EFFECTS OF SEX AND COMPUDOSE® IMPLANTATION ON PORCINE MUSCLE HISTOCHEMISTRY

by

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Organization of Thesis

This thesis is written in a series of chapters. An introduction to the work is in Chapter I. Chapter II includes a review of the literature. Chapter III describes the research written in the format for publication in the Journal of Animal Science.

Chapter I

GENERAL INTRODUCTION

Diet conscious consumers are demanding lean, palatable and nutritious meat products. Production of intact males can help meet these goals. Boars grow more rapidly with less feed and yield a carcass with less fat and more red meat than do barrows. Boars, however, present a variety of management problems and more importantly, their cooked meat sometimes emits a "boar odor" which is quite objectionable. Boar taint is the reason most often cited for not producing boars.

Beef producers have used growth promoting implants for years to modify growth and development patterns and to increase meat production. Increased production efficiencies through use of exogenous hormone treatments may also be beneficial to the pork industry.

Muscle, fat and bone are the major carcass constituents, with muscle being the most important economically. Muscle is composed primarily of muscle cells that contain the contractile units which allows muscle to perform work. There are two basic types of muscle fibers, red and white. Red fibers are smaller, have tonic contraction and use oxidative metabolism. White fibers are larger, have phasic contraction and use glycolytic metabolism.

Production of boars rather than barrows for market and the use of exogenous hormone modification may have an impact on fiber type composition of porcine muscle and subsequently on muscle metabolism. Undesirable alteration of muscle metabolic potential is related to the porcine stress syndrome and to the etiology of

pale, soft and exudative pork muscle. However, the amount of biological variation in porcine muscle fiber histochemistry due to sex per se is unknown.

The purpose of this study was to characterize the effects of sex and hormonal influence on muscle fiber type and size in porcine longissimus and semimembranosus muscles.

Chapter II

REVIEW OF LITERATURE

RED and WHITE MUSCLE

Overview

Skeletal muscle mass comprises 30 to 50% of body weight; hence skeletal muscle cells (fibers) constitute the largest mass of cells in the body which have similar morphological and physiological properties (Cardinet, 1971). Early work (Ranvier, 1874, cited by Needham, 1926) revealed that skeletal muscle could be categorized as either red or white. Muscle differentiation has been recognized for a long time and early observations have been reviewed by Needham (1926). The terms red and white were introduced to distinguish between muscles based on their gross coloration. Subsequently, the terms red and white have come to imply more specific meaning relating to physiological properites and populations of muscle fibers types within a muscle (Cardinet, 1971). Fibers have since been classified individually by many anatomical, physiological, biochemical, histological and histochemical properties with nomenclature systems based on these characteristics.

Properties and Identification

Anatomical. That skeletal muscle may vary in color, not only from species to species, but in the muscles of the same animal, has long been known (Needham, 1926). Many early workers in the eighteenth century, believed that the red color was due to a greater supply of blood to a particular muscle (Needham, 1926). Romanul (1964) found greater capillary localization in areas with a higher red fiber

content. Moreover, red fibers are designed to contract for prolonged periods of time. In studies with anesthetized as compared with unanesthetized animals, the preservation of a threefold higher blood flow through the red soleus compared with the gastrocnemius indicates that this "differentiated" blood flow is not due to the greater activity of the soleus in maintaining posture. The greater blood flow of red muscle, therefore, is appropiately matched to its different function and metabolism (Beatty and Bocek, 1970).

Researchers have characterized muscle fibers into red (dark), white (light), (Beecher et al., 1965) and intermediate types (Padykula and Gauthier, 1967; Ogata, 1958; Rowe, 1973; Tomanek, 1976) based on differences in myoglobin content, Z-line thickness, mitochondrial properties and other anatomical characteristics. Myoglobin is the major pigment in muscle and has a higher content in red than white muscle (Cassens and Cooper, 1971). Lawrie (1950) found .280% myoglobin (wet weight) in porcine longissimus muscle and .435% in porcine psoas major muscle. The more myoglobin there is in skeletal muscle, the greater its capacity for respiratory metabolism and the less its ability to carry out glycolytic processes (Lawrie, 1950). Muscle color is dependent upon the proportion of red fibers which the muscle contains (Beecher et al., 1965). They reported that the myoglobin content of porcine muscles increased as the percentage of red fibers increased. Additionally, red fibers contained significantly higher concentrations of myoglobin than white muscles (Beecher et al., 1965). Red muscle also contains a higher lipid content (Cassens and Cooper, 1971; Beatty and Bocek, 1970) and larger and more numerous mitochondria, (Padykula and Gauthier,1967; Close, 1972) which are more irregularly distributed (Pellegrino and Franzini, 1963; Yellin and Guth, 1970). Red fibers have larger and more irregular sarcoplasmic spaces and a less well developed sarcoplasmic reticulum at the A-band level (Pellegrino and Franzini, 1963). Marsh (1977) reported that the

sarcoplasmic reticulum of white muscle is more able to retain its calcium at low temperatures than can red muscle. He also suggested that the free calcium which initiates cold shortening comes from mitochondria responding to postmortem anoxia, and not from the sarcoplasmic reticulum reacting to cold. Since the sarcoplasmic reticulum is less able to sequester calcium ions at low temperature, then only in the red tissue are there enough mitochondria to release sufficient calcium to trigger a length change.

Red fibers also are characterized by their smaller size (Ashmore, 1972; Padykula and Gauthier, 1967), thicker Z-lines, (Padykula and Gauthier, 1967; Pellegrino and Franzini, 1963; Gauthier 1969; Rowe, 1973) and less calcium binding by troponin (Furukawa and Peter, 1971; Streter, 1964) while both red and white types have very similar α-actinin properties (Suzuki et al., 1972). Red fiber types, which are adapted for repetitive contraction, require continual energy production, and therefore, a more constant source of oxygen, nutrients and waste removal which could be best supplied to a small fiber size (Ashmore 1972). White fibers derive a greater proportion of their energy anaerobically from the metabolism of endogenous glycogen stores, thus lowering the requirements for blood supply (Ashmore 1972).

Physiological. Apparently, highly specific attributes of the nerve regulate the speed of contraction of slow and fast muscle (Guth et al., 1968). However, nerve impulses as such do not influence contraction speed, but some neurotropic substances bring about muscle transformation in the normal processes of muscle differentation (Buller et al., 1960; Cassens and Cooper, 1971). Guth et al. (1968), experimenting with neural regulation, stated that one cannot explain all of the observations solely on the basis of the electrical activity of the nerves, nor can one explain them entirely by some hypothetical neurotrophic chemical transmitted from

nerve to muscle. Guth et al. (1968) tried to alter red and white muscle by cross-reinnervation. In comparison with self-reinnervated controls, the cross-reinnervated slow muscles exhibited many of the histochemical, quantitative and electrophoretic characteristics of fast muscle, although the conversion was not complete. The cross-reinnervated white muscles showed very little histochemical or quantitative evidence of conversion. The incompleteness of the conversions observed in these studies indicate that the degree of conversion is probably determined by an interaction between specific neural factors and other physiological influences. It seems likely that the amount of work performed by a reinnervated muscle could affect the rate or degree of its conversion (Guth et al., 1968).

