothesis.

In addition to having a greater total number of myofibers, the Charolais calves had heavier mean muscle weights (table VI, appendix C) in both seasons than the Angus calves (with the exception of the TBL muscle in season I which had the same mean weight for both Angus and Charolais calves). A greater total number of myofibers in already heavier muscles indicated that even at this early stage of postnatal development, the Charolais calves possessed the potential for greater overall muscle growth

than the Angus calves (assuming that the individual myofibers of the Angus and Charolais subsequently hypertrophy to similar degrees).

<u>Myofiber Number by Type - ATPase Stain</u>

As can be seen in table VII, appendix C, the LD muscle of Charolais calves in season I tended to have a greater number of both $\alpha$ white and $\beta$ red fibers and fewer $\alpha$ red fibers than the Angus calves. Results of season II in the same muscle showed a different trend in that the Charolais calves had a greater number of myofibers of each fiber type than the Angus calves. This number was significantly greater ($P < .05$) for the $\alpha$ red fibers.

Upon examination of the ST muscle of season I (table VIII, appendix C) it can be seen that the Charolais calves tended to have a greater number of myofibers of each fiber type than the Angus calves. Although breed differences in $\alpha$ red fibers were only very slight, the breed differences in $\beta$ red fibers were significant at the $P < .05$ level. Season II results show a similar tendency for $\alpha$ white fibers and $\alpha$ red fibers, however, the number of $\beta$ red fibers was slightly greater for the Angus calves than for the Charolais.

Results for the TBL muscle (table IX, appendix C) indicated the same trends in both seasons as the LD muscle;

the Charolais calves had greater myofiber numbers for each myofiber type except the $\alpha$ red fibers in season I.

Myofiber Number by Type - NADH-TR Stain

In both season I and season II for the LD muscle the Charolais calves had a greater number of myofibers of each fiber type than the Angus calves (table VII, appendix C). Fiber number was significantly greater ($P < .05$) for all fiber types except the $\alpha$ white fibers of season I.

In the ST muscle the trend was the same (table VIII, appendix C). In both seasons, for each myofiber type the Charolais calves had a greater number of myofibers. However, in this muscle only the number of $\beta$ red fibers of both seasons was significantly greater in the Charolais calves ($P < .05$).

Results for the TBL muscle (table IX, appendix C) were similar to those of the LD and ST muscles. For both seasons and each myofiber type the Charolais calves possessed a greater number of myofibers than the Angus calves. The $\beta$ red fibers of season I and the $\alpha$ red fibers of season II were significantly greater ($P < .05$) for the Charolais calves.

Discussion

Reviewing the above results, it can be seen that the Charolais calves had a greater number of myofibers of each

fiber type in both seasons with these exceptions: the ATPase stained $\alpha$ red fibers of season I in the LD and TBL muscles and the ATPase stained $\beta$ red fibers of season II in the ST muscle.

Possible reasons for the observed greater number of $\alpha$ red fibers in Angus calves follow:

Exact birth dates were unavailable on some of the calves examined (especially the "25 day old" Angus calves). Considering this and the fact that $\alpha$ red myofibers purportedly differentiate to $\alpha$ white myofibers with increasing age (Ashmore et al., 1972); it may be possible that slight age differences between breeds in the two seasons was responsible for the observed greater number of $\alpha$ red fibers in the LD and TBL muscles of the Angus calves of season I.

Noting the differences in staining procedures and the myofibers which they are more likely to identify clearly, the difference observed between stains in counting $\alpha$ red fibers could be due to the subjective assignment of myofiber type based on staining intensity. It must be noted that in determining myofiber number by type, a different microscopic field was counted for each stain. Therefore, it might be that the counts for the ATPase and the NADH-TR stains would not be the same due to random error and/or animal to animal variation. Finally, perhaps there is a real difference in the prenatal development of beef calves

born in the spring vs those born in the fall.

Possible reasons for the observed greater number of $\beta$ red fibers in the ST muscle of Angus calves of season II follow:

In taking cores for tissue sectioning and myofiber typing it is possible that a random distribution of myofibers was not achieved due to sampling error since according to Beermenn et al. (1977) and others, the inner and outer portions of the ST muscle contain different proportions of myofiber types. It is also possible that a particular field counted happened to have a greater number of $\beta$ red myofibers.

With the above exceptions it can be noted that most of the data indicated that the Charolais calves had a greater number of myofibers of each fiber type. This, in addition to the earlier stated greater total number of myofibers in each muscle for the Charolais calves indicates a greater muscle growth potential in the Charolais calves.

## Summary

Live weight, carcass weight, muscle weight for each muscle examined, and total myofiber number in each muscle examined all appear to be greater in 25 day old Charolais calves than in 25 day old Angus calves. The greater total myofiber number compounded with larger muscle size and

larger body size at a constant age all point to a greater growth potential in the Charolais calves which is evident even at this early age.

The somewhat inconsistant results between stains for myofiber typing indicates (as previously suggested by several authors) the necessity for simultaneous determination of myofiber type with more than one stain using the same microscopic field on serial sections. Even with the above inconsistancies, however, a trend was detected indicating a greater number of myofibers of each fiber type in the Charolais calves.

Cost factors involved in purchasing animals at this young age led to a limited sample size available for this study. This, in addition to problems in determining exact animal age and the staining inconsistancies above, may limit the conclusions that can be drawn from these data. However, further study in this area is indeed indicated if muscle sampling at an early age is to be developed into a useful technique in determining potential muscle growth in cattle.

CHAPTER V

# DNA AND RNA IN MUSCLE OF TWENTY-FIVE DAY OLD BEEF CALVES

## Introduction

Muscle DNA and RNA are important components in the study of muscle growth. Because muscle cells are multinucleated, DNA and RNA play a special role in muscle tissue during growth and development.

Muscle DNA is accumulated very quickly in prenatal muscle, but seems to increase more slowly in postnatal development (Winick and Noble, 1965; Burleigh, 1974). Postnatal increase in DNA concentration in muscles is thought to occur via satellite cell incorporation into existing myofibers thus increasing the DNA content (or nuclear number) of these cells (Cheek et al., 1971; Burleigh, 1974; Allen et al., 1979).

It is generally accepted that the amount of DNA per diploid nucleus is a constant value which can be used to calculate nuclear number in multinucleated muscle tissue. Enesco and LeBlond (1962) used the following equation for the calculation of nuclear number in muscle tissue:

$$\text{nuclear number (millions)} = \frac{\text{mg DNA in whole organ or tissue} \times 10^3}{6.2 \times 10^{-12} \text{ g}}$$

Many authors have reported an increase in nuclear number in muscle tissue postnatally.

Burleigh (1974) and Harbison et al. (1976) have reported that nuclear number determines ultimate muscle mass. In addition, Enesco and LeBlond (1962) observed a steady rise in muscle weight per nucleus with increasing age. Some authors (Robinson, 1971; Cheek et al., 1971; Allen et al., 1979) have stated that muscle tissue reaches an ultimate maximum size because each nucleus can support only a given amount of tissue, and Harbison et al. (1976) have related increased muscling in pigs, mice and cattle to increase in total muscle DNA.

The RNA:DNA ratio has been related to the protein synthetic capacity of muscle. Topel (1971), Ezekwe and Martin (1975) and Harbison et al. (1976) reported that an increase in the RNA:DNA ratio in muscle is an indication of increased protein synthesis. Futhermore, it has been noted that an increase in the ratio of cellular nitrogen or protein to DNA is an indication of cellular hypertrophy (Cheek, 1968; Robinson and Lambourne, 1970a; Robinson, 1971; Cheek et al., 1971; Ezewke and Martin, 1975). In an attempt to further characterize muscle differences between two breed-types of cattle, DNA and RNA were extracted and compared from muscle tissue of 25 day old Angus and

Charolais calves.

## Materials and Methods

For DNA and RNA analysis, a transverse section was taken from each muscle at 50 percent of its length in the frozen state (adjacent to the section used for myofiber type determination, figure 1). Then, four random cores were taken from the muscle section to insure enough tissue for duplicate extractions. Nucleic acids were extracted and DNA and RNA concentrations were determined according to procedures described by Escoubas (1977) (appendix B).

## Results and Discussion

### DNA Concentration per Muscle

Data in table X, appendix C, show that in each muscle in both seasons, the DNA concentration was significantly greater (P .01) in the Charolais calves than in the Angus calves. In addition, in blth breeds and in both seasons the DNA concentration was greatest in the LD muscle and lowest in the TBL muscle with the ST intermediate.

### RNA Concentration per Muscle

Results of the RNA concentration per muscle show the same trends as the DNA concentration per muscle. It can be seen in table XI, appendix C, that the Charolais calves had significantly greater ($P<.01$) RNA concentrations for

each muscle in both seasons. Furthermore, the ranking of muscles from high to low RNA concentrations were similar to the DNA concentrations: LD>ST>TBL.

Number of Nuclei per Muscle

As can be seen in table XII, appendix C, in all muscles in both seasons, the Charolais calves had a greater number of nuclei than the Angus calves. In season I, in both the LD and ST muscles this difference was significant at the $P<.01$ level. In season II, in the LD and ST muscles the difference was significant at the $P<.05$ level. In both seasons in both breeds the ranking of muscles from highest to lowest nuclear number was LD>ST>TBL.

Grams Tissue Supported per Nucleus

In season I, it appears that in all muscles the Angus calves had a greater amount of tissue supported per nucleus than the Charolais calves (table XIII, appendix C). However, in season II it appeared that the Charolais calves had a greater amount of tissue supported per nucleus. Indeed, in the ST muscle this difference was statistically significant ($P<.05$).

