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Influence of Selection for Breast Muscle Mass on pH and Metabolism of Supracoracoideus Muscle from Male and Female Turkey

John Yost

Thesis submitted to the
College of Agriculture, Forestry, and Consumer Sciences
at West Virginia University
in partial fulfillment of the requirements
for the degree of

Masters of Science
in
Animal and Veterinary Sciences

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Division of Animal and Veterinary Sciences

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ABSTRACT

Influence of Selection for Breast Muscle Mass on pH and Metabolism of Supracoracoideus Muscle from Male and Female Turkeys

John Yost

The objective of this study was to determine if selection for increased breast muscle mass has affected the supracoracoideus muscle's ability to regulate pH during physical activity in turkeys. The lightweight male line (LM) had the greatest breast muscle mass as a percent of body weight ($p < 0.0001$), a lower average pH (6.68; $p < 0.0001$), and greater Lactate Dehydrogenase ($293 \text{ mmol NADH min}^{-1} \mu\text{g}^{-1}$; $p < 0.0001$) and Glyceraldehyde Phosphate Dehydrogenase ($0.4452 \text{ mmol NADH min}^{-1} \mu\text{g}^{-1}$; $p < 0.05$) activities than the HM and FL lines. The LM line had the highest ratio (2.33:1) ($p < 0.05$) of adult to neonatal myosin. Hens had greater breast muscle mass as a percent of body weight than males ($p < 0.0001$), and a lower average pH (6.62; $p < 0.05$). This study showed that genetic selection for breast muscle mass resulted in an increased ratio of adult to neonatal myosin and an increased anaerobic capacity, which may predispose birds to a lower ultimate pH during physical activity.

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Review of Literature

I. Introduction:

The United States turkey industry has experienced tremendous growth, facilitated by steady market prices coupled with advances in genetic selection and nutrition. In 1988, 242 million turkeys were produced at a value of 1.9 billion dollars. This number steadily grew each year until, in 1995, there were over 292 million turkeys produced worth more than 2.7 billion dollars. Similar growth was seen in West Virginia's turkey industry. West Virginia's industry grew from 2.3 million birds, worth 16 million dollars in 1988, to 4.8 million birds, worth 40.6 million dollars in 1995. Unfortunately, selection for larger, faster growing birds has led to quality problems in turkey breast muscle. The physiological function of these faster growing birds has not kept pace with the rapid increase in muscle mass, predisposing these animals to muscle disorders such as Deep Pectoral Myopathies. In addition, abnormalities of post-mortem metabolism, most frequently observed as pale, soft, and exudative muscle, have increased. This review addresses development and function of pectoralis muscles of turkey, and the consequences of growth on muscle quality.

II. Myology and Vascular Supply:

The *Muscularis pectoralis superficialis*, or superficial pectoral muscle, is the largest muscle of the turkey (Harvey et al., 1970). Lying just under the skin, the superficial pectoral is broad, flat, and fusiform in shape. It's origin is: A) the furcula on its lateral border, where it is attached by fascia; B) the keel on the ventral crest, where it is attached by fascia; and C) the sternal ribs, where it is attached by fibers. The superficial pectoral converges and inserts by fibers, typically surrounded by a dense

tendenous envelope, on the ventral surface of the distal crest of the humerus. The *M. pectoralis superficialis* serves to adduct the brachium and omobrachium ventromedially.

The *Muscularis pectoralis profundus*, also known as the supracoracoideus or deep pectoral muscle, is a long and fusiform muscle that is mostly surrounded by the pectoralis superficialis. The origin of this muscle is: A) the lateral surface of the keel where it is attached by fibers, B) the cranial carinal margin of the sternum where it is attached by fibers, and C) the lateral distal aspect of the furcula where it is attached by fascia. The fibers of the supracoracoideus pass upward and converge on one or more large tendons, which pass dorsal through the triosseal canal. The tendon turns outward to insert on the dorsal surface of the humerus, usually just distal to its articular head. The action of the *M. pectoralis profundus* is to abduct the brachium.