Dark colored muscles contain high proportions of red fibers, have a high degree of tonus, contract slowly, perform postural functions and resist fatigue. Light colored muscles contain mostly white fibers, contract quickly, have greater peak strength than red muscles and fatigue more rapidly (Aberle et al., 1975). Burke (1971) designed a nomenclature system for muscles based on: 1) their resistance to fatigue during prolonged repetitive stimulation and , 2) the shape of the tension output during unfused tetani. The fibers were called: 1) S - slow contracting, 2) FR - fast contracting, fatigue resistant, 3) FF - fast contracting, fast fatigue. Fibers were considered S if their tension output after 2 min of continuous stimulation was not at least 25% of the original tension produced. Fibers were considered FR when their tension rose to a maximum and maintained that tension. FF fibers achieved maximum tension and experienced a "sag" in tension output, but never fell below 75% of original tension (Burke, 1971). Barnard et al. (1971) used two criteria to classify muscle fibers; contraction time and mitochondrial content. Based on their study, they suggested that fibers be classified as fast-twitch red, fast-twitch white

and slow-twitch intermediate. They showed that the generalization that red muscles have slow contraction times is not valid.

Biochemical, Histochemical and Histological. Peter et al. (1972) examined the classification system suggested by Barnard et al. (1971) of fast-twitch red, fast-twitch white and slow-twitch intermediate fibers and determined the metabolic profiles for these three different physiological types. Peter et al. (1972) separated all muscles by 1) relative contraction times within the same animal, 2) glycolytic capacity and 3) oxidative capacity. They proposed a descriptive alternative to the Barnard et al. (1971) classification calling them fast-twitch glycolytic, fast-twitch oxidative-glycolytic and slow-twitch oxidative, respectively.

Fast-twitch glycolytic type fibers are anaerobic and have high glycogen concentration and high phosphorylase, lactate dehydrogenase and α -glycerophosphate dehydrogenase activities. Fast-twitch oxidative-glycolytic fibers were mostly aerobic due to high concentrations of succinate dehydrogenase (SDH), cytochrome, glycogen and mitochondrial α -glycerolphosphate dehydrogenase. Slow-twitch oxidative fibers were low in glycogen, phosphorylase, lactate dehydrogenase and mitochondrial α -glycerolphosphate dehydrogenase, but moderate in cytochrome and succinate dehydrogenase activities (Peter et al. 1972). Romanul (1964) used eight enzyme activity measures and derived eight fiber types which he put in three major groups (group I, II or III). There was an obvious inverse relationship between the activities of esterase and phosphorylase enzymes. Stein and Padykula (1962) proposed three fiber types (A, B and C) on the basis of cytochemical distribution of succinic dehydrogenase. Type A represents the classical "white" fiber type and type B and C are both "red" fiber types.

Histochemical classification dealing with fiber adenosine 5'-triphophatase (ATPase) activity is well documented (Padykula and Herman, 1955; Engel, 1962; Guth et al., 1970; Brooke and Kaiser, 1970; Dubowitz and Brooke, 1973; Susuki and Cassens, 1980a). Engel (1962) first used ATPase activity to classify fibers as type I and type II. Type II are high in phosphorylase and ATPase activity (Engel, 1962; Cooper et al., 1970). The fibers of mammalian muscle differ qualitatively in the pH lability of their myosin ATPase (Guth et al., 1970). Therefore, they classified fibers as , ß and ß. The ATPase activity of the fibers is acid-labile, that of the ß fibers is alkali-labile, and that of the ß fibers is intermediate in pH lability (Guth et al., 1970; Samaha et al., 1970). Brooke and Kaiser (1970) based their nomenclature system on pH lability plus pH sensitivity and classified fibers which had low ATPase activity at pH 9.4 as type I. Type II fibers were positive in activity at 9.4, but were subdivided on the basis of their susceptibility to preincubation for five minutes at acid pH's. Type IIA fibers are almost completely inhibited below pH 4.5. Type IIB fibers are inhibited below pH 4.3, and type IIC below 3.9 (Brooke and Kaiser, 1970). These authors also have noted that classification of striated muscle into different types has always been somewhat confusing, but recently has shown an alarming trend toward the incomprehensible. They described three attributes that any nomenclature system should have: 1) it must be useful when applied to experimental or pathological situations, 2) it should be based on properties being examined, not derived by inference from other properties of that fiber, and 3) the categories should be easily differentiated, without a gradual transition from one fiber type to another or any indeterminate fibers (Brooke and Kaiser, 1970). Ashmore (1974) agreed with Brooke and Kaiser (1970) for the first two attributes, but did not agree with the third attribute. Ashmore (1974) concluded that there is, in fact, an indeterminate number of "types" of fibers and that no cytochemical

techniques would allow discrete partitioning of fibers into a limited number of sub-groups. Ashmore and Doerr (1971a) developed a system of nomenclature for chick muscle and proved it applicable (Ashmore and Doerr, 1971b) to other species. They used αW, αR and ßR terminology. Both αW and αR exhibited high ATPase activity at pH 10.0 while ßR exhibited a low ATPase activity. αW and αR fibers are differentiated on their SDH/phosphorylase ratio since αR generally have higher SDH and lower phosphorylase enzyme activities (Ashmore and Doerr, 1971b). Suzuki and Cassens (1980a) developed a nomenclature system also based on oxidative and glycolytic capacities of individual muscle fibers. They combined a very pH sensitive ATPase activity measurement with a nicotinamide-adeninedinucleotide-tetrazolium reductase (NADH-TR) activity. Fibers were classified as type I if the myofibers reacted strong for acid-stable myosin ATPase and weak for alkali-stable ATPase. Type II fibers showed the reciprocal relationship. Type II fibers were then subdivided. Myofibers unstained for ATPase after pre-incubation at pH 4.4 were identified as type IIA_1 if the NADH-TR activity was high and type IIA_2 if the NADH-TR activity was moderate. Myofibers which stained weakly or moderately after acid pre-incubation were classified into type IIB_1 with moderate to high NADH-TR activity and type IIB_2 with low NADH-TR activity. Intermediate fibers were also subdivided based on ATPase characteristics. Myofibers which stained strongly for ATPase after acid pre-incubation and moderately after alkali pre-incubation ATPase were termed SM. Myofibers were called MS if they were moderate in acid pre-incubation ATPase reaction and a strong for alkali-stable ATPase reaction. Fibers which stained moderately or strongly for ATPase after both acid and alkaline pre-incubation were named SS (Suzuki and Cassens, 1980a; Suzuki and Cassens, 1980b). Suzuki and Cassens (1983) while working with sheep muscle, subclassified types I and II into groups (IC, ID, IIA and IIB) on the basis of intensity

and pattern of staining from the NADH-TR reaction. Type IC displayed diformazan particles in a striped pattern, while type ID had a reticular and striped pattern. Both IC and ID were strong in NADH-TR activity. Type IIA had strong NADH-TR activity and type IIB possessed weak NADH-TR activity (Suzuki and Cassens, 1983).

SEX DIFFERENCES in MUSCLE FIBER TYPES, SIZES and NUMBER

Bovine

Holmes and Ashmore (1972) showed that after puberty males (bulls) had larger fibers for all fiber types than females, with the largest difference being an 18% increase in the average size of αW fibers. They hypothesized a more rapid growth rate occurred in males near puberty. Percentage of αW fibers was greater in the male (48.2%) than the female (43.3%) for the triceps longus muscle, but no differences were found in the percentage of αW fibers in the abdominal cutaneous or semitendinosus muscles at 15 mo of age (Holmes and Ashmore, 1972). West (1974) reported that steers tended to have more αW fibers in the longissimus muscle and more βR fibers in the semimembranosus muscle than heifers at a similar live weight (454 kg). Moreover, steers tended to have larger fibers for all fiber types than heifers. In contrast, Johnston et al. (1981) reported that fiber diameter of longissimus for steers and heifers were not different for any of the fiber types. They also reported that steers had more βR fibers per square centimeter, while heifers had a higher percent of αW fibers.