Grams Protein per Gram DNA

Results from season I indicate that the Angus calves had a greater protein:DNA ratio than the Charolais calves

in each muscle. In season II, on the other hand, in the LD and ST muscles the Charolais calves had a greater protein:DNA ratio while in the TBL muscle the Angus calves had a greater protein:DNA ratio. None of these differences, however, were statistically significant (table XIV, appendix C).

RNA:DNA Ratio

The RNA:DNA ratio in season I appeared greater for the Angus calves in the ST and TBL muscles and greater for the Charolais calves in the LD muscle. In season II, the RNA:DNA ratio was greater for the Charolais calves in each muscle. None of these differences were statistically significant (table XV, appendix C).

Discussion

From the above results, it appears that the Charolais calves had a greater capacity for muscle growth than the Angus calves. This statement is supported by the greater number of nuclei per muscle in the Charolais calves which is based on the greater DNA concentration per muscle in this breed. If each nucleus is capable of supporting only a given amount of tissue (Robinson, 1971; Cheek et al., 1971; Allen et al., 1979) then the muscles with the greatest number of nuclei should reach the largest ultimate size. The current data suggests that those muscles would

be from the Charolais calves.

Upon examination of data for grams of tissue supported per nucleus it appeared in season I that the Angus calves might be further along in muscle development than the Charolais calves, since they appeared to have more tissue associated with each nucleus in each muscle than the Charolais calves. In season II, however, the Charolais calves tended to have more tissue associated with each nucleus, but they also had a greater number of nuclei, so again there appears to be support for greater muscle growth potential in the Charolais calves.

Examination of individual muscles indicated both through DNA concentration and nuclear number that the LD muscle in both breeds had potential for the greatest growth, followed by the ST and the TBL muscles respectively. The grams tissue supported per nucleus followed a similar trend with several exceptions (the ST muscle of both breeds in season I and the ST of the Charolais calves in season II). These exceptions may have been caused by differences in removing the muscles from the carcass. Since the entire LD muscle was not removed, it is possible that weight differences in LD muscles due to excisement technique caused the above discrepancies.

Examination of the muscle RNA concentration data showed that the Charolais calves appeared to have a greater capacity for protein synthesis than the Angus calves.

This is based on the proposal by Munro (1969) that the RNA concentration in a tissue is related to that tissue's capacity for protein synthesis. With two exceptions (ST and TBL muscles of season I), the RNA:DNA ratio data supported the above conclusion. It has been stated by several authors that increase in the ratio of RNA to DNA in muscle is an indication of increased protein synthesis. The presence of increased protein synthesis in the Charolais calves is especially apparent in season II which might account for the observed greater grams tissue supported per nucleus in table XIII, appendix C.

The grams protein per gram DNA data indicated that in season I the Angus calves had larger muscle cells and in season II the cells appeared to be much the same size between breeds. This is supported by the greater grams of tissue per nucleus observed in table XIII, appendix C. Eventhough the Charolais calves in season I had more nuclei, they had smaller cells; this further enhanced their greater growth potential. In season II it appeared that the increased protein synthetic activity of the Charolais calves, as previously discussed, caused the similar cell size observed in table XIV, appendix C, however, a greater nuclear number clearly indicated that even at a similar cell size at this time, the Charolais calves had the potential for greater cellular growth than the Angus calves. (Examination of myofiber width data /table XVI, appendix

C] supports the above conclusions about season I, however, in season II the Charolais calves had significantly larger myofiber widths for each muscle [$P < .01$ for LD and ST muscles and $P < .05$ for TBL muscle] than the Angus calves.)

## Summary

The results discussed in this chapter seem to point to a greater growth potential in the Charolais calves than in the Angus calves. The Charolais calves appear to have the potential to accumulate a greater amount of cytoplasmic material in entire muscles based on their greater nuclear number and higher DNA concentration. On the other hand, the muscles of the Angus calves appeared in a more advanced state of maturity than the Charolais as indicated by the greater amount of tissue per nucleus at a similar age in season I and less tissue per nucleus but fewer nuclei in season II. If nuclei support only a given amount of tissue and if tissue accumulation continues at a similar rate then it would follow that the muscles of the Angus calves would reach the maximum tissue supported per nucleus state more quickly than the Charolais calves.

These data appear to be quite useful in predicting growth potential and possibly even ultimate size. With muscle biopsy as a method for collecting tissue for DNA and RNA analysis, samples might be taken with minimum damage to the animal. Data could then be useful to

determine the future use for breeding or marketing of individual animals.

Again, it must be pointed out that a very small number of animals was used in this study and further work in this area is needed.

# CHAPTER VI

## GENERAL SUMMARY

Results have been presented here on live weight, carcass weight, muscle weight, myofiber number, myofiber type, myofiber width and DNA and RNA analyses on three muscles of 25 day old Angus and Charolais calves born in different seasons.

It was apparent from these data that live weight, carcass weight, muscle weight and total myofiber number all appeared to be greater in the 25 day old Charolais calves than in the 25 day old Angus calves (though rarely was a statistically significant difference observed). Although there were some inconsistancies in myofiber typing results, there also appeared to be a tendency for the Charolais calves to exhibit a greater number of myofibers for each myofiber type in the three muscles examined.

Based on the DNA and RNA analyses, it appeared that the 25 day old Charolais calves had the potential to accumulate a greater amount of cytoplasmic material in their muscles than the 25 day old Angus calves. Furthermore, the Angus calves tended to be in a more advanced state of maturity than the Charolais calves at this early stage of development.

The above results suggested that at 25 days of age the Charolais calves showed a greater potential for muscle growth and ultimate muscle mass than the Angus calves. Thus, with respect to the original goals of this research, results indicated that the two breed-types did differ with respect to the various muscle parameters examined. These differences can be detected at an early age and myofiber number; myofiber type; myofiber size; live body weight; carcass weight; muscle weight; total body lean, fat and bone; total lean and individual muscle protein, moisture, fat and ash; and muscle DNA and RNA concentrations might be used in systems analysis equations to predict ultimate muscle mass of the animal. Regarding ease of use as a selection tool; as used in this study, none of these methods would be practical for use as a selection tool at the current time. But, perhaps biopsy sampling using one or more of the parameters examined here could prove to be an effective selection tool.

Finally, it must be remembered that due to costs associated in working with large animals the sample size for this study was limited. This coupled with the obvious large degree of animal to animal variation in the data no doubt limited the attainment of statistical significance of some of the results. Further study with a larger number of animals would certainly seem to be in order.

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# APPENDIX A

# MODIFICATION OF STAINING PROCEDURES

## MODIFICATION OF DPNH-TR STAINING PROCEDURE OF ENGEL AND BROOKE (1966)

Nicotinamide Adenine Dinucleotide-Tetrazolium Reductase (NADH-TR) was used in place of Reduced Diphosphopyridine Nucleotide-Tetrazolium Reductase (DPNH-TR).

## MODIFICATION OF ALKALINE ATPase STAINING PROCEDURE OF GUTH AND SAMAHA (1969)

Frozen sections were immediately affixed onto microscope slides and fixed for five minutes in buffered formaldehyde (sodium acetate replaced sodium cacodylate in the buffered formaldehyde solution).

The fixative was not removed (only blotted) before sections were placed in the alkali preincubation solution of 0.1 M Tris containing 0.018 M calcium chloride at pH 10.3 for 15 minutes.

From the preincubation solution, slides were blotted and transferred to the staining solution which consisted of 0.1 M Tris containing 0.018 M calcium chloride and 0.027 M ATP at pH 9.4. Sections were incubated in this solution for 30 minutes at 36°C.

Following the three, 30 second rinses in 0.07 M calcium chloride, the sections were placed in two percent cobalt chloride for six to eight minutes. They were then

rinsed in 0.1 M Tris buffer at pH 9.4 for approximately two minutes and placed in one percent dilute yellow ammonium sulfide for three minutes.

Final procedures consisted of washing sections for three to five minutes in distilled water followed by mounting in glycerogel.

# APPENDIX B

## TOTAL DNA AND RNA PROCEDURE (ESCOUBAS, 1977)

## TOTAL DNA AND RNA

Nucleic acids were isolated and quantitated by modified procedures of Schneider (1945), Ceriotti (1955), and Burton (1956). Duplicate muscle tissue samples (0.4 to 0.6 grams) were sectioned from frozen muscle homogenates, weighed and immersed immediately into ten volumes (5 milliliters) of ice cold deionized, glass distilled water. The samples were homogenized at $0^{o}C$ for 10 minutes at a low speed in the Sorvall Omni-mixer, micro attachment, apparatus. The resulting suspension was acidified with appropriate quantities of 0.6 N perchloric acid (PCA) to a final concentration of 0.2 N PCA. The acidified suspension was mixed thoroughly on the Vortex mixer and centrifuged for 15 minutes in a clinical centrifuge at a Variad setting of 75. The supernatant was discarded and the precipitate re-extracted in 0.2 N PCA and centrifuged as above for 10 minutes. The resulting precipitate was extracted in the following solvents, in the indicated order and centrifuged in the clinical centrifuge at a Variad setting of 75 after each extraction.

1. 95 Percent Ethanol: Extracted 10 minutes, Centrifuged 15 minutes
2. Ethanol:Chloroform (3:1): Extracted 10 minutes,

Centrifuged 10 minutes

3. Ethanol:Ether (3:1): Extracted 10 minutes, Centrifuged 10 minutes

4. Ether: Extracted 10 minutes, Centrifuged 10 minutes

The resulting precipitate from the ether extraction and centrifugation was extracted in 0.2 N PCA and centrifuged, after the extraction, for 10 minutes as noted above. This procedure was performed twice. The final precipitate was air dried under convection in the laboratory hood for 15 minutes. Note should be made that all extractions, mixings and centrifugations were accomplished in a 1.1°C environment.