In a description of deep pectoral myopathy, Orr and Riddell (1977) discussed the vascular supply to the pectoral muscles of the turkey. They reported that the cranial pectoral artery, caudal pectoral artery, 2 small branches from the pectoral trunk, and the small branch from the auxiliary artery supply the pectoral muscle. The caudal pectoral artery and the sternoclavicular artery supply the supracoracoideus. The arteries enter the muscle at the anterior region and then transverse the muscle to the posterior region

III. Muscle Development and Myosin Isoform Transitions:

Myogenic development can be characterized in three stages: 1) commitment of a multipotential stem cell into a myoblast, 2) differentiation during which myoblasts fuse into multinucleated structures expressing muscle specific proteins, and 3) maturation of the contractile unit via isoform expression for specialized function (Young and Brown, 1990). All living organisms start life as a single multipotential stem cell that eventually

gives rise to specific stem cells (Konieczny, 1991). Somites, precursors of muscle fibers, are derived from paraxial mesoderm (McLennan, 1994; Christ and Ordahl, 1995). Myoblasts originate from somatic mesoderm, where tendons and muscular fascia arises from somatopleural mesoderm (Chevallier et al., 1977; Chevallier, 1979; Sassoon, 1993). Ordahl and LeDouarin (1992) reported that precursors of limb muscles originate from the lateral half of the somite, whereas axial muscles arise exclusively from the medial portion. Somites 12 through 22 give rise to pectoral muscles (Chevallier, 1979). As cells migrate from somites to wing buds they become committed to give rise to muscle tissue (Wachtler et al., 1981). Somites may use vascular elements for migration at the 24-somite stage, or they are simply obstacles that somites must transverse during their migration (Solursh et al., 1987). Premuscle masses of the wing bud become distinct from nonmuscle masses at stages 21 through 23, of embryonic development (Schramm and Solursh, 1990). Cleavage into muscles of the pectoral girdle from the anterior limb bud occurs about embryonic day (ED) 5.5, (Butler et al., 1982).

At least four isoforms of myosin heavy chain (MHC) are expressed during development of muscle in poultry (Gauthier and Orfanos, 1993; Winkelmann et al. 1983). The terminology is based on the first appearance of the isoform (i.e. embryonic, fetal, neonatal, and adult). Embryonic myosin is the major isoform present at 10 days of incubation (Lowey et al. 1983, Bandman et al. 1982).

Mouly et al. (1987) found that chick hindlimb myoblasts express stage-specific phenotypes when differentiated in culture. Myoblasts that express the early phenotype are short and contain no more than 20 nuclei, and appear around ED 3. These myotubes synthesize a myosin, the light chains of which are LC1_F and LC2_S. The later

phenotype (ED 9) is characterized by long myotubes containing more than 100 nuclei and synthesizes a myosin with light chains LC1_F and LC2_F. Lowey et al. (1986) reported that two, fast-type, embryonic isoforms are present during development (embryonic and fetal). Van Horn and Crow (1989) reported that the embryonic appears at ED 3 to ED 5, and fetal appears at ED 5 to ED 12. The late embryonic, or fetal, isoform is replaced by neonatal myosin, beginning at ED 13, and it is predominant at one week post-hatch (Hofmann et al., 1988). The adult isoform is predominant by 26 weeks post-hatch. The different developmental isoforms are fast-twitch, and have distinct enzymatic characteristics, as well as differing primary structures (Stockdale and Miller, 1987; Bader et al., 1982). The different MHC isoforms are due to differing mRNA's and not post-transcriptional modifications (Umeda et al., 1983). All muscles undergo the same transition of embryonic to neonatal (Crow and Stockdale, 1986a). This process is 90% complete in the pectoralis major (PM) by 2 weeks, but is only 65% complete in mixed muscles of the leg. The PM will completely change from neonatal to the adult isoform by 26 weeks of age; whereas the muscles of the leg retain the neonatal along with the adult.

In analyzing the distribution of fast MHC isoforms in the thick filaments of developing chicken pectoral muscle, Taylor and Bandman (1989) stated that two different isoforms were present in the same thick filaments during isoform transitions. From 19-day old muscle, they found three types of filaments, those that contain only embryonic, those that contain embryonic and neonatal, and those that contain only neonatal. At day 44, they found similar results, but the three types of filaments contained only neonatal, neonatal and adult, or only adult. Turkey PM exhibits isoform

transitions similar to those in the chicken (Maruyama and Kamemaki, 1991). An isoform comparable, if not identical, to chicken neonatal MHC was identified at 7 days post-hatch in turkeys along with an embryonic isoform. By 14 days, an adult isoform was isolated.