Greater cross-sectional areas of white fibers were observed in Angus and Hereford steers than in their respective heifers at 6 of 7 biopsy periods between 28 and 392 days of age (Spindler, 1980). Holstein steers had a smaller percentage of

white fiber area, and higher percentage of red type fibers than Holstein heifers. Generally, heifers tended to have the larger cross-sectional area of intermediate fibers (Spindler, 1980).

Dreyer (1977) reported that in both light and dark areas of the semitendinosus of bulls there was a larger percentage of red fibers and a smaller percentage of white fibers than in steers. Bulls had 33% red, 52% white and 15% intermediate fibers, while steers had 29, 58 and 13%, respectively. The average muscle fiber diameter was larger for all the muscles (light and dark semimembranosus and semitendinosus) of the bull than those of the steer, yet the bulls had a lower percentage of white fibers (Dreyer, 1977).

Cornforth (1973) found no sex differences between fiber type or size in the major muscles of the four primal cuts of Hereford and Holstein steers and heifers. However, there was a tendency for Holsteins and heifers to have a greater percentage of red fibers in the round, loin and rib samples than the Herefords and steers, respectively (Cornforth, 1973). Jeremiah et al. (1977) reported that steers generally had smaller average longissimus fiber diameters than bulls or heifers from Charolais, Simmental and Chianina sired calves.

Overall, bulls seem to have a higher percentage of ßR fibers and larger average fiber diameters than steers and heifers. No consistent pattern of fiber type differences are apparent between steers and heifers.

Ovine

Hammond (1932) showed that at five months of age, fibers of rams (39.7 µm) were slightly larger than those of the ewe (37.9 µm) corresponding to its larger body size and weight of muscle while those of the wether (38.4 µm) are intermediate. Moody et al. (1970) found that fiber diameter in the longissimus of

wethers did not increase with increasing slaughter weight (36, 45 and 54 kg). However, the fiber diameter of the same muscle in rams increased from 33.5 μm at 36 kg to 42.3 μm at 54 kg. They hypothesized that this effect was probably due to the rapid early gaining ability of the rams making them younger at any given slaughter weight than comparable wethers. The rams had, therefore, attained less of their potential muscular growth at each weight. Conversely, fiber diameters of the semitendinosus increased with increasing weight in both wethers and rams. This suggests that the semitendinosus is a slower maturing muscle than the longissimus and fibers in the semitendinosus reach their maximum diameter later in life. In this experiment, rams tended to have smaller fiber diameters in the semitendinosus, but no fiber size differences occurred in the longissimus (Moody et al., 1970). Moody et al. (1980) conducted two experiments, one comparing ewes with wethers, and another comparing wethers with rams. Fiber type percentages were similar for ewes and wethers. However, rams had more βR and less αR fibers than wethers. Wethers had larger βR fibers than ewes and rams had larger βR fibers than wethers. In contrast, Johnson (1974, cited by Moody et al., 1980) found that muscle fibers in ewe lambs were larger than those in wethers. Solomon et al. (1981) reported that wethers had fewer αR fibers than did ewe lambs in the semimembranosus muscle. Ewe lambs had larger αR and αW fibers in the semimembranosus muscle than did wether lambs. No sex effect was found for fiber type percent or fiber diameter in the longissimus (Solomon et al., 1981).

Sex (wethers and ewes) and testosterone injections failed to significantly effect average muscle fiber diameter in the longissimus (MacDonald and Slen, 1959). Wethers that received estradiol had larger muscle fiber diameters than those of control or testosterone treated wethers.

Porcine

Miller et al. (1975) studied 131 gilts and 136 barrows and found gilts possessed larger average fiber diameters in the longissimus than barrows while none of the measures of fiber type (Sudan Black-B) were affected by sex.

No differences in muscle longissimus fiber diameter were found between gilts, boars and castrated boars (Staun, 1963). However, there was a trend for gilts to have larger fiber diameters.

Clausen et al. (1960, cited by Staun, 1963) also found no difference in longissimus fiber diameter between gilts, boars and boars castrated at 20, 30, 40, 50, 60 or 70 kg and all slaughtered at 90 kg. They hypothesized that the similarity in fiber sizes was because all pigs were fed alike and were the same weight and age when slaughtered.

Neseni and Muller (1955, cited by Staun, 1963) studied the ileopsoas muscle and found fiber diameter differences between boars (49.5 μm), gilts (34.0 μm) and castrates (30.2 μm).

Other Species

Sex did not influence either number of muscle fibers or the cross-sectional area of individual fibers in male and female white rats (Eliot et al., 1943). Rowe and Goldspink (1969) found no differences in total number of fibers between male and female mice. They also found no difference in fiber diameter in the tibalis anterior, biceps brachii, extensor digitorum longus and soleus. However, in the sternomastoideus, the diameter of the female fibers (15 μm) were much smaller than male fibers (45 μm). They hypothesized that subsequent fiber development is dependent on the work load per fiber and sex has an indirect influence only by hormonal control of the growth of body tissues in general (Rowe and Goldspink,

1969). Rowe (1968) working with the same five muscles compared male, castrated male and female mice. Mean fiber diameter was largest for males, smallest for females and intermediate for castrates in all muscles, except for the soleus which had no differences. Males were always significantly different than castrates and females. Castrates were significantly different from females in two of the four muscles and in all cases the castrates tended to be more like the females than the males (Rowe, 1968).

Vaughan (1974) used the soleus muscle of male and female brown mice (group 1); male, castrated male and female, albino mice (group 2); and male and castrated male, albino mice (group 3) to study sex effects on ATPase labile fibers. In each of these three groups of animals, males had significantly fewer alkali-labile ATPase (red type fibers) fibers than females or castrate males. In group 2, females did not differ significantly from castrates, although both had more alkali-labile fibers than intact males. Since females and castrates have very low androgen levels compared with intact males, it appears that androgen levels influence the proportion of fibers in the muscle (Vaughan et al., 1974). Mice castrated at 22 (group 2) and those castrated at 4 mo of age (group 3) had more alkali-labile ATPase fibers than intact males when all were slaughtered at 6.5 mo of age. Thus, 2.5 mo after castration the percentage of fibers with alkali-labile ATPase had increased. It appears that continuously high androgen levels are required to maintain the low proportion of alkali-labile ATPase fibers observed in intact males (Vaughan et al., 1974).

Gutmann et al. (1970) also experimented with androgen (testosterone) injections into female guinea pigs and compared them with control males and female guinea pigs. There was a clear sexual differentiation of the temporal muscle as the "male" muscle had more glycolytic activity and the "female" muscle was high in mitochondrial and oxidative enzymes. Female guinea pigs injected with testosterone

for 1.5 mo gave the appearance of "male" muscle. Muscle weight and muscle fiber size both increased considerably under the effect of testosterone. They rationalized that sexual differentiation resulting in the "male" temporal muscle being "white", and the "female" muscle being "red" occurs postnatally under the influence of testosterone. Sex hormones are presumed to act on the central nervous system, particularly the hypothalamus. It appears that androgenic substances secreted by the developing testis act on neural tissues and organize the "male" type muscle. Females receiving testosterone can also develop the "male" type muscle. Thus, the male sex hormone has an important role in the differentiation of muscles sensitive to the sex hormones (Gutmann and Hanzlikova, 1970).