The air dried precipitate was incubated at 90°C for 46 minutes in seven milliliters of 0.2 N PCA with periodic mixing. After incubation, the tubes and contents were chilled in icewater for 10 minutes and centrifuged at 1.1° C in a clinical centrifuge at a Variad setting of 75. The supernatants obtained were decanted through glass wool into 10 milliliter acid washed volumetric flasks. The residues were washed with 1.5 milliliters, each, of 0.2 N PCA and the suspensions centrifuged according to the above indicated method for 10 minutes. The supernatants were decanted through glass wool into the appropriate volumetric flasks and the glass wool washed with several drops, each, of 0.2 N PCA. The combined supernatants and washes

were finally made to 10 milliliters.

RNA concentrations were determined on the solutions by a modified procedure of Ceriotti (1955) at a wavelength of 660 millimicrons using the orcinol system. DNA was also quantitated from these solutions by modified procedures of Burton (1956) using a wavelength of 600 millimicrons and utilizing the diphenylamine system. Standard stock solutions were prepared at a concentration of 1.0 milligram DNA and RNA per milliliter in 0.01 N potassium hydroxide.

Working standards of 100 micrograms per milliliter DNA and RNA were prepared from the stock solutions using 0.01 N potassium hydroxide. The DNA was obtained from Sigma Chemical Company as the highly polymerized sodium salt from calf thymus and the RNA was also obtained from Sigma Chemical Company as baker's yeast core RNA, Type II C. Calculations for the quantification of RNA and DNA were made based on an assay curve of the standard stock solutions.

# APPENDIX C

## TABLES OF MEANS AND STANDARD ERRORS

TABLE I

ANIMAL AGES AT SACRIFICE AND SACRIFICE DATES

| | Season I (Spring Born) | | | Season II (Fall Born) | | |
|---|---|---|---|---|---|---|
| Breed | Animal No. | Age (days) | Sacrifice date | Animal No. | Age (days) | Sacrifice date |
| Angus | 106 | ~25 | 4/26/78 | 97 | ~25 | 9/28/78 |
| | 76 | ~25 | 4/26/78 | 89 | ~25 | 9/28/78 |
| | 113 | ~25 | 4/26/78 | 72 | ~25 | 9/28/78 |
| Charolais | 75 | ~25 | 4/28/78 | 8M11 | 39 | 10/19/78 |
| | 866 | ~25 | 4/28/78 | 8M10 | 41 | 10/19/78 |
| | 58 | ~25 | 4/28/78 | 8B124 | 31 | 10/19/78 |
| Angus | 32 | ~240 | 11/27/78 | 141 | ~310 | 7/10/79 |
| | 13 | ~240 | 11/27/78 | 139 | ~310 | 7/10/79 |
| | 23 | ~240 | 11/27/78 | 127 | ~310 | 7/10/79 |
| | 6 | ~240 | 11/27/78 | 137 | ~310 | 7/10/79 |
| | 21 | ~240 | 11/27/78 | 114 | ~310 | 7/10/79 |
| | 2 | ~240 | 11/27/78 | 116 | ~310 | 7/10/79 |
| Charolais | 55 | 222 | 12/ 4/78 | 94 | 257 | 6/18/79 |
| | 45 | 214 | 12/ 4/78 | W72 | 259 | 6/18/79 |
| | 51 | 230 | 12/ 4/78 | 88 | 220 | 6/18/79 |
| | 41 | ~ 225 | 12/ 4/78 | 108 | ~239 | 6/18/79 |
| | 50 | ~225 | 12/ 4/78 | 74 | 244 | 6/18/79 |
| | W58 | 224 | 12/ 4/78 | 90 | ~239 | 6/18/79 |
| Angus | 25 | ~648 | 1/ 8/80 | 117 | ~604 | 4/29/80 |
| | 12 | ~648 | 1/ 8/80 | 131 | ~604 | 4/29/80 |
| | 16 | ~662 | 1/22/80 | 142 | ~606 | 5/ 1/80 |
| | 22 | ~662 | 1/22/80 | 138 | ~606 | 5/ 1/80 |
| | 28 | ~662 | 1/22/80 | | | |
| | 20 | ~662 | 1/22/80 | | | |
| Charolais | 59 | 626 | 1/ 8/80 | 73 | 573 | 5/20/80 |
| | 62 | 624 | 1/ 8/80 | M76 | ~575 | 5/20/80 |
| | 44 | 628 | 1/22/80 | 105 | ~575 | 5/20/80 |
| | 35 | 716 | 4/ 8/80 | 83 | 613 | 5/27/80 |
| | 52 | ~716 | 4/ 8/80 | 87 | 595 | 5/27/80 |

TABLE II

EFFECT OF STAIN, SEASON AND BREED ON TOTAL MYOFIBER COUNT[a] IN LD MUSCLE OF 25 DAY OLD BEEF CALVES

| | ATPase Stain | | NADH-TR Stain | |
|---|---|---|---|---|
| Breed | Season I | Season II | Season I | Season II |
| Angus | | | | |
| Mean | 2.503 | 1.720[b] | 2.475[c] | 1.628[d] |
| ±SEM | (0.115) | (0.099) | (0.116) | (0.089) |
| Charolais | | | | |
| Mean | 2.732 | 2.582[b] | 2.982[c] | 2.664[d] |
| ±SEM | (0.151) | (0.195) | (0.170) | (0.196) |

[a]Mean count in millions.

[b,c,d]Means with same superscript in same column are statistically different ($P < .05$).

TABLE III

EFFECT OF STAIN, SEASON AND BREED ON TOTAL MYOFIBER COUNT[a] IN ST MUSCLE OF 25 DAY OLD BEEF CALVES

| | ATPase Stain | | NADH-TR Stain | |
|---|---|---|---|---|
| Breed | Season I | Season II | Season I | Season II |
| Angus | | | | |
| Mean | 1.676 | 1.340 | 1.562 | 1.184 |
| ±SEM | (0.070) | (0.043) | (0.051) | (0.038) |
| Charolais | | | | |
| Mean | 2.020 | 1.665 | 1.895 | 1.533 |
| ±SEM | (0.084) | (0.075) | (0.072) | (0.068) |

[a]Mean count in millions.

TABLE IV

EFFECT OF STAIN, SEASON AND BREED ON TOTAL MYOFIBER COUNT[a] IN TBL MUSCLE OF 25 DAY OLD BEEF CALVES

| | ATPase Stain | | NADH-TR Stain | |
|---|---|---|---|---|
| Breed | Season I | Season II | Season I | Season II |
| Angus | | | | |
| Mean | 1.217 | 1.018 | 1.172 | 0.832 |
| ±SEM | (0.050) | (0.042) | (0.041) | (0.035) |
| Charolais | | | | |
| Mean | 1.485 | 1.317 | 1.464 | 1.209 |
| ±SEM | (0.076) | (0.078) | (0.049) | (0.085) |

[a]Mean count in millions.

TABLE V

EFFECT OF SEASON AND BREED ON LIVE WEIGHT[a] AND CARCASS WEIGHT[b] IN 25 DAY OLD BEEF CALVES

| | Season I | | Season II | |
|---|---|---|---|---|
| Breed | Live Wt. | Carcass Wt. | Live Wt. | Carcass Wt. |
| Angus | | | | |
| Mean | 44.6 | 26.7 | 39.0[c] | 24.2[d] |
| ±SEM | (5.1) | (4.0) | (3.3) | (1.8) |
| Charolais | | | | |
| Mean | 52.6 | 32.7 | 60.6[c] | 39.4[d] |
| ±SEM | (3.2) | (1.9) | (3.2) | (2.8) |

[a]Mean live weight expressed in kilograms.

[b]Mean hot carcass weight expressed in kilograms.

[c,d]Weights with same superscript in same column are statistically different ($P < .05$).

TABLE VI

EFFECT OF SEASON AND BREED ON MUSCLE WEIGHTS[a] IN 25 DAY OLD BEEF CALVES

| | Season I | | | Season II | | |
|---|---|---|---|---|---|---|
| Breed | LD | ST | TBL | LD | ST | TBL |
| Angus | | | | | | |
| Mean | 300.8 | 204.7 | 81.0 | 222.8[b] | 174.7[c] | 58.0 |
| ±SEM | (40.1) | (23.1) | (7.4) | (15.0) | (15.5) | (2.7) |
| Charolais | | | | | | |
| Mean | 313.2 | 240.2 | 81.0 | 330.0[b] | 301.2[c] | 92.7 |
| ±SEM | (5.0) | (3.7) | (2.5) | (22.1) | (13.7) | (4.5) |

[a]Mean weight (in grams) of right and left muscle for all animals in breed group.

[b,c]Means with same superscript in same column are significantly different ($P < .01$).