IV. Fiber Type Classification and Characteristics:

Fiber types were originally classified in humans as type I, type IIA, type IIB, and IIC, based on staining intensity for oxidative enzymes (Brooke and Kaiser, 1970). Characterization has been based on motor unit type in the cat gastrocnemius (Burke et al., 1971), and their classification denotes physiological features of each motor unit type; FF, fast contracting, fast fatigue; FR, fast contracting, fatigue resistant; and S, slow contracting. Barnard et al. (1971) classified fibers of guinea pig muscle as fast-twitch red, fast-twitch white, and slow-twitch intermediate. Ashmore and Doerr (1971a) used α -white, α -red, and β -red to classify fibers of the chicken pectoralis. Peter et al. (1972) noted that the fast twitch fibers of guinea pigs and rabbits had an increased activity of myosin ATPase and the slow twitch, oxidative fibers have negligible ATPase activity. Khan et al. (1973) characterized rabbit fibers as type I red, type II red, and white. All classification methods utilized three basic fiber types: 1) a slow contracting oxidative fiber (type I, S, slow-twitch intermediate, β -red, type I red), 2) an intermediate fiber (type IIB, FR, fast-twitch red, α -red, type II red), and 3) a fast contracting glycolytic fiber (type IIA, FF, α -white, white).

According to the classification of Ashmore and Doerr (1971a), α -white fibers stain darkly for ATPase at pH 10.0, and they have a low mitochondrial content. They are fast-twitch/fast fatigue, the largest in diameter, have a sparse capillary supply, and are

rich with glycogen. The α -red fiber also stains darkly for ATPase at alkaline pH, and it has high sarcolemmal accumulation of mitochondria. They are fast-twitch/fatigue resistant, intermediate in diameter, liberally supplied with capillaries, and have a rich glycogen supply. The β -red fibers stain lightly for ATPase at pH 10.0, and they have a high concentration of mitochondria. In addition, the β -red fibers are the smallest in diameter, have very little glycogen, and are liberally supplied with capillaries. A single motoneuron contacts α -white and α -red fibers; whereas, β -red fibers are multiply innervated (Barnard et al., 1982). Muscles with low ATPase activity have low lactate dehydrogenase (LDH) activity, while those with strong ATPase activity have strong LDH activity (Briand et al., 1981).

V. Fiber Type Development:

Muscle fiber formation proceeds in two distinct phases (McLennan, 1994). Primary myotubes are first formed by the end-to-end fusion of myoblasts. Following a period of no new fiber formation, secondary myotubes form on the sides of the primary myotubes. In cultures of PM myotubes, only the fast isoform of MHC was produced at ED 8 and beyond (Miller et al., 1985). In ED 5 to ED 6 myotubes, both fast and slow myosin could be found. However, of the myotubes produced, only 4-8% contained slow MHC. During early development of the avian hindlimb, two types of muscle fibers were identified (Crow and Stockdale, 1986b; Stockdale et al., 1986). Fibers contained either fast MHC or both fast and slow MHC. Miller and Stockdale (1986a) found fibers that produced fast MHC, fast and slow MHC, and slow MHC from myoblasts of ED 5 cell cultures. Only fast MHC was found in fibers formed from myoblasts of ED 12 cell cultures. When single cells from the hindlimb cultures were cloned, ED 4 to ED 5

colonies produced three types of cells. Seventy-five percent of the colonies contained only fast MHC, 20% contained fast and slow MHC, and 3% contained slow only. Cloned colonies of ED 10 to ED 12 contained only fast MHC. In ED 12 breast muscle cultures, only fast MHC was found (Miller and Stockdale, 1986b).

The question has arisen as to what controls expression of MHC. Ashmore et al. (1978) found that α -white fibers are innervated by a single endplate, whereas, α -red and β -red fibers are multiply innervated. Kikuchi and Ashmore (1976) observed nerve fibers between cells of the *m. complexus* and *m. biceps femoris* at ED 7. Initial thickening of the sarcolemal membrane occurred at ED 10. Premature neuromuscular junctions were seen on β fibers at ED 12, and on α fibers at ED 14. When somites that form the wing were transplanted to the pelvic region, fiber type expression was unchanged (Laing and Lamb, 1983). In embryos treated to stop neural influence, there was no difference in cross-sectional area and fiber number of treated and untreated ED 9 embryos (McLennan, 1983). By ED 17, treated embryos had fewer, smaller fibers than nontreated embryos. It was concluded that production of primary fibers is independent of innervation, but formation of secondary fibers is under neural influence. Condon et al. (1990) found that embryos treated to block neural input had MHC isoform expression similar to that of untreated embryos at ED 20. He also found that treated embryos had less muscle mass, due to smaller fiber diameter, delays in secondary myogenesis, and delays in expression of different myosin isoforms. Secondary myotubes have the ability to express neonatal MHC, but not slow MHC when neural input is stopped. Feldman and Stockdale (1991) found that there were multiple types of satellite cells, those that produced fast myosin and those that produced both fast and