Rayne and Crawford (1975) counted total fiber numbers in whole, transverse histological sections in two small muscles of Lister rats. Adult females had about 15% fewer fibers in the medial pterygoid than males, but no sex difference occurred in the lateral pterygoid.

Beermann (1983) found no sex differences for muscle fiber numbers in the soleus and extender digitorum longus muscles of rats.

While working with 12 mo old dogs, Ihemelander (1980) found no difference between males and females in the number of type I, type II or intermediate myofibrillar ATPase staining fibers in the pectineus muscle.

Human biceps muscle contains approximately 37% type I and 63% type II fibers (Brooke and Engel, 1969; Brooke and Kaiser, 1970). Type I fibers have an average diameter of 58 μm in women and 62 μm in men (Brooke and Kaiser, 1970). The average diameter of type II fibers in women is 50 μm and in men 69 μm. Thus, type II fibers in the males are larger than the type I muscle fibers, while the reverse is true for females.

Sex and other hormonal influences appear to have some effect on muscle fiber type composition and on fiber type development. However, there are inconsistencies in the literature among species and across muscles which make conclusions about sex affects on muscle histochemistry difficult.

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Chapter III

EFFECTS OF SEX AND COMPUDOSE® IMPLANTATION ON PORCINE MUSCLE HISTOCHEMISTRY

Summary

Muscle fiber types and sizes were determined for six treatment groups: 1) boars, 2) boars implanted with Compudose®, 3) barrows, 4) gilts, 5) boars, penned with as post pubertal gilt (BG) and 6) implanted boars, penned with a post pubertal gilt (IBG). Muscle fibers from the longissimus and semimembranosus samples were classified as either ATPase type I (acid stable, alkali labile), SM, SS, MS (pH sensitive intermediate type fibers), IIA (acid labile, alkali stable plus NADH-TR positive) and IIB (acid labile, alkali stable plus NADH-TR negative). Percentage of type IIB fibers were higher ($P<.05$) and type IIA and NADH-TR red fibers were lower ($P<.05$) in the longissimus than the semimembranosus muscle. Red-white fiber ratios were lower ($P<.05$) for the longissimus muscle. Type I and IIB fiber diameter were larger ($P<.05$) for longissimus than semimembranosus muscle. For longissimus muscles, implanted boar and BG groups had higher ($P<.05$) percentages of type IIA fibers than boars, gilts and IBG. Fiber diameter of type IIA fibers was smaller ($P<.05$) in implanted boars than for the boar, BG and IBG groups. Gilts also had smaller ($P<.05$) IIA fibers than boars. Semimembranosus percent fiber types and percent fiber areas were not different ($P>.05$) for all fiber types across treatment groups. Type IIB fibers were smaller ($P<.05$) in boars, implanted boars, barrows and gilts than in the IBG group and IIB fibers in gilts and implanted boars were smaller ($P<.05$) than those in the BG group. Boars, implanted boars and barrows had smaller ($P<.05$) type

I fibers than IBG and BG groups. Although significant differences occurred for some fiber traits, neither sex nor Compudose® implantations had little effect on longissimus and semimembranosus fiber type composition.

(Key Words: Porcine Muscle, Histochemistry, Fiber Types, Fiber Diameter, Sex Effects.)

Introduction

Early muscle fiber work (Needham, 1926) established that muscle is composed of a heterogeneous mixture of red and white fibers. Numerous muscle properties, such as visual appearance and physiological and biochemical characteristics, reflect the myofiber type proportions which are present in muscle (Cassens, 1977). Utilizing different methodology and fiber type nomenclature, Cooper et al. (1970), Davies (1972), Swatland and Cassens (1973) and Beermann et al. (1978) have demonstrated at least three fiber type populations in porcine muscle. Suzuki and Cassens (1980a) used acid and alkali labile ATPase and NADH-TR activity to distinguish eight types of fibers.

The role that these fiber types contribute to the understanding of animal growth and development (Ashmore et al., 1972; Johnston et al. 1981) and to variations in postmortem changes (Beecher et al., 1965; Cassens, 1977) is not totally clear. Fiber type populations appear to be determined primarily by muscle function (Needham, 1926) and innervation (Beerman et al., 1978), but the effects of other factors such as time of fiber type formation, level of nutrition, proximity of blood circulation and hormonal effects are not well established (Ashmore, 1974). Data for the bovine (Holmes and Ashmore, 1972; Dreyer 1977) and the ovine (Moody et al., 1980) suggest part of the variation in muscle fiber type percentage is attributable to sex. Miller et al. (1975), however, indicates there were no significant differences in fiber populations in porcine muscle for barrows and gilts.

The purpose of this study was to characterize the effects of sex and Compudose® implantation on muscle fiber type and size in porcine longissimus and semimembranosus muscle.

Materials and Methods

Animals, Treatments and Sampling. Boars, barrows and gilts of 14 litters were allotted to six treatment groups: 1) boars, 2) boars implanted with Compudose® (24 mg estradiol controlled release implant, Elanco Products Company, Indianapolis, Indiana), 3) barrows, 4) gilts, 5) boars, penned with a post-pubertal gilt (BG) and 6) boars implanted with Compudose® and penned with a post-pubertal gilt (IBG). Animals in groups 1 through 4 were littermates, while some animals in groups 5 and 6 were from different litters than those in the first four groups. Implants were administered and post-pubertal gilts were placed in pens when the average group weight was 45.4 kg. Animals were fed ad libitum and slaughtered at an average pen weight of 109 kg. Performance, hormone and carcass data are presented elsewhere (Timmis 1984).

Longissimus (LD) muscle samples were studied for 9 boars, 8 implanted boars, 6 barrows, 6 gilts, 6 BG and 5 IBG. Semimembranosus (SMB) muscle samples were studied for 11 boars, 10 implanted boars, 9 barrows, 5 gilts, 6 BG and 5 IBG. Approximately 80% of the individuals were represented with both muscles.

Tissue samples were collected 24 h postmortem. Longissimus muscle was removed between the 10th - 12th thoracic vertebrae and histochemical samples were removed from the center of each muscle. Semimembranosus samples were removed from the muscle's longitudinal center, about 2.5 cm deep from the medial edge of the muscle. Conscious efforts were made to sample from the same location in each carcass.

Histochemical Procedure. Samples 1 cm^3 were removed, frozen in liquid nitrogen and stored at -80C. Serial, transverse frozen sections (12μm) were

incubated for acid and alkaline myosin ATPase activity by the method of Padykula and Herman (1955) as modified by Guth and Samaha (1970). Pre-incubation of ATPase sections at 32C was according to Suzuki and Cassens (1980a). Optimum pH's (acid, 5 min at 4.1 and 4.14; alkaline, 15 min at 10.1, 10.2 and 10.4) were slightly lower than theirs. The most fiber differentiating section for both the acid and alkali incubation was used per animal. Sections were also stained for reduced nicotinamide-adenine dinucleotide tetrazolium reductase (NADH-TR) according to the procedure of Engel and Brooke (1966).

Two serial microphotographs were taken for each of the NADH-TR and acid and alkaline ATPase sections (figure 1) and enlarged 150X for fiber typing of 500 to 800 fibers per muscle. Fibers were classified (table 1) according to Suzuki and Cassens (1980a, 1983) as type I (acid ATPase, positive; alkaline negative), type II (acid ATPase negative; alkaline positive), SM (acid ATPase positive; alkaline moderate), SS (acid ATPase positive; alkaline positive) and MS (acid ATPase moderate; alkaline positive). Type II fibers were sub-divided into Type IIA (NADH-TR, positive) and Type IIB (NADH-TR, negative). NADH-TR enlargements were classified as red (including intermediate staining fibers) and white (NADH negative).