TABLE VII

EFFECT OF STAIN, SEASON AND BREED ON MYOFIBER TYPE COUNT[a] IN THE LD MUSCLE OF 25 DAY OLD BEEF CALVES

| | | ATPase Stain | | NADH-TR Stain | |
|---|---|---|---|---|---|
| Breed | Myofiber Type | Season I | Season II | Season I | Season II |
| Angus | α White | | | | |
| | Mean | 1.433 | 1.064 | 1.203 | 0.782[e] |
| | ±SEM | (0.066) | (0.076) | (0.063) | (0.046) |
| | α Red | | | | |
| | Mean | 0.496 | 0.262[b] | 0.459[c] | 0.212[f] |
| | ±SEM | (0.030) | (0.016) | (0.023) | (0.020) |
| | β Red | | | | |
| | Mean | 0.574 | 0.394 | 0.812[d] | 0.634[g] |
| | ±SEM | (0.033) | (0.021) | (0.049) | (0.038) |
| Charolais | α White | | | | |
| | Mean | 1.609 | 1.299 | 1.369 | 1.057[e] |
| | ±SEM | (0.102) | (0.097) | (0.079) | (0.086) |
| | α Red | | | | |
| | Mean | 0.437 | 0.667[b] | 0.518[c] | 0.422[f] |
| | ±SEM | (0.026) | (0.054) | (0.031) | (0.028) |
| | β Red | | | | |
| | Mean | 0.686 | 0.615 | 1.094[d] | 1.185[g] |
| | ±SEM | (0.044) | (0.054) | (0.074) | (0.092) |

[a]Mean count in millions.

[b,c,d,e,f,g]Counts with same superscript in same column are statistically different ($P < .05$).

TABLE VIII

EFFECT OF STAIN, SEASON AND BREED ON MYOFIBER TYPE COUNT[a] IN THE ST MUSCLE OF 25 DAY OLD BEEF CALVES

| | | ATPase Stain | | NADH-TR Stain | |
|---|---|---|---|---|---|
| Breed | Myofiber Type | Season I | Season II | Season I | Season II |
| Angus | $\alpha$White | | | | |
| | Mean | 0.899 | 0.770 | 0.820 | 0.630 |
| | ±SEM | (0.040) | (0.020) | (0.028) | (0.019) |
| | $\alpha$Red | | | | |
| | Mean | 0.386 | 0.192 | 0.365 | 0.145 |
| | ±SEM | (0.026) | (0.010) | (0.023) | (0.011) |
| | $\beta$Red | | | | |
| | Mean | 0.391[b] | 0.378 | 0.378[c] | 0.409[d] |
| | ±SEM | (0.025) | (0.027) | (0.022) | (0.023) |
| Charolais | $\alpha$White | | | | |
| | Mean | 1.013 | 0.986 | 0.910 | 0.659 |
| | ±SEM | (0.046) | (0.045) | (0.040) | (0.023) |
| | $\alpha$Red | | | | |
| | Mean | 0.389 | 0.322 | 0.385 | 0.188 |
| | ±SEM | (0.020) | (0.023) | (0.018) | (0.013) |
| | $\beta$Red | | | | |
| | Mean | 0.618[b] | 0.358 | 0.600[c] | 0.686[d] |
| | ±SEM | (0.044) | (0.028) | (0.042) | (0.050) |

[a]Mean count in millions.

[b,c,d]Counts with same superscript in same column are statistically different ($P < .05$).

TABLE IX

EFFECT OF STAIN, SEASON AND BREED ON MYOFIBER TYPE COUNT[a] IN THE TBL MUSCLE OF 25 DAY OLD BEEF CALVES

| | | ATPase Stain | | NADH-TR Stain | |
|---|---|---|---|---|---|
| Breed | Myofiber Type | Season I | Season II | Season I | Season II |
| Angus | $\alpha$ White | | | | |
| | Mean | 0.519 | 0.513 | 0.681 | 0.438 |
| | ±SEM | (0.036) | (0.030) | (0.037) | (0.030) |
| | $\alpha$ Red | | | | |
| | Mean | 0.202 | 0.077[b] | 0.214 | 0.070[d] |
| | ±SEM | (0.014) | (0.009) | (0.014) | (0.006) |
| | $\beta$ Red | | | | |
| | Mean | 0.497 | 0.428 | 0.277[c] | 0.324 |
| | ±SEM | (0.029) | (0.021) | (0.021) | (0.022) |
| Charolais | $\alpha$ White | | | | |
| | Mean | 0.673 | 0.571 | 0.705 | 0.506 |
| | ±SEM | (0.065) | (0.035) | (0.045) | (0.030) |
| | $\alpha$ Red | | | | |
| | Mean | 0.182 | 0.253[b] | 0.246 | 0.199[d] |
| | ±SEM | (0.017) | (0.028) | (0.014) | (0.026) |
| | $\beta$ Red | | | | |
| | Mean | 0.630 | 0.493 | 0.513[c] | 0.505 |
| | ±SEM | (0.044) | (0.037) | (0.030) | (0.042) |

[a]Mean count in millions.

[b,c,d]Counts with same superscript in same column are statistically different ($P < .05$).

TABLE X

EFFECT OF BREED AND SEASON ON DNA CONCENTRATION[a] PER MUSCLE IN 25 DAY OLD BEEF CALVES

| | Season I | | | Season II | | |
|---|---|---|---|---|---|---|
| Breed | LD | ST | TBL | LD | ST | TBL |
| Angus | | | | | | |
| Mean | $251^{b}$ | $165^{c}$ | $76^{d}$ | $204^{e}$ | $154^{f}$ | $55^{g}$ |
| ±SEM | (20) | (3) | (3) | (14) | (5) | (4) |
| Charolais | | | | | | |
| Mean | $360^{b}$ | $277^{c}$ | $99^{d}$ | $255^{e}$ | $211^{f}$ | $84^{g}$ |
| ±SEM | (11) | (26) | (7) | (22) | (11) | (4) |

[a] Milligrams DNA per muscle.

[b,c,d,e,f,g] DNA concentrations with same superscript in same column are statistically different ($P < .01$).

TABLE XI

EFFECT OF BREED AND SEASON ON RNA CONCENTRATION[a] PER MUSCLE IN 25 DAY OLD BEEF CALVES

| | Season I | | | Season II | | |
|---|---|---|---|---|---|---|
| Breed | LD | ST | TBL | LD | ST | TBL |
| Angus | | | | | | |
| Mean | 628[b] | 348[c] | 178[d] | 571[e] | 360[f] | 163[g] |
| ±SEM | (62) | (46) | (14) | (86) | (24) | (7) |
| Charolais | | | | | | |
| Mean | 961[b] | 532[c] | 207[d] | 1053[e] | 712[f] | 350[g] |
| ±SEM | (82) | (39) | (17) | (49) | (21) | (18) |

[a]Milligrams RNA per muscle.

[b,c,d,e,f,g]RNA concentrations with same superscript in same column are statistically different ($P < .01$).

TABLE XII

EFFECT OF BREED AND SEASON ON THE NUMBER OF NUCLEI PER MUSCLE IN 25 DAY OLD BEEF CALVES

| | Season I | | | Season II | | |
|---|---|---|---|---|---|---|
| | LD | ST | TBL | LD | ST | TBL |
| Angus | | | | | | |
| Mean | $4.049\times10^{10}$ [a] | $2.666\times10^{10}$ [b] | $1.234\times10^{10}$ | $3.290\times10^{10}$ [c] | $2.492\times10^{10}$ [d] | $0.885\times10^{10}$ |
| ±SEM | ($0.317\times10^{10}$) | ($0.050\times10^{10}$) | ($0.052\times10^{10}$) | ($0.232\times10^{10}$) | ($0.082\times10^{10}$) | ($0.072\times10^{10}$) |
| Charolais | | | | | | |
| Mean | $5.807\times10^{10}$ [a] | $4.467\times10^{10}$ [b] | $1.592\times10^{10}$ | $4.107\times10^{10}$ [c] | $3.407\times10^{10}$ [d] | $1.364\times10^{10}$ |
| ±SEM | ($0.174\times10^{10}$) | ($0.425\times10^{10}$) | ($0.109\times10^{10}$) | ($0.364\times10^{10}$) | ($0.185\times10^{10}$) | ($0.064\times10^{10}$) |

[a,b] Nuclear numbers with the same superscript in same column are statistically different ($P<.01$).

[c,d] Nuclear numbers with the same superscript in same column are statistically different ($P<.05$).

TABLE XIII

EFFECT OF BREED AND SEASON ON THE GRAMS OF TISSUE SUPPORTED PER NUCLEUS IN 25 DAY OLD BEEF CALVES

| | Season I | | | Season II | | |
|---|---|---|---|---|---|---|
| | LD | ST | TBL | LD | ST | TBL |
| Angus | | | | | | |
| Mean | $7.249x10^{-9}$ | $7.686x10^{-9}$ | $6.516x10^{-9}$ | $7.120x10^{-9}$ | $6.982x10^{-9}$[a] | $6.765x10^{-9}$ |
| ±SEM | $(0.480x10^{-9})$ | $(0.871x10^{-9})$ | $(0.440x10^{-9})$ | $(1.024x10^{-9})$ | $(0.510x10^{-9})$ | $(0.633x10^{-9})$ |
| Charolais | | | | | | |
| Mean | $5.417x10^{-9}$ | $5.540x10^{-9}$ | $5.162x10^{-9}$ | $8.121x10^{-9}$ | $8.881x10^{-9}$[a] | $6.793x10^{-9}$ |
| ±SEM | $(0.183x10^{-9})$ | $(0.347x10^{-9})$ | $(0.222x10^{-9})$ | $(0.257x10^{-9})$ | $(0.362x10^{-9})$ | $(0.086x10^{-9})$ |

[a]Grams tissue with same superscript in same column are statistically different ($P < .05$).