slow myosin. From the above-mentioned results, it can be concluded that the expression, and growth, of the first generation of muscle fibers is independent of neural input. However, influence of the nervous system is necessary for the development and growth of the second generation of fibers due to support of the continued expression of slow myosin isoforms and ordered expression of MHC isoforms.

The majority of chicken PM fibers are the α type, at hatching, and no β fibers are present (Ashmore and Doerr, 1971a). Fiber size in the PM is considerably smaller than fibers located in the leg. By four days, PM fiber size has noticeably increased but does not equal the diameter of those in the leg. Mitochondria rich fibers are clearly in the minority, and there is no change in ATPase activity. By 6 to 10 days post-hatch, the fibers of the PM have equaled those of the leg. Adult enzyme patterns are obtained by three weeks of age, and the major change that subsequently occurs is the rapid increase in fiber diameter.

Livestock species exhibit fiber differentiation patterns comparable to that of the chicken. In newborn swine, lambs, and calves all fibers have high succinate dehydrogenase activity and therefore are classified as red (Ashmore et al., 1972). Assay for myosin ATPase reveals α and β populations. However, α -white fibers are the major fiber type found in the body. Turkey PM contains 90-100% α -white fibers with the remainder being α -red fibers; no β -red fibers are present (Wiskus et al., 1976). Wiskus also reported that no glycogen staining was apparent in any of the α -white fibers. This was attributed to ATP production via anaerobic glycolysis, which would rapidly deplete glycogen stores. This explanation supports the findings of Ashmore and Doerr (1971b) who noted that α -red fibers have a higher glycogen content than the α -white fibers but

exhibit less phosphorylase activity than the α -white. Rosser and George (1986) reported that, in birds capable of flight, the majority of fibers are the α type.

VI. Muscle pH and its' regulation:

Lactic acid formed from glucose and glycogen metabolism is the major source of acid in skeletal muscle. Sahlin et al. (1978) reported that a decrease in muscle pH was linearly related to an accumulation of lactate and pyruvate. The lactate accumulation in skeletal muscles that have been stimulated to fatigue may reach 40-50 mmol/L in cell water within minutes, and internal pH may decrease to 6.5 (Juel et al., 1990). Aickin and Thomas (1977) first suggested that the Na^+/H^+ exchange system was the main mechanism responsible for initial pH regulation and that the $\text{Cl}^-/\text{HCO}_3^-$ system was involved in adjusting intracellular pH of mouse soleus muscle. A similar conclusion was reached in frog semitendinosus muscle (Putnam et al., 1986). The Na^+/H^+ exchange system was characterized by Vigne et al. (1982). They found that: 1) uptake of Na^+ is dependent on extracellular Na^+ concentration; 2) this system is controlled by the internal Na^+ and H^+ concentrations; 3) this system is inhibited by the diuretic, amiloride; and 4) the amiloride-sensitive Na^+ influx is accompanied by a amiloride-sensitive H^+ efflux. In fast-twitch muscle both the Na^+/H^+ exchange and $\text{Cl}^-/\text{HCO}_3^-$ systems were found to function together until pH rises to 7.0-7.1; thereafter, Na^+/H^+ exchanger rate is negligible whereas the $\text{Cl}^-/\text{HCO}_3^-$ system raises that pH to 7.2 (Grossie et al., 1988).

Lactate can be removed from muscle in three ways: 1) metabolism to glycogen, 2) passive diffusion, and 3) via a lactate transporter (Jeul, 1996). Bangsbo et al. (1991) found that approximately 27% of lactate produced in muscle is converted to glycogen, while the remaining 73% is released into the blood stream. Both L- and D-lactate were

found to transverse the cell membrane in the undissociated form (Roos, 1975).

Immediately after exhaustive exercise, venous blood pH can decrease to 6.95 in man (Sahlin et al., 1978), but it can drop to 6.00 in rabbit (Cooke et al., 1988). Venous blood lactate concentration can increase from 1.0 mmol/l to 11 mmol/l a few minutes after exercise (Bangsbo et al., 1993). The amount of lactate produced in a muscle and the speed at which it is removed can be influenced by type of training (Medbo and Sejersted, 1985).