Fibers on alkaline ATPase photographs were counted and diameters measured using the Zeiss TGZ-3 Partical Size Analyzer.

Statistical Analysis. Data were analyzed by analysis of variance using the Statistical Analysis System (SAS, 1982). Least Significant Difference (LSD) was used to separate treatment differences when the analysis of variance F-test was significant ($P<.05$). Simple correlation coefficients were calculated between

TABLE 1. HISTOCHEMICAL BASIS FOR MYOFIBER NOMENCLATURE SYSTEM[a]

Fiber type	Histochemical reaction[b]		
	ATPase		
	Acid	Alkaline	NADH-TR
I	+++	-	+++
SM[c]	+++	++	++
SS[c]	+++	+++	++
MS[c]	++	+++	++
IIA[d]	-	+++	++
IIB[d]	-	+++	-

[a] Suzuki and Cassens (1980a and 1983).

[b] Very positive (+++), moderately positive (++) and weak or negative (-) reaction intensity.

[c] SM=strong acid, moderate alkaline ATPase, SS=strong acid and alkaline ATPase, MS=moderate acid, strong alkaling ATPase intensities.

[d] Differentiated into A and B based on NADH-TR intensity.

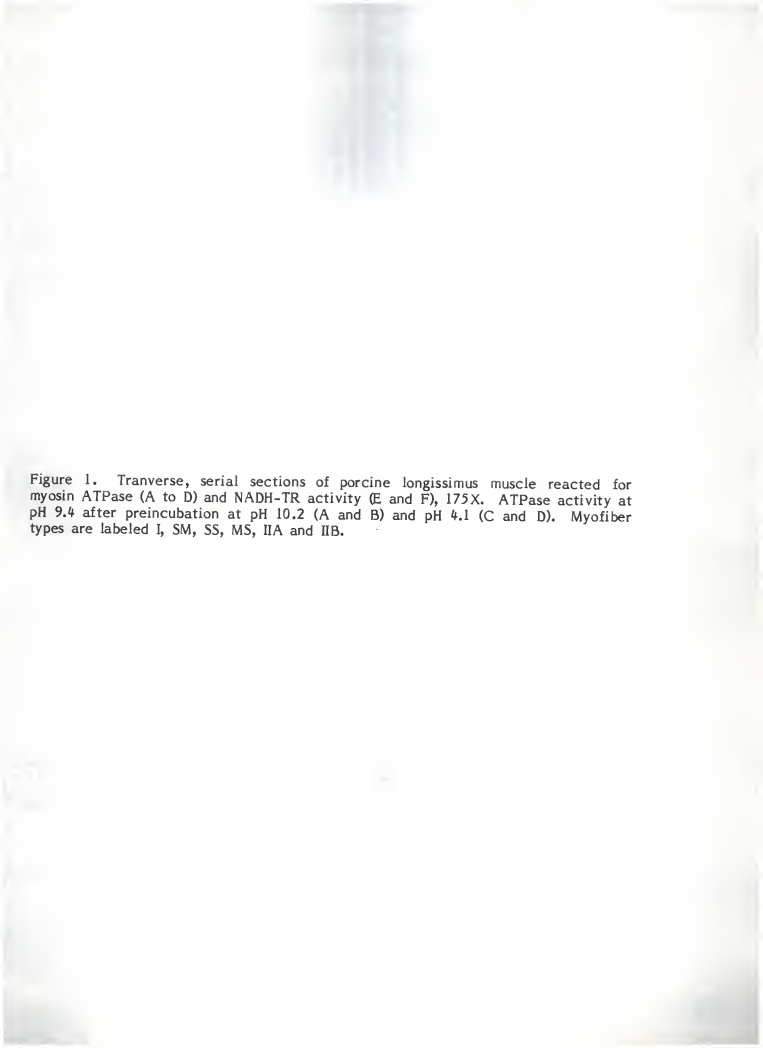
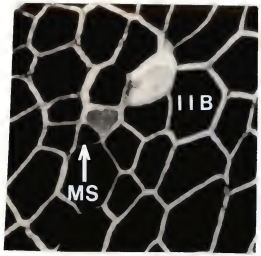
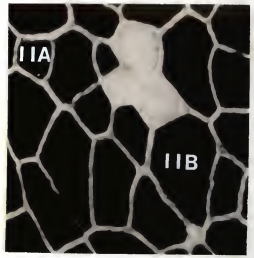
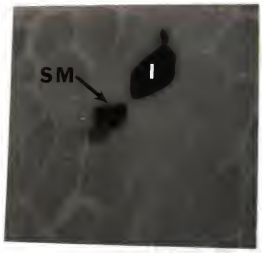


Figure 1. Tranverse, serial sections of porcine longissimus muscle reacted for myosin ATPase (A to D) and NADH-TR activity (E and F), 175X. ATPase activity at pH 9.4 after preincubation at pH 10.2 (A and B) and pH 4.1 (C and D). Myofiber types are labeled I, SM, SS, MS, IIA and IIB.


IIB
MS


IIA
IIB


SM
I


SS
I

histological characteristics and growth and carcass traits pooled across all treatment groups.

Results

Muscle Comparisons. Percentage of type IIB fibers was higher (P<.05) and the percentage of type IIA was lower (P<.05) in the LD muscle than in the SMB (table 2). No differences (P>.05) were found between muscles for percentage of types MS, SS, SM and I fibers. The LD was a whiter muscle than the SMB sample (taken approximately 2.5 cm from medial surface) as there were fewer (P<.05) NADH red fibers and both of the ATPase fiber ratios, indicating red-white fiber composition, were lower (P<.05) for the LD muscle.

Fiber diameters for the LD were larger (P<.05) for types IIB and I, but LD diameters were smaller (P<.05) for SM type fibers than in the SMB sample. Since type I and IIB fibers represent over 85% of all fibers, their larger diameters indicate an overall fiber diameter difference between muscles. No difference (P>.05) was found between muscles for mean fiber diameter in types IIA, MS and SS. Percent area of type IIB was greater (P<.05) and area for types IIA and SM lower (P<.05) in the LD than the SMB muscle. Types MS, SS and I were not different (P>.05) for percent area between muscles.

Treatment Groups. Implanted boars and the BG group had a higher (P<.05) percentage of type IIA fibers in the LD muscle than boars, gilts and IBG group (table 3). No differences for sex treatment group (P>.05) in LD fiber type percentage were found for all the other ATPase and NADH-TR fiber types.