TABLE XIV

EFFECT OF BREED AND SEASON ON GRAMS PROTEIN PER GRAM DNA IN 25 DAY OLD BEEF CALVES

| | Season I | | | Season II | | |
|---|---|---|---|---|---|---|
| | LD | ST | TBL | LD | ST | TBL |
| Angus | | | | | | |
| Mean | 242.97 | 255.33 | 211.45 | 250.66 | 242.48 | 228.16 |
| ±SEM | (18.38) | (29.99) | (14.33) | (35.37) | (19.91) | (22.03) |
| Charolais | | | | | | |
| Mean | 169.98 | 173.96 | 160.79 | 272.21 | 293.27 | 213.48 |
| ±SEM | (5.40) | (10.20) | (7.81) | (8.50) | (10.51) | (3.11) |

TABLE XV

EFFECT OF BREED AND SEASON ON THE RNA:DNA RATIO IN 25 DAY OLD BEEF CALVES

| | Season I | | | Season II | | |
|---|---|---|---|---|---|---|
| Breed | LD | ST | TBL | LD | ST | TBL |
| Angus | | | | | | |
| Mean | 2.51 | 2.13 | 2.32 | 2.74 | 2.35 | 3.10 |
| ±SEM | (0.17) | (0.31) | (0.14) | (0.27) | (0.20) | (0.31) |
| Charolais | | | | | | |
| Mean | 2.68 | 1.97 | 2.09 | 4.27 | 3.43 | 4.14 |
| ±SEM | (0.26) | (0.17) | (0.06) | (0.33) | (0.23) | (0.12) |

TABLE XVI

EFFECT OF BREED AND SEASON ON MYOFIBER WIDTH[a] IN 25 DAY OLD BEEF CALVES

| | Season I | | | Season II | | |
|---|---|---|---|---|---|---|
| Breed | LD | ST | TBL | LD | ST | TBL |
| Angus | | | | | | |
| Mean | 32.8 | 32.1 | 33.0 | 37.4[b] | 35.7[c] | 35.3[d] |
| ±SEM | (1.1) | (1.3) | (1.0) | (0.8) | (0.9) | (1.0) |
| Charolais | | | | | | |
| Mean | 28.8 | 29.1 | 28.5 | 41.8[b] | 42.8[c] | 37.5[d] |
| ±SEM | (0.7) | (0.7) | (0.6) | (0.6) | (0.9) | (0.4) |

[a]Mean myofiber width in microns.

[b,c]Means with same superscript in same column are significantly different ($P < .01$).

[d]Means with same superscript in same column are significantly different ($P < .05$).

APPENDIX D

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST

TABLE XVII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR TOTAL MYOFIBER NUMBER PER MUSCLE 25 DAYS OLD, SEASON I, ATPase STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 40,537,734,000 | .7166 |
| Breed x Side | 1 | 915,626,260,000 | .1395 |
| Animal x Side (Breed) | 4 | 271,564,550,000 | |
| Muscle | 2 | 39,102,679,000,000 | .0001 |
| Breed x Muscle | 2 | 81,977,380,000 | .8979 |
| A x M (B)[a] | 8 | 755,696,200,000 | |
| Side x Muscle | 2 | 171,492,420,000 | .7422 |
| B x S x M[b] | 2 | 395,879,320,000 | .5172 |
| A x S x M (B)[c] | 8 | 546,821,060,000 | |
| Core (B A S M)[d] | 108 | 433,303,680,000 | |
| Duplicate (B A S M C)[e] | 144 | 37,277,393,000 | |
| Corrected Total | 287 | 528,288,520,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 2.732 | 2.503 | 2.020 | 1.676 | 1.485 | 1.217 |
| | a | a | b | bc | cd | d |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c,d]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XVIII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR TOTAL MYOFIBER NUMBER PER MUSCLE 25 DAYS OLD, SEASON II, ATPase STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 942,323,830,000 | .5651 |
| Breed x Side | 1 | 203,162,100,000 | .7042 |
| Animal x Side (Breed) | 4 | 1,235,144,300,000 | |
| Muscle | 2 | 24,018,973,000,000 | .0013 |
| Breed x Muscle | 2 | 2,415,441,600,000 | .2075 |
| A x M (B)[a] | 8 | 1,256,399,700,000 | |
| Side x Muscle | 2 | 754,447,470,000 | .7283 |
| B x S x M[b] | 2 | 1,652,556,500,000 | .5133 |
| A x S x M (B)[c] | 8 | 2,254,927,300,000 | |
| Core (B A S M)[d] | 108 | 270,856,270,000 | |
| Duplicate (B A S M C)[e] | 144 | 28,848,981,000 | |
| Corrected Total | 287 | 502,537,640,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 2.582 | 1.720 | 1.665 | 1.340 | 1.317 | 1.018 |
| | a | b | b | bc | bc | c |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

a,b,cMeans with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XIX

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR TOTAL MYOFIBER NUMBER PER MUSCLE 25 DAYS OLD, SEASON I, NADH-TR STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 297,353,300,000 | .5797 |
| Breed x Side | 1 | 993,234,660,000 | .1733 |
| Animal x Side (Breed) | 4 | 364,381,650,000 | |
| Muscle | 2 | 50,487,312,000,000 | .0001 |
| Breed x Muscle | 2 | 310,774,290,000 | .5795 |
| A x M (B)[a] | 8 | 525,194,420,000 | |
| Side x Muscle | 2 | 5,146,235,000 | .9903 |
| B x S x M[b] | 2 | 461,797,200,000 | .5763 |
| A x S x M (B)[c] | 8 | 478,761,800,000 | |
| Core (B A S M)[d] | 108 | 420,392,800,000 | |
| Duplicate (B A S M C)[e] | 144 | 29,348,435,000 | |
| Corrected Total | 287 | 613,100,960,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 2.982 | 2.475 | 1.895 | 1.562 | 1.464 | 1.172 |
| | a | b | c | cd | de | e |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c,d,e]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XX

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR TOTAL MYOFIBER NUMBER PER MUSCLE 25 DAYS OLD, SEASON II, NADH-TR STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 1,379,224,700,000 | .2540 |
| Breed x Side | 1 | 585,945,430,000 | .5619 |
| Animal x Side (Breed) | 4 | 779,622,250,000 | |
| Muscle | 2 | 31,992,677,000,000 | .0003 |
| Breed x Muscle | 2 | 3,634,015,700,000 | .0653 |
| A x M (B)[a] | 8 | 933,178,720,000 | |
| Side x Muscle | 2 | 1,162,012,200,000 | .5830 |
| B x S x M[b] | 2 | 1,725,515,100,000 | .5415 |
| A x S x M (B)[c] | 8 | 1,986,552,100,000 | |
| Core (B A S M)[d] | 108 | 294,445,760,000 | |
| Duplicate (B A S M C)[e] | 144 | 21,043,302,000 | |
| Corrected Total | 287 | 579,575,850,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $TBL_C$ | $ST_A$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 2.664 | 1.628 | 1.533 | 1.209 | 1.184 | 0.832 |
| | a | b | b | bc | bc | c |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXI

ANALYSIS OF VARIANCE FOR LIVE WEIGHT
25 DAYS OLD, SEASON I

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 96.320 | <.1000 |
| Animal (Breed) | 4 | 55.322 | |
| Corrected Total | 5 | 63.521 | |

TABLE XXII

ANALYSIS OF VARIANCE FOR HOT CARCASS WEIGHT
25 DAYS OLD, SEASON I

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 53.402 | <.1000 |
| Animal (Breed) | 4 | 30.208 | |
| Corrected Total | 5 | 34.847 | |

TABLE XXIII

ANALYSIS OF VARIANCE FOR LIVE WEIGHT
25 DAYS OLD, SEASON II

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 700.920 | <.0100 |
| Animal (Breed) | 4 | 31.729 | |
| Corrected Total | 5 | 165.567 | |

TABLE XXIV

ANALYSIS OF VARIANCE FOR HOT CARCASS WEIGHT
25 DAYS OLD, SEASON II

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 345.194 | <.0250 |
| Animal (Breed) | 4 | 16.858 | |
| Corrected Total | 5 | 82.525 | |

TABLE XXV

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR MUSCLE WEIGHT, 25 DAYS OLD, SEASON I

| ANALYSIS OF VARIANCE | | | |
|---|---|---|---|
| Source | df | Mean Square | OSL |
| Breed | 1 | 2,288.028 | .6891 |
| Animal (Breed) | 4 | 12,414.528 | |
| Side | 1 | 30.250 | .1815 |
| Breed x Side | 1 | 23.361 | .2290 |
| Animal x Side (Breed) | 4 | 11.639 | |
| Muscle | 2 | 156,458.028 | .0001 |
| Breed x Muscle | 2 | 974.528 | .6507 |
| A x M (B)[a] | 8 | 2,116.278 | |
| Side x Muscle | 2 | 210.583 | .0019 |
| B x S x M[b] | 2 | 11.194 | .5458 |
| A x S x M (B)[c] | 8 | 12.722 | |
| Corrected Total | 35 | 10,982.485 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

| DUNCAN'S MULTIPLE RANGE TEST* | | | | | | |
|---|---|---|---|---|---|---|
| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
| Mean** | 313.2 | 300.8 | 240.2 | 204.7 | 81.0 | 81.0 |
| | a | ab | bc | c | d | d |

*($\alpha$ tested at .05 and .01 levels)

**Mean muscle weights in grams.

[a,b,c,d]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXVI

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR MUSCLE WEIGHT, 25 DAYS OLD, SEASON II