Of the lactate removed from muscle after exercise, one-half of the efflux is via a lactate carrier (Jeul, 1988). The facilitated diffusion of lactate, via the lactate carrier, is the major transport mechanism for movement of lactate across the sarcolemma (Jeul et al., 1994). Lactate transport is a saturable, stereoselective process that is sensitive to a pH gradient, inhibited by other monocarboxylates, and inhibited by protein modifying agents (McDermott and Bonen, 1993). Transport is enhanced by an acidic intracellular pH. L(-) lactate has a higher transport rate than D(-) lactate (Roth and Brooks, 1990; Jeul, 1991). L(-) lactate showed saturation kinetics, whereas D(-) lactate was linear. Mean lactate removal is 39-50% greater in red muscles than in white muscles of rabbits and rats (Jeul et al., 1991). Muscles from younger animals also had a higher transport capacity. The lactate transporter is actually a co-transporter, carrying lactate and proton across the sarcolemmal membrane in a 1:1 manner (Jeul, 1995; Pilegaard and Jeul, 1995). The co-transporter has approximately twice the capacity as the Na^+/H^+ and $\text{Cl}^-/\text{HCO}_3^-$ exchange systems combined. The total capacity to remove protons is greater than the ability to remove lactate (ratio = 1.3).

VII. Effects of Muscle Growth on Meat Quality:

A. Pale, Soft, and Exudative (PSE) Muscle:

As muscle matures, the percentage of red fibers decreases with a concomitant increase in white fibers (Ashmore and Doerr, 1971a; Ashmore and Doerr, 1971b; and Ashmore et al., 1972). With the number of individual muscle fibers predetermined at birth, it is the ability of the muscle to make this transition from red to white fibers that is responsible for the large muscle mass of today's meat animals. In early turkey breeding programs, different lines were used for the production of male and female breeding stock. Male lines were selected for muscle mass and quality; whereas female lines were selected for reproductive performance. In order to meet consumer demand for poultry, female lines are now selected for muscle attributes, similar to male lines. This shift in emphasis for female lines has produced dramatic increases in breast muscle mass and growth characteristics. However, these increases in growth rate and muscle mass have produced muscle disorders such as deep pectoral myopathies (DPM) and anomalies of postmortem muscle metabolism such as pale, soft, and exudative (PSE) muscle.

Malignant Hyperthermia (MH) in pigs and humans is caused by a recessive allele (nn). In the live animal, stress due to handling and/or environmental conditions induces prolonged muscle contraction and an associated hypermetabolism, elevated body temperature, and cellular ion imbalance (for review: MacLennan and Phillips, 1992). Mickelson et al. (1986; 1989) found that the sarcoplasmic reticulum (SR) of pigs susceptible to MH released significantly more calcium upon stimulation than that of normal pigs. They also observed (Mickelson et al., 1988) that calcium was released at

a faster rate from MH susceptible SR than normal SR. O'Brien (1990) determined that MH susceptible swine are hypersensitive to the contracture producing effects of caffeine and halothane. It was later determined that because of a defect in the ryanodine receptor of the Ca^{2+} releasing channels from the SR of nn pigs, that the channels remained open longer than those from normal pigs (Fill et al., 1990). The defect in the Ca^{2+} releasing channel is not fiber type specific (Ervasti et al., 1991). In humans susceptible to MH, a defective ryanodine receptor was related to a mutation of the ryanodine receptor (RYR) gene on chromosome 19 (MacLennan et al., 1991). In pigs, Fugii et al. (1991) discovered that substitution of a thymidine for a cytosine at position 1843 of the RYR1 gene resulted in the insertion of a cysteine at position 615 instead of an arginine, producing the mutation.

Post-mortem the condition results in muscle with an unacceptable light color, soft texture, and watery appearance. Homo- and heterozygotes for the recessive gene have superior carcass characteristics (Jones et al., 1988; Simpson and Webb, 1989; Pommier et al., 1992; Leach et al., 1996). Pigs with the nn and Nn alleles will have higher carcass weights with a greater percentage of lean than NN pigs. In addition, pigs positive for the gene will have a greater proportion of ham and loin. This advantage in growth is offset by a decrease in muscle quality (Pommier and Houde, 1993). Carriers of the gene have lighter colored loins with more exudate and lower ultimate pH, typical of pale soft and exudative (PSE) muscle, than pigs negative for the gene. There have been no reports of a significant genotype X gender interaction (Sather et al., 1991; Jones et al., 1988). However, Pommier et al. (1992) reported that gilts exhibited darker loins than barrows.