Type IIA fiber diameter was larger (P<.05) for boars, BG and IBG than implanted boars in the LD muscle. Boars had larger (P<.05) type IIA fiber diameters

TABLE 2. FIBER CHARACTERISTICS FOR PORCINE LONGISSIMUS (LD) AND SEMIMEMBRANOSUS (SMB) MUSCLES

Fiber type[f]	Fiber type percentage[a]		Mean fiber diameter, μm[a]		Percent fiber area[a]	
	Muscle		Muscle		Muscle	
	LD	SMB	LD	SMB	LD	SMB
ATPase						
IIB	78.6[b] ±.7	73.3[c] ±.9	67.3[b] ±1.1	64.6[c] ± .9	85.4[b] ±.5	81.6[c] ±.6
IIA	9.5[c] ±.6	13.8[b] ±.6	50.3 ±1.0	51.4 ± .7	5.7[c] ±.4	9.5[b] ±.4
MS	.3 ±.04	.2 ±.03	43.2 ±1.7	45.3 ±2.8	.1 ±.02	.1 ±.02
SS	.5 ±.07	.4 ±.06	44.7 ±1.8	47.0 ±1.7	.2 ±.03	.2 ±.04
SM	.6 ±.07	.7 ±.09	38.4[c] ±1.3	43.5[b] ±1.7	.2[c] ±.03	.4[b] ±.05
I	10.9 ±.5	12.0 ±.5	58.0[c] ±.9	51.8[b] ±.7	8.6 ±.4	8.4 ±.3
IIA/II[d]	10.9[c] ±.6	15.9[b] ±.7				
R/W[e]	27.5[c] ±1.2	37.3[b] ±1.7				
NADH-TR Red fibers	29.6[c] ±1.0	40.5[b] ±.9				

[a]Longissimus N=40, semimembranosus N=46; means ± standard error.

[bc]Means in a row within a fiber characteristic with different superscript letters are different ($P<.05$).

[d]Proportion of type IIA fibers to all type II (IIA + IIB) fibers.

[e]Proportion of red fibers (type I + SM + SS + MS + type IIA) to white fibers (IIB).

[f]Fibers classified according to Suzuki and Cassens (1980a).

TABLE 3. LONGISSIMUS MUSCLE FIBER CHARACTERISTICS FOR SEX TREATMENTS

Fiber type	Treatment group: Boars	Implanted boars	Barrows	Gilts	BG[a]	IBG[a]
	Fiber type percentage					
ATPase						
IIB	78.1[b]	78.0	79.3[bc]	81.3[b]	76.6	79.2[b]
IIA	7.9[b]	11.7[c]	9.1[bc]	7.8[b]	12.4[c]	7.8[b]
MS	.3	.4	.3	.3	.2	.2
SS	.4	.5	.4	.4	.5	.6
SM	.7	.6	.3	.6	.9	.5
I	12.9	9.3	11.3	10.1	9.7	11.9
NADH-TR						
Red fibers	29.3	26.0	31.8	28.7	29.2	34.8
	Mean fiber diameter, µm					
ATPase						
IIB	70.1	63.1[b]	66.0[bcd]	64.0[bd]	70.9[cd]	69.9
IIA	54.7[c]	45.4[b]	50.3[bcd]	46.6[bcd]	52.2[d]	52.7[cd]
MS	39.8[bd]	41.0[bd]	48.6[bc]	41.2[bcd]	37.1[d]	51.8[c]
SS	48.5	41.5	39.4	39.9	44.3	47.8
SM	40.6	36.0	31.6	35.9	42.8	40.7
I	58.9	57.8	59.5	54.0	58.2	59.2
	Percent fiber area					
ATPase						
IIB	84.8	84.9	84.7	87.9	84.9	85.6
IIA	5.1	6.5	5.7	4.4	7.5	4.6
MS	.1	.1	.1	.1	.1	.2
SS	.2	.2	.1	.2	.2	.3
SM	.3	.2	.1	.2	.3	.2
I	9.7	8.3	9.5	7.4	7.1	9.3

[a]Boars (BG) and implanted boars (IBG) penned with a cycling gilt.

[bcd]Means in a row with different superscript letter are different ($P<.05$).

[e]Proportion of red fibers (type I + SM + SS + MS + type IIA) to white fibers (IIB).

than gilts. Fiber diameters for MS type fibers were larger ($P<.05$) for IBG than boar, implanted boar and BG groups in the LD. Barrows fiber diameter for LD type MS was larger ($P<.05$) than those in BG.

Percent fiber area was not different ($P>.05$) across all treatments for any fiber type in the LD muscle because of the inverse relationship of percentage fiber type and mean fiber diameter.

Semimembranosus percentage fiber types and percent fiber areas were not different ($P>.05$) for all fiber types across the treatment groups (table 4). Semimembranosus type IIB fiber diameter was larger ($P<.05$) in IBG than boars, implanted boars, barrows and gilts, while type IIB fiber diameter for BG was larger ($P<.05$) than those in implanted boars and gilts. Diameter of the type IIA SMB fibers for the IBG group was larger ($P<.05$) than for boars, implanted boars, and gilts, while type IIA fiber diameter in the BG group were larger ($P<.05$) than those in gilts. Fibers from the IBG and BG groups had larger ($P<.05$) SMB type I fibers than boars, implanted boars and barrows. Fiber diameters of types MS, SS, and SM fibers were not different ($P>.05$) for treatment groups in the SMB muscle.

Treatment group differences also were analyzed by categorizing fibers into three fiber types (table 5) using two different nomenclature systems. No differences were found when fibers were categorized as types I, II and intermediate. When fibers were classified according to Cooper et al., 1970 (similar to Ashmore and Doerr, 1971) the following results were obtained. Longissimus intermediate fiber percentage for the BG group was considerably higher ($P<.05$) than all other treatment groups. Implanted boars also had a higher ($P<.05$) intermediate fiber percentage than the IBG group. Types I and II were not different ($P>.05$) across all treatments for the LD muscle. No fiber type differences ($P>.05$) were found in the SMB muscle across all treatment groups for this nomenclature system.

TABLE 4. SEMIMEMBRANOSUS MUSCLE FIBER CHARACTERISTICS FOR SEX TREATMENTS

Fiber type	Treatment group					
	Boars	Implanted boars	Barrows	Gilts	BG[a]	IBG[a]
	Fiber type percentage					
ATPase						
IIB	74.4	76.0	72.5	73.1	70.1	71.2
IIA	14.4	11.3	14.1	15.2	15.0	13.9
MS	.1	.4	.3	.2	.2	.3
SS	.5	.4	.3	.4	.4	.6
SM	.7	.5	.4	.6	1.2	.9
I	10.3	12.0	12.9	11.0	13.4	13.4
NADH-TR						
Red fibers	39.2	40.1	38.7	43.2	43.8	40.4
	Mean fiber diameter, μm					
ATPase						
IIB	65.0^{bc}	60.8^{b}	64.1^{bc}	59.8^{b}	69.1^{cd}	71.8^{d}
IIA	50.6^{bc}	49.9^{bc}	51.2^{bcd}	48.0^{b}	54.4^{cd}	56.3^{d}
MS	51.3	41.9	44.7	41.2	37.1	54.7
SS	46.5	43.8	46.7	39.9	52.9	48.9
SM	45.2	43.6	38.8	32.1	49.2	49.5
I	49.4^{b}	50.2^{b}	50.9^{b}	52.5^{bc}	55.8^{c}	56.3^{c}
	Percent fiber area					
ATPase						
IIB	83.4	82.9	81.1	79.8	79.1	80.7
IIA	9.5	8.1	9.9	10.6	10.5	9.3
MS	.1	.2	.2	.1	.1	.2
SS	.3	.2	.1	.2	.3	.3
SM	.4	.3	.2	.2	.6	.5
I	6.6	8.5	8.8	9.3	9.6	9.2

[a]Boars (BG) and implanted boars (IBG) penned with a cycling gilt.

[bcd]Means in a row with different superscript letters are different ($P < .05$).

[e]Proportion of red fibers (type I + SM + SS + MS + type IIA) to white fbers (IIB).