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 72,002.778 | .0270 |
| Animal (Breed) | 4 | 6,080.194 | |
| Side | 1 | 5.444 | .8636 |
| Breed x Side | 1 | 0.000 | 1.0000 |
| Animal x Side (Breed) | 4 | 179.139 | |
| Muscle | 2 | 136,700.194 | .0001 |
| Breed x Muscle | 2 | 7,031.694 | .0196 |
| A x M (B)[a] | 8 | 1,052.319 | |
| Side x Muscle | 2 | 11.194 | .9410 |
| B x S x M[b] | 2 | 7.583 | .9601 |
| A x S x M (B)[c] | 8 | 183.181 | |
| Corrected Total | 35 | 11,269.454 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $ST_C$ | $LD_A$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 330.0 | 301.2 | 222.8 | 174.7 | 92.7 | 58.0 |
| | a | a | b | c | d | d |

*($\alpha$ tested at .05 and .01 levels)

**Mean muscle weights in grams.

[a,b,c,d]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXVII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF $\alpha$ WHITE MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON I, ATPase STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 16,075,636,000 | .7073 |
| Breed x Side | 1 | 139,531,290,000 | .3036 |
| Animal x Side | 4 | 100,084,630,000 | |
| Muscle | 2 | 20,873,969,000,000 | .0001 |
| Breed x Muscle | 2 | 24,145,874,000 | .9424 |
| A x M (B)[a] | 8 | 404,626,470,000 | |
| Side x Muscle | 2 | 38,209,158,000 | .8631 |
| B x S x M[b] | 2 | 20,986,545,000 | .9214 |
| A x S x M (B)[c] | 8 | 255,343,920,000 | |
| Core (B A S M)[d] | 108 | 184,934,330,000 | |
| Duplicate (B A S M C)[e] | 144 | 17,097,846,000 | |
| Corrected Total | 287 | 252,101,910,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 1.609 | 1.433 | 1.013 | 0.899 | 0.673 | 0.519 |
| | a | a | b | bc | cd | d |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c,d]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXVIII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF $\alpha$ WHITE MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON II, ATPase STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 305,186,160,000 | .3634 |
| Breed x Side | 1 | 19,118,068 | .9900 |
| Animal x Side (Breed) | 4 | 288,409,880,000 | |
| Muscle | 2 | 9,825,721,500,000 | .0006 |
| Breed x Muscle | 2 | 223,765,000,000 | .5874 |
| A x M (B)[a] | 8 | 388,327,700,000 | |
| Side x Muscle | 2 | 285,851,520,000 | .6364 |
| B x S x M[b] | 2 | 316,635,340,000 | .6076 |
| A x S x M (B)[c] | 8 | 588,817,780,000 | |
| Core (B A S M)[d] | 108 | 95,635,976,000 | |
| Duplicate (B A S M C)[e] | 144 | 12,798,726,000 | |
| Corrected Total | 287 | 160,424,900,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 1.299 | 1.064 | 0.986 | 0.770 | 0.571 | 0.513 |
| | a | ab | b | bc | c | c |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXIX

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF $\alpha$ WHITE MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON I, NADH-TR STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 288,251,620,000 | .2133 |
| Breed x Side | 1 | 376,040,540,000 | .1663 |
| Animal x Side (Breed) | 4 | 132,257,810,000 | |
| Muscle | 2 | 8,937,755,800,000 | .0001 |
| Breed x Muscle | 2 | 119,977,210,000 | .5215 |
| A x M (B)[a] | 8 | 167,973,280,000 | |
| Side x Muscle | 2 | 29,107,468,000 | .7489 |
| B x S x M[b] | 2 | 176,160,690,000 | .2198 |
| A x S x M (B)[c] | 8 | 95,833,741,000 | |
| Core (B A S M)[d] | 108 | 127,868,530,000 | |
| Duplicate (B A S M C)[e] | 144 | 15,362,510,000 | |
| Corrected Total | 287 | 134,546,230,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 1.369 | 1.203 | 0.910 | 0.820 | 0.705 | 0.681 |
| | a | a | b | bc | bc | c |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXX

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF $\alpha$ WHITE MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON II, NADH-TR STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 202,140,900,000 | .3404 |
| Breed x Side | 1 | 81,833,691,000 | .5316 |
| Animal x Side (Breed) | 4 | 171,929,590,000 | |
| Muscle | 2 | 4,890,962,500,000 | .0012 |
| Breed x Muscle | 2 | 421,059,900,000 | .2407 |
| A x M (B)[a] | 8 | 246,493,390,000 | |
| Side x Muscle | 2 | 199,098,490,000 | .5894 |
| B x S x M[b] | 2 | 323,206,370,000 | .5640 |
| A x S x M (B)[c] | 8 | 347,760,730,000 | |
| Core (B A S M)[d] | 108 | 56,946,076,000 | |
| Duplicate (B A S M C)[e] | 144 | 5,262,355,800 | |
| Corrected Total | 287 | 90,661,350,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 1.057 | 0.782 | 0.659 | 0.630 | 0.506 | 0.438 |
| | a | b | bc | bc | c | c |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXXI

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF $\alpha$ RED MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON I, ATPase STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 75,361,146,000 | .1192 |
| Breed x Side | 1 | 126,268,520,000 | .0630 |
| Animal x Side (Breed) | 4 | 19,367,196,000 | |
| Muscle | 2 | 1,917,700,900,000 | .0001 |
| Breed x Muscle | 2 | 23,606,327,000 | .5235 |
| A x M (B)[a] | 8 | 28,710,444,000 | |
| Side x Muscle | 2 | 22,652,333,000 | .3878 |
| B x S x M[b] | 2 | 24,189,915,000 | .3660 |
| A x S x M (B)[c] | 8 | 21,092,822,000 | |
| Core (B A S M)[d] | 108 | 22,085,140,000 | |
| Duplicate (B A S M C)[e] | 144 | 4,346,747,600 | |
| Corrected Total | 287 | 27,819,650,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_A$ | $LD_C$ | $ST_C$ | $ST_A$ | $TBL_A$ | $TBL_C$ |
|---|---|---|---|---|---|---|
| Mean** | 0.496 | 0.437 | 0.389 | 0.386 | 0.202 | 0.182 |
| | a | ab | b | b | c | c |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXXII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF $\alpha$ RED MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON II, ATPase STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 106,256,840,000 | .2489 |
| Breed x Side | 1 | 318,956,280,000 | .0796 |
| Animal x Side (Breed) | 4 | 58,554,697,000 | |
| Muscle | 2 | 2,271,857,000,000 | .0005 |
| Breed x Muscle | 2 | 521,365,070,000 | .0198 |
| A x M (B)[a] | 8 | 78,362,238,000 | |
| Side x Muscle | 2 | 52,362,355,000 | .6632 |
| B x S x M[b] | 2 | 192,280,200,000 | .2577 |
| A x S x M (B)[c] | 8 | 119,240,700,000 | |
| Core (B A S M)[d] | 108 | 18,962,933,000 | |
| Duplicate (B A S M C)[e] | 144 | 3,083,108,500 | |
| Corrected Total | 287 | 52,731,821,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $ST_C$ | $LD_A$ | $TBL_C$ | $ST_A$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 0.667 | 0.322 | 0.262 | 0.253 | 0.192 | 0.077 |
| | a | b | b | b | bc | c |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXXIII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF $\alpha$ RED MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON I, NADH-TR STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 83,532,817,000 | .0171 |
| Breed x Side | 1 | 60,252,780,000 | .0278 |
| Animal x Side (Breed) | 4 | 5,195,265,200 | |
| Muscle | 2 | 1,616,607,300,000 | .0001 |
| Breed x Muscle | 2 | 9,436,591,700 | .5448 |
| A x M (B)[a] | 8 | 14,231,343,000 | |
| Side x Muscle | 2 | 1,308,849,400 | .9529 |
| B x S x M[b] | 2 | 16,611,280,000 | .5659 |
| A x S x M (B)[c] | 8 | 26,832,924,000 | |
| Core (B A S M)[d] | 108 | 21,469,905,000 | |
| Duplicate (B A S M C)[e] | 144 | 4,734,369,300 | |
| Corrected Total | 287 | 24,479,133,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 0.518 | 0.459 | 0.385 | 0.365 | 0.246 | 0.214 |
| | a | b | c | c | d | d |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c,d]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXXIV

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF $\alpha$ RED MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON II, NADH-TR STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 30,198,414,000 | .3559 |
| Breed x Side | 1 | 72,401,751,000 | .1798 |
| Animal x Side (Breed) | 4 | 27,579,409,000 | |
| Muscle | 2 | 912,717,080,000 | .0002 |
| Breed x Muscle | 2 | 169,183,960,000 | .0144 |
| A x M (B)[a] | 8 | 22,370,291,000 | |
| Side x Muscle | 2 | 4,628,586,100 | .8786 |
| B x S x M[b] | 2 | 24,742,572,000 | .5279 |
| A x S x M (B)[c] | 8 | 35,352,054,000 | |
| Core (B A S M)[d] | 108 | 14,012,797,000 | |
| Duplicate (B A S M C)[e] | 144 | 3,165,183,600 | |
| Corrected Total | 287 | 21,649,263,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $TBL_C$ | $ST_C$ | $ST_A$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 0.422 | 0.212 | 0.199 | 0.188 | 0.145 | 0.070 |
| | a | b | bc | bc | c | d |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c,d]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXXV

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF $\beta$ RED MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON I, ATPase STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 39,987,893,000 | .2048 |
| Breed x Side | 1 | 51,984,881,000 | .1594 |
| Animal x Side (Breed) | 4 | 17,523,497,000 | |
| Muscle | 2 | 379,989,460,000 | .0364 |
| Breed x Muscle | 2 | 91,170,971,000 | .3422 |
| A x M (B)[a] | 8 | 73,968,634,000 | |
| Side x Muscle | 2 | 11,460,077,000 | .9077 |
| B x S x M[b] | 2 | 156,127,820,000 | .3186 |
| A x S x M (B)[c] | 8 | 117,785,540,000 | |
| Core (B A S M)[d] | 108 | 63,372,730,000 | |
| Duplicate (B A S M C)[e] | 144 | 7,511,978,100 | |
| Corrected Total | 287 | 45,570,559,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $TBL_C$ | $ST_C$ | $LD_A$ | $TBL_A$ | $ST_A$ |
|---|---|---|---|---|---|---|
| Mean** | 0.686 | 0.630 | 0.618 | 0.574 | 0.497 | 0.391 |
| | a | ab | ab | ab | bc | c |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXXVI

ANALYSIS OF VARIANCE FOR NUMBER OF $\beta$ RED MYOFIBERS PER MUSCLE - 25 DAYS OLD, SEASON II, ATPase STAIN

| ANALYSIS OF VARIANCE | | | |
|---|---|---|---|
| Source | df | Mean Square | OSL |
| Side | 1 | 8,524,179,209 | .7976 |
| Breed x Side | 1 | 12,024,143,901 | .7637 |
| Animal x Side (Breed) | 4 | 121,350,836,862 | |
| Muscle | 2 | 469,291,735,646 | .0982 |
| Breed x Muscle | 2 | 361,872,392,086 | .1504 |
| A x M (B)[a] | 8 | 149,733,864,363 | |
| Side x Muscle | 2 | 24,164,849,634 | .8642 |
| B x S x M[b] | 2 | 103,675,212,702 | .5576 |
| A x S x M (B)[c] | 8 | 162,965,285,915 | |
| Core (B A S M)[d] | 108 | 37,728,264,751 | |
| Duplicate (B A S M C)[e] | 144 | 8,773,803,816 | |
| Corrected Total | 287 | 37,895,400,016 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

TABLE XXXVII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF $\beta$ RED MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON I, NADH-TR STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 78,741,990,000 | .6151 |
| Breed x Side | 1 | 19,023,507,000 | .6563 |
| Animal x Side (Breed) | 4 | 82,104,692,000 | |
| Muscle | 2 | 8,575,970,500,000 | .0001 |
| Breed x Muscle | 2 | 23,562,433,000 | .8555 |
| A x M (B)[a] | 8 | 148,158,550,000 | |
| Side x Muscle | 2 | 40,017,167,000 | .7069 |
| B x S x M[b] | 2 | 53,051,614,000 | .6352 |
| A x S x M (B)[c] | 8 | 108,799,130,000 | |
| Core (B A S M)[d] | 108 | 78,406,040,000 | |
| Duplicate (B A S M C)[e] | 144 | 10,817,647,000 | |
| Corrected Total | 287 | 125,119,170,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $TBL_C$ | $ST_A$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 1.094 | 0.812 | 0.600 | 0.513 | 0.378 | 0.277 |
| | a | b | c | cd | de | e |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c,d,e]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXXVIII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF β RED MYOFIBERS PER MUSCLE 25 DAYS OLD, SEASON II, NADH-TR STAIN

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Side | 1 | 303,629,900,000 | .2520 |
| Breed x Side | 1 | 44,238,333,000 | .6383 |
| Animal x Side (Breed) | 4 | 169,952,320,000 | |
| Muscle | 2 | 6,295,298,600,000 | .0007 |
| Breed x Muscle | 2 | 883,337,260,000 | .0871 |
| A x M (B)[a] | 8 | 263,698,550,000 | |
| Side x Muscle | 2 | 329,949,390,000 | .5208 |
| B x S x M[b] | 2 | 403,204,270,000 | .5439 |
| A x S x M (B)[c] | 8 | 460,948,360,000 | |
| Core (B A S M)[d] | 108 | 77,085,836,000 | |
| Duplicate (B A S M C)[e] | 144 | 7,129,705,500 | |
| Corrected Total | 287 | 140,652,300,000 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

[d]Core (Breed Animal Side Muscle)

[e]Duplicate (Breed Animal Side Muscle Core)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $ST_C$ | $LD_A$ | $TBL_C$ | $ST_A$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 1.185 | 0.686 | 0.634 | 0.505 | 0.409 | 0.324 |
| | a | b | bc | bcd | cd | d |

*($\alpha$ = .05)

**Mean myofiber count per muscle in millions.

[a,b,c,d]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XXXIX

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR DNA CONCENTRATION PER MUSCLE 25 DAYS OLD, SEASON I

| ANALYSIS OF VARIANCE | | | |
|---|---|---|---|
| Source | df | Mean Square | OSL |
| Breed | 1 | 0.058987326 | .0168 |
| Animal (Breed) | 4 | 0.003625905 | |
| Side | 1 | 0.000468631 | .2437 |
| Breed x Side | 1 | 0.001036880 | .1116 |
| Animal x Side (Breed) | 4 | 0.000251624 | |
| Muscle | 2 | 0.144919680 | .0001 |
| Breed x Muscle | 2 | 0.007777441 | .0522 |
| A x M (B)[a] | 8 | 0.001788026 | |
| Side x Muscle | 2 | 0.000991395 | .1652 |
| B x S x M[b] | 2 | 0.000536698 | .3435 |
| A x S x M (B)[c] | 8 | 0.000437104 | |
| Corrected Total | 35 | 0.011492984 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

| DUNCAN'S MULTIPLE RANGE TEST* | | | | | | |
|---|---|---|---|---|---|---|
| Muscle | $LD_C$ | $ST_C$ | $LD_A$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
| Mean** | 360 | 277 | 251 | 165 | 99 | 76 |
| | a | b | c | d | e | f |

*($\alpha$ = .01)

**Mean DNA concentration per muscle in milligrams.

a,b,c,d,e,f Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XL

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR DNA CONCENTRATION PER MUSCLE 25 DAYS OLD, SEASON II

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 0.018787530 | .0703 |
| Animal (Breed) | 4 | 0.003131646 | |
| Side | 1 | 0.000029440 | .8059 |
| Breed x Side | 1 | 0.000057515 | .7377 |
| Animal x Side (Breed) | 4 | 0.000459165 | |
| Muscle | 2 | 0.080829711 | .0001 |
| Breed x Muscle | 2 | 0.000602386 | .6341 |
| A x M (B)[a] | 8 | 0.001230531 | |
| Side x Muscle | 2 | 0.000129740 | .6606 |
| B x S x M[b] | 2 | 0.000168443 | .5877 |
| A x S x M (B)[c] | 8 | 0.000292601 | |
| Corrected Total | 35 | 0.005968096 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $ST_C$ | $LD_A$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 255 | 211 | 204 | 154 | 84 | 55 |
| | a | b | c | d | e | f |

*($\alpha$ = .01)

**Mean DNA concentration per muscle in milligrams.

[a,b,c,d,e,f]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XLI

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR RNA CONCENTRATION PER MUSCLE 25 DAYS OLD, SEASON I

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 0.29728897 | .1088 |
| Animal (Breed) | 4 | 0.07056734 | |
| Side | 1 | 0.00051871 | .5730 |
| Breed x Side | 1 | 0.00000033 | .9855 |
| Animal x Side (Breed) | 4 | 0.00135770 | |
| Muscle | 2 | 1.09844718 | .0001 |
| Breed x Muscle | 2 | 0.06898909 | .0520 |
| A x M (B)[a] | 8 | 0.01583280 | |
| Side x Muscle | 2 | 0.00160371 | .5879 |
| B x S x M[b] | 2 | 0.00001697 | .9946 |
| A x S x M (B)[c] | 8 | 0.00278734 | |
| Corrected Total | 35 | 0.08778809 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $LD_A$ | $ST_C$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 961 | 628 | 532 | 348 | 207 | 178 |
| | a | b | c | d | e | f |

*($\alpha$ = .01)

**Mean RNA concentration per muscle in milligrams.

[a,b,c,d,e,f]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XLII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR RNA CONCENTRATION PER MUSCLE 25 DAYS OLD, SEASON II

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 0.04132149 | .0082 |
| Animal (Breed) | 4 | 0.03971757 | |
| Side | 1 | 0.00194033 | .2408 |
| Breed x Side | 1 | 0.00299707 | .1620 |
| Animal x Side (Breed) | 4 | 0.00102655 | |
| Muscle | 2 | 0.92628474 | .0001 |
| Breed x Muscle | 2 | 0.06581470 | .0808 |
| A x M (B)[a] | 8 | 0.01886260 | |
| Side x Muscle | 2 | 0.00030186 | .8590 |
| B x S x M[b] | 2 | 0.00069133 | .7155 |
| A x S x M (B)[c] | 8 | 0.00195146 | |
| Corrected Total | 35 | 0.09605523 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $ST_C$ | $LD_A$ | $ST_A$ | $TBL_C$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 1053 | 712 | 571 | 360 | 350 | 163 |
| | a | b | c | d | e | f |

*($\alpha$ = .01)

**Mean RNA concentration per muscle in milligrams.

[a,b,c,d,e,f]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XLIII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF NUCLEI PER MUSCLE 25 DAYS OLD, SEASON I