TABLE 5. LONGISSIMUS AND SEMIMEMBRANOSUS MUSCLE FIBER TYPE PERCENT FOR SEX TREATMENTS

Fiber type	Treatment groups					
	Boars	Implanted boars	Barrows	Gilts	BG[a]	IBG[a]
Longissimus[b]						
II	86.1	89.7	88.4	89.1	88.9	86.9
Intermediate	1.7	1.6	1.4	1.2	1.9	1.3
I	12.9	9.3	11.3	10.1	9.7	11.9
Semimembranosus[b]						
II	88.8	87.3	86.7	88.2	85.1	85.1
Intermediate	1.6	1.2	1.2	.5	1.5	1.6
I	10.3	11.9	12.9	11.0	13.4	13.4
Longissimus[c]						
II	78.1[df]	78.0[d]	79.3[df]	81.3[df]	76.6[e]	79.2[f]
Intermediate	8.4	12.8	8.5	9.4	17.4	8.2
I	12.9	9.3	11.2	10.1	9.7	11.9
Semimembranosus[c]						
II	74.4	76.0	72.5	73.1	70.0	71.1
Intermediate	15.5	11.7	20.3	14.3	16.9	16.4
I	10.3	11.9	12.9	11.0	13.4	13.4

[a]Boars (BG) and implanted boars (IBG) penned with a cycling gilt

[b]Fiber nomenclature: II=IIA + IIB, Intermediate=SM + SS + MS, I=I.

[c]Fiber nomenclature: II=IIB, Intermediate=IIA + SM + SS + MS, I=I; similar to Cooper et al., 1971 and Ashmore and Doerr, 1971 where type II αW, intermediate αR and I βR.

[def]Means in a row with different letter superscripts are different (P<.05)

Correlations. Correlations were obtained between all fiber type characteristics and slaughter weight, testosterone level, average daily gain, loineye area, average backfat, muscling score, color score, marbling score, percent muscle and Warner-Bratzler shear force values. Selected correlations ($P<.10$) are summarized in tables 6 and 7. Fiber type correlations were low to moderate and followed no distinct pattern, although the SM type fiber tended to be related to a greater number of traits. In general, correlations between fiber type traits and growth and hormonal characteristics were nonsignificant ($P>.10$) and were low in magnitude.

Discussion

Muscle Comparisons. Both ATPase and NADH-TR histochemical data show that the SMB muscle is a redder muscle than the LD. Based on the criteria presented by Beecher et al. (1965), the SMB would be classified a red muscle (>40% red fibers) and the LD would be classified a white muscle (<30% red fibers). Beecher et al. (1965) reported 26% red fibers (Sudan Black B) and Moody and Cassens (1968) reported 30% red fibers (DPNH-TR) for the porcine LD muscle.

Total number of ATPase type II fibers (IIA plus IIB) did not differ between the LD (88.1%) and SMB (87.1%) muscles. However, when type II fibers were divided into IIA and IIB, based on their NADH-TR staining, a significant difference existed between muscles. Apparently, the SMB muscle has more oxidative capacity than the LD in the population of alkali stable ATPase fibers. The ratio of type IIA fibers to total type II fibers in the LD muscle agrees with the ratio (11.0) reported by Suzuki and Cassens (1980b).

TABLE 6. CORRELATIONS[a] BETWEEN LONGISSIMUS FIBER TYPE CHARACTERISTICS AND CARCASS TRAITS

	Fiber Characteristics				
	Percent fiber type		Percent fiber area		Average Diameter
Carcass trait	IIA	SS	SM	I	SM
Loineye area	—	—	.31*	—	—
Muscling score	—	—	—	.34**	—
Color score[b]	.31**	.46**	—	—	—
Percent muscle	—	—	.36**	—	.44***

* P<.10.
** P<.05.
*** P<.01.
[a]Omitted values were low and non-significant (P>.10).
[b]1=extremely pale; 5=extremely dark.

TABLE 7. CORRELATIONS[a] BETWEEN SEMIMEMBRANOSUS FIBER TYPE CHARACTERISTICS AND CARCASS TRAITS

	Fiber Characteristics							
	Percent fiber type			Percent fiber area		Average fiber diameter		
Carcass trait	SS	SM	I	SM	I	IIB	IIA	SM
Back fat	—	—	—	-.36**	—	-.35**	-.24*	-.46***
Loineye area	—	.37**	—	.31*	—	—	—	—
Muscling score	.44***	—	—	—	—	—	—	.39**
Marbling score[b]	—	-.30*	.33**	-.40**	.34**	—	—	-.33*
Percent muscle	—	.46***	—	.53***	—	.31**	.32**	.48***

* P<.10.
** P<.05.
*** P<.01.
[a]Omitted values were low and non-significant (P>.10).
[b]1=devoid; 5=abundant.

Intermediate fiber types (SM, SS, MS) were each less than .7% for both LD and SMB muscles. Suzuki and Cassens (1980b) reported that intermediate fibers decreased from 1.6% at 2 wk of age to less than .2% at 16 wk of age in the LD muscle. Hence, they suggested that the proportion of fibers in this transformational stage may be indicative to muscle maturity at birth. Others (Anderson and Parrish, 1972; Davies, 1972) reported 6 to 14 % ATPase intermediate staining fibers in the longissimus, but they used different fiber type nomenclature than in the present study.

Treatment Groups. Boars, barrows and gilts in this study had growth, carcass and hormonal differences indicative of each of their respective sex groups (Timmis 1984). In general, treatment group effects on fiber type percentage and percent fiber area were small and inconsistent for both the LD and SMB muscles. This agrees with Miller et al. (1975) who found that fiber type percentages were not different for barrows and gilts. However, in bovine (West, 1974; Dreyer et al., 1977), ovine (Moody et al., 1980) and mice (Vaughn et al., 1974) sex did alter some fiber type percentages.

MacDonald and Slen (1959) reported that estradiol administered to either ewes or wethers caused a significant increase in LD fiber diameter. Similarily, Miller et al. (1975) reported that gilts had larger red and intermediate fibers than barrows. However, our fiber diameter data are similar to Staun (1963) as no consistent pattern for sex effects for fiber diameter was found between gilts, boars and barrows.

The primary objective of our study was to relate sex and Compudose® implantation of boars to muscle fiber type characteristics. Even though muscle differences were demonstrated, no definitive trends within muscles were found across treatment groups for the fiber type characteristics measured. Thus, we conclude that neither sex and nor Compudose® implantation of boars has little effect on porcine muscle fiber type composition of the LD and SM muscles.

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APPENDIX 1

Histochemical Procedures

The histochemical reaction for myosin adenosine triphophatase (ATPase) is useful for characterizing different myofiber types in skeletal muscle. Skeletal muscle consists, in general, of type I (high acid-stable ATPase activity) and type II (high alkali stable ATPase activity) fibers. Suzuki's classification system (1980) (discussed earlier) uses a cross-comparison of an alkali pre-incubated and acid pre-incubated serial section. He also stained NADH-TR sections for fiber typing purposes.

Species variability and muscle variability exist for pH sensitivity, in the ATPase reactions. Following is the procedure for myosin ATPase by Guth and Samaha (1970), which we modified for this experiment. The NADH-TR procedure of Engel and Brooke (1966) is presented also.