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 1.5345298 E+21 | .0168 |
| Animal (Breed) | 4 | 9.4326356 E+19 | |
| Side | 1 | 1.2191245 E+19 | .2437 |
| Breed x Side | 1 | 2.6973991 E+19 | .1116 |
| Animal x Side (Breed) | 4 | 6.5458779 E+18 | |
| Muscle | 2 | 3.7700229 E+21 | .0001 |
| Breed x Muscle | 2 | 2.0232678 E+20 | .0522 |
| A x M (B)[a] | 8 | 4.6514722 E+19 | |
| Side x Muscle | 2 | 2.5790712 E+19 | .1652 |
| B x S x M[b] | 2 | 1.3961957 E+19 | .3435 |
| A x S x M (B)[c] | 8 | 1.1371080 E+19 | |
| Corrected Total | 35 | 2.9898500 E+20 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $LD_C$ | $ST_C$ | $LD_A$ |
|---|---|---|---|
| Mean** | $5.807 \times 10^{10}$ | $4.467 \times 10^{10}$ | $4.049 \times 10^{10}$ |
| | a | b | b |
| Muscle | $ST_A$ | $TBL_C$ | $TBL_A$ |
| Mean** | $2.666 \times 10^{10}$ | $1.592 \times 10^{10}$ | $1.234 \times 10^{10}$ |
| | c | d | d |

*($\alpha$ tested at .05 and .01 levels)

**Mean number of nuclei per muscle.

[a,b,c,d]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XLIV

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR NUMBER OF NUCLEI PER MUSCLE 25 DAYS OLD, SEASON II

| ANALYSIS OF VARIANCE | | | |
|---|---|---|---|
| Source | df | Mean Square | OSL |
| Breed | 1 | 4.8874947 E+20 | .0703 |
| Animal (Breed) | 4 | 8.1468425 E+19 | |
| Side | 1 | 7.6585529 E+17 | .8059 |
| Breed x Side | 1 | 1.4962376 E+18 | .7377 |
| Animal x Side (Breed) | 4 | 1.1944990 E+19 | |
| Muscle | 2 | 2.1027500 E+21 | .0001 |
| Breed x Muscle | 2 | 1.5670813 E+19 | .6341 |
| A x M (B)[a] | 8 | 3.2011727 E+19 | |
| Side x Muscle | 2 | 3.3751230 E+18 | .6606 |
| B x S x M[b] | 2 | 4.3819783 E+18 | .5877 |
| A x S x M (B)[c] | 8 | 7.6118895 E+18 | |
| Corrected Total | 35 | 1.5525743 E+20 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

| DUNCAN'S MULTIPLE RANGE TEST* | | | |
|---|---|---|---|
| Muscle | $LD_C$ | $ST_C$ | $LD_A$ |
| Mean** | $4.107 \times 10^{10}$ | $3.407 \times 10^{10}$ | $3.290 \times 10^{10}$ |
| | a | ab | b |
| Muscle | $ST_A$ | $TBL_C$ | $TBL_A$ |
| Mean** | $2.492 \times 10^{10}$ | $1.364 \times 10^{10}$ | $0.885 \times 10^{10}$ |
| | c | d | d |

*($\alpha$ tested at .05 and .01 levels)

**Mean number of nuclei per muscle.

[a,b,c,d]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XLV

ANALYSIS OF VARIANCE FOR GRAMS TISSUE SUPPORTED PER NUCLEUS - 25 DAYS OLD, SEASON I

| ANALYSIS OF VARIANCE | | | |
|---|---|---|---|
| Source | df | Mean Square | OSL |
| Breed | 1 | 2.8425325 E-17 | .1317 |
| Animal (Breed) | 4 | 7.9938747 E-18 | |
| Side | 1 | 2.8929260 E-20 | .6131 |
| Breed x Side | 1 | 4.9680535 E-19 | .0840 |
| Animal x Side (Breed) | 4 | 9.5176499 E-20 | |
| Muscle | 2 | 1.8433535 E-18 | .1857 |
| Breed x Muscle | 2 | 4.7751871 E-19 | .6059 |
| A x M (B)[a] | 8 | 8.8261389 E-19 | |
| Side x Muscle | 2 | 3.2932796 E-19 | .0533 |
| B x S x M[b] | 2 | 1.8219312 E-19 | .1536 |
| A x S x M (B)[c] | 8 | 7.6467443 E-20 | |
| Corrected Total | 35 | 2.1327057 E-18 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

TABLE XLVI

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR GRAMS TISSUE SUPPORTED PER NUCLEUS 25 DAYS OLD, SEASON II

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 8.5726959 E-18 | .5629 |
| Animal (Breed) | 4 | 1.1354690 E-17 | |
| Side | 1 | 6.8978674 E-21 | .8552 |
| Breed x Side | 1 | 4.1048357 E-19 | .2255 |
| Animal x Side (Breed) | 4 | 2.0077877 E-19 | |
| Muscle | 2 | 4.2686164 E-18 | .0486 |
| Breed x Muscle | 2 | 2.6301842 E-18 | .1211 |
| A x M (B)[a] | 8 | 9.4889791 E-19 | |
| Side x Muscle | 2 | 3.3547995 E-19 | .0878 |
| B x S x M[b] | 2 | 2.5106049 E-19 | .1431 |
| A x S x M (B)[c] | 8 | 1.0058133 E-19 | |
| Corrected Total | 35 | 2.2450990 E-18 | |

[a]Animal x Muscle (Breed)

[b]Breed x Side x Muscle

[c]Animal x Side x Muscle (Breed)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $ST_C$ | $LD_C$ | $LD_A$ |
|---|---|---|---|
| Mean** | $8.881 \times 10^{-9}$ a | $8.121 \times 10^{-9}$ ab | $7.120 \times 10^{-9}$ b |
| Muscle | $ST_A$ | $TBL_C$ | $TBL_A$ |
| Mean** | $6.982 \times 10^{-9}$ b | $6.793 \times 10^{-9}$ b | $6.765 \times 10^{-9}$ b |

*($\alpha$ = .05)

**Mean grams tissue supported per nucleus.

[a,b]Means with same letter are not significantly different.

A = Angus, C = Charolais

TABLE XLVII

ANALYSIS OF VARIANCE FOR MYOFIBER WIDTH
25 DAYS OLD, SEASON I

| ANALYSIS OF VARIANCE | | | |
|---|---|---|---|
| Source | df | Mean Square | OSL |
| Breed | 1 | 6555.125 | .1896 |
| Animal (Breed) | 4 | 2641.708 | |
| Side | 1 | 21.125 | .5758 |
| Breed x Side | 1 | 17.014 | .6136 |
| Animal x Side (Breed) | 4 | 56.153 | |
| Muscle | 2 | 7.542 | .8395 |
| Breed x Muscle | 2 | 92.625 | .1726 |
| Animal x Muscle (Breed) | 8 | 42.104 | |
| Side x Muscle | 2 | 218.375 | .1299 |
| B x S x M[a] | 2 | 464.847 | .0293 |
| A x S x M (B)[b] | 8 | 82.257 | |
| Duplicate (B A S M)[c] | 36 | 115.514 | |
| Residual | 1728 | 58.293 | |
| Corrected Total | 1799 | 69.391 | |

[a]Breed x Side x Muscle

[b]Animal x Side x Muscle (Breed)

[c]Duplicate (Breed Animal Side Muscle)

TABLE XLVIII

ANALYSIS OF VARIANCE AND DUNCAN'S MULTIPLE RANGE TEST FOR MYOFIBER WIDTH - 25 DAYS OLD, SEASON II

ANALYSIS OF VARIANCE

| Source | df | Mean Square | OSL |
|---|---|---|---|
| Breed | 1 | 9430.222 | .0374 |
| Animal (Breed) | 4 | 994.903 | |
| Side | 1 | 144.500 | .3704 |
| Breed x Side | 1 | 8.000 | .8165 |
| Animal x Side (Breed) | 4 | 140.958 | |
| Muscle | 2 | 1898.792 | .0004 |
| Breed x Muscle | 2 | 890.514 | .0029 |
| Animal x Muscle (Breed) | 8 | 63.444 | |
| Side x Muscle | 2 | 36.292 | .8084 |
| B x S x M[a] | 2 | 11.292 | .9341 |
| A x S x M (B)[b] | 8 | 165.000 | |
| Duplicate (B A S M)[c] | 36 | 167.611 | |
| Residual | 1728 | 84.824 | |
| Corrected Total | 1799 | 96.852 | |

[a]Breed x Side x Muscle

[b]Animal x Side x Muscle (Breed)

[c]Duplicate (Breed Animal Side Muscle)

DUNCAN'S MULTIPLE RANGE TEST*

| Muscle | $ST_C$ | $LD_C$ | $TBL_C$ | $LD_A$ | $ST_A$ | $TBL_A$ |
|---|---|---|---|---|---|---|
| Mean** | 42.8 | 41.8 | 37.5 | 37.4 | 35.7 | 35.3 |
| | a | a | b | b | c | c |

*($\alpha$ tested at .05 and .01 levels)

**Mean myofiber width per muscle in microns.

[a,b,c]Means with same letter are not significantly different.

A = Angus, C = Charolais

VITA 2

Jane Elizabeth Bartlett Link

Candidate for the Degree of

Master of Science

Thesis: EVALUATION OF SEVERAL MUSCLE CHARACTERISTICS IN THREE MUSCLES OF TWENTY-FIVE DAY OLD BEEF CALVES OF TWO BREED-TYPES

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Personal Data: Born in Lansing, Michigan, October 2, 1956, the daughter of James P. Bartlett and Billie E. Ehrlich. Married Barry C. Link, July 12, 1980. Son, Jason D. Link, born February 27, 1984.

Education: Graduated from Fraser High School, Fraser, Michigan, June 6, 1974; received Bachelor of Science Degree in Animal Husbandry from Michigan State University in June 1978; completed requirements for Master of Science Degree at Oklahoma State University in May 1984.

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