ATPase Muscle Fiber Staining

Procedure

	Alkaline	Acid
1. Section frozen muscle	12 µm	12 µm
2. Mount on coverslips; air dry	30-240 min.	30-240 min.
3. Place in .5% $CaCl^*$	5 min.	5 min.
4. Pre-incubate solution 37C**	15 min. pH - 10.1, 10.2, 10.4	5 min. pH - 4.1, 4.14
5. Distilled water rinse	2 min.	2 min.
6. Incubation solution 37C	30 min pH 9.4	30 min pH 9.4
7. Rinse in 1% CaCl	2 min.	2 min.
8. Place in 2% $CoCl_2$	3 min.	3 min.
9. Rinse with distilled water, thoroughly	2.5 min.	2.5 min.
10. Place in 1% yellow ammonium sulfate (under hood)	3 min.	3 min.
11. Rinse with distilled water thoroughly	2.5 min.	2.5 min.
12. Dehydrate in alcohol		
80%	2 min.	2 min.
95%	2 min.	2 min.
95%	2 min.	2 min.
100%	2 min.	2 min.
100%	2 min.	2 min.
13. Clear with toluene	2 min.	2 min.

14. Mount with permount

15. Identify by animal number, date, muscle, pH pre-incubation and stain.

*This calcium rinse was added to help prevent some wrinkling of some sections and to help prevent precipitate collection on the section.

**Use 2 acid + 3 alkali pH's to allow for animal variation. These pH's may vary depending on the tissue, treatment etc,

Reagents

1. Alkaline pre-incubation solution:*

Sigma No. 221 buffer (2-amino-2 methyl-1-propanol)	3.35 ml
2% $CaCl_2$	5.00 ml
Distilled water	40.00 ml

2. Acid pre-incubation solution:

2% CaCl	100.00 ml
Glacial acetic acid	3.00 ml
Distilled water	900.00 ml

3. Incubation solution:*

Sigma 221 buffer (1.5M)	3.35 ml
2% $CaCl_2$	5.00 ml
ATP, disodium (MW 551.2)	76.00 ml
Distilled water	38.00 ml

Adjust the previous solutions with 2N KOH or 2N HCl by using a pH meter just prior to use.

4. .5% $CaCl_2$:

$CaCl_2$	.5 g
Distilled water	100.0 ml

5. 1% $CaCl_2$:

$CaCl_2$	1.0 g
Distilled water	100.0 ml

6. 2% $CaCl_2$:

$CaCl_2$	2.0 g
Distilled water	100.0 ml

7. 2% Cobaltous chloride:

$CoCl_2$	2.0 g
Distilled water	100.0 ml

8. 1% Yellow Ammonium Sulfate:*

Ammonium Sulfate	.25 ml
Distilled water	25.0 ml

*Made fresh daily (other reagents made as needed).

NADH Muscle Fiber Staining

	NADH-TR
1. Section frozen muscle	12 µm
2. Mount on coverslips, air dry	30-240 min
3. Incubate at 37C	60 min
Solution: 0.2M Tris buffer (pH 7.4)	10 ml
Nitro blue tetrazolium	10 mg
ß-NADH	8 mg
4. Rinse with distilled water	2 min
5. Fix in 10% formol saline	10 min
6. Rinse with distilled water	2 min
7. Air dry	15-20 min

8. Mount in glycerol gel.
   Solution: Heat 10 g gelatin in 60 ml distilled water until it dissolves. Add 1 ml phenol.

9. Identify animal, number, date and incubation time.

APPENDIX 2

Statistical Method

Data

Fibers on the alkali ATPase photograph were compared with serial photographs of the acid ATPase and NADH-TR stained fibers and typed according to Suzuki and Cassens (1980). The different fiber types were marked in different colors on the alkali ATPase photograph which was used for counting on the Zeiss Partical Size Analyzer. This instrument places each fiber counted in one of 48 size categories. Data were placed on the computer by the number of fibers in each size category. Between 500 and 800 fibers were counted on two 150X enlargements for each muscle for each animal.

Fiber percentage for each fiber type

Percentage of each fiber type was calculated by taking the total number of each fiber type and dividing by the total number of fibers for that animal.

Average diameter for each fiber

The average fiber diameter for each size category was taken times the number of fibers in that category. All size categories were then totalled and divided by the total number of fibers counted for that animal. This produces the average diameter for that fiber type. The average diameter is then multiplied by 1000 (to place in μm) and divided by 150 to correct for photo enlargement. This is

actual fiber diameter in μm. Average diameter for enlargement times 1000/Picture enlargement (150) = Actual Average Fiber Diameter.

Average area for each fiber type

Each fiber size category must be done separately for each fiber type to determine percent area. First, to figure total area for each fiber size category take: number of fibers in the size category times π (3.1416)/2 times (fiber diameter for that category/2)^2 times photograph correction factor (1000/150). Sum all of the areas for all size categories to get a total area for each fiber type. Total area for each fiber type is then divided by the total area for that animal which yields the percent area of each fiber type.

Statistical Analysis

All averages were then statistally analyzed by Analysis of Variance by SAS. Least Significant Differences (LSD) were used to separate treatment differences when the analysis of variance F-test was significant ($P<.05$). This is called a protected LSD.

EFFECTS OF SEX AND COMPUDOSE® IMPLANTATION ON PORCINE MUSCLE HISTOCHEMISTRY

by

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Muscle fiber types and sizes were determined for six treatment groups: 1) boars, 2) boars implanted with Compudose® (24 mg estradiol), 3) barrows, 4) gilts, 5) boars penned with a post-pubertal gilt (BG), and 6) boars implanted with Compudose® penned with a post-pubertal gilt (IBG). Longissimus and semimembranosus samples were reacted for ATPase activity to classify fibers as type I (acid positive; alkali negative) or type II (alkali positive; acid negative). Type II fibers were subdivided on NADH-TR activity into IIA (NADH positive) or IIB (NADH negative). ATPase subtypes (SM, SS and MS) were intermediate to types I and II and were classified on the basis of pH sensitivity (4.1 and 10.2) to the ATPase reaction.

Percentages of type IIB fibers were higher ($P<.05$) and types IIA and NADH-TR red were lower ($P<.05$) in the longissimus than in the semimembranosus muscle. Red-white fiber ratios also were lower ($P<.05$) for the longissimus muscle. Fiber diameters were larger ($P<.05$) for longissimus types I and IIB fibers and fiber diameters were smaller ($P<.05$) for type SM fibers than those in the semimembranosus muscle. Percent area of type IIB fibers was larger ($P<.05$) and areas for types IIA, MS and SM were smaller ($P<.05$) in the longissimus than the semimembranosus muscle.

For longissimus muscles, implanted boars and BG had higher ($P<.05$) percentages of type IIA fibers than boars, gilts and IBG. Type IIA fiber diameters were larger ($P<.05$) in boars, BG and IBG than implanted boars and type IIA fibers also were larger ($P<.05$) in boars than gilts. Type MS fiber diameters were larger ($P<.05$) in IBG than implanted boars, boars and BG, and were also larger ($P<.05$) in barrows than in BG. Percent fiber area was not different ($P>.05$) across all treatments for any fiber type.

Semimembranosus fiber type percentages and percent fiber areas were not different ($P>.05$) for any fiber types across treatment groups. Semimembranosus type IIB fiber diameters were larger ($P<.05$) in IBG than boars, implanted boars, barrows and gilts, and diameters in BG were larger ($P<.05$) than implanted boars and gilts. Type IIA fibers were larger ($P<.05$) in IBG than boars, implanted boars and gilts, and IIA fibers in BG also were larger ($P<.05$) than gilts. IBG and BG type I fibers were larger ($P<.05$) than boars, implanted boars and barrows.

Although some significant differences occurred for some fiber traits, sex and Compudose® implantation had little consistent effect on muscle fiber type composition of porcine longissimus and semimembranosus muscle.