From early work on postmortem metabolism in swine (Bendell et al., 1963), pale and watery meat was attributed to a combination of high temperature and low pH soon after exsanguination. Animals with pale and watery muscle reached a final pH of 5.4 in 150 minutes at a rate of 1.05 units/min; the normal group attained a final pH of 5.6 in 270 minutes at a rate of 0.65 units/min. Carcasses that exhibit the PSE condition reach an ultimate pH in the range of 4.8-5.0 instead of the normal 5.4-5.7 (Bendell, 1988). However, the pH at 45 minutes post-mortem can be highly variable, producing varying degrees of severity for the PSE condition (Fernandez et al., 1994). The severe PSE group had a 45 minute pH of 5.49; whereas, the 45 minute readings of the PSE and normal groups was 5.75 and 6.35, respectively. Enfalt et al. (1993) reported that pH decline during the first 50 minutes was nonlinear for PSE muscle and that the initial pH was well below normal carcasses or those exhibiting the dark, firm, and dry (DFD) characteristics. The lower initial pH was attributed to elevated levels of lactate before slaughter. Klont et al. (1993) reported that there was no difference in resting metabolism at the moment of slaughter between halothane genotypes. Lundstrom et al. (1989) found that nn pigs had higher lactate and glucose-6-phosphate levels in combination with lower glycogen, creatine phosphate, and ATP levels at slaughter. However, there was no difference in glycolytic and mitochondrial oxidation activities between genotypes. Pyruvate kinase activity of PSE pigs is four times greater than normal pigs (Schwagele et al., 1996). In addition, the enzyme can operate at a lower pH and has a ten time greater affinity for phosphoenal pyruvate (PEP).

The incidence of PSE in poultry breast meat has been attributed to the predominately anaerobic metabolism of the muscle, sedentary growing conditions

coupled with the large size of birds, preslaughter handling conditions, and fast pH declines along with an accelerated onset of rigor mortis (Sosnicki, 1993).

Environmental conditions and preslaughter handling dramatically affect the occurrence of PSE in poultry. McCurdy et al. (1996) reported that the appearance of light-colored breast meat was the highest in the summer months and lowest in the winter.

Approximately 19% of birds can be effected in the summer months (Barbut, 1996). PSE can be found in 5% to 30% of chicken (Barbut, 1997a) and mature turkey hen breast meat (Barbut, 1997b). Ma and Addis (1973) found that birds allowed to struggle during slaughter exhibited a faster rate of glycolysis and that the decline in pH was rapid enough to denature muscle proteins. Heat stressed birds and birds allowed to struggle freely during exanguination had lower pH values and greater expressed fluid than birds that were cold stressed or anesthetized (Froning et al., 1978). Birds slaughtered by a modified kosher technique exhibited faster rates of post-mortem pH decline and higher L* values than birds that were electrically stunned before exsanguination (Kenney et al., 1996). Amount of transportation and rest after transportation can have detrimental effects on ultimate pH and water holding capacity of turkey breast muscle (Van Hoof, 1979). Handling associated with transport causes a depletion of glycogen stores in breast muscle. During transport to the processing plant and storage at the plant, birds continue to exhaust glycogen reserves. This results in a lack of available substrate for muscle metabolism and decrease time to ultimate pH.

After slaughter, high muscle temperature and low pH result in denaturation of myosin that leads to excess moisture loss, soft texture, and light color in breast meat. White meat from poultry was found to have greater water retention capacity and slightly

lower pH than dark meat (Froning and Norman, 1966). As pH decreases and temperature increases, the degree of myosin denaturation increases (Arteaga and Nakai, 1992). Denaturation of myosin, due to a low pH and high temperature, results in the denaturation on the myosin head with some denaturation seen in the rod portion (Stabursvik et al., 1984). Thermal preconditioning of broilers was found to increase survival but had no effect on muscle characteristics post mortem (Northcutt et al., 1994). Mckee et al. (1998) reported that PSE is associated with rapid postmortem metabolism. As post-mortem pH approaches 6.0, muscle exudate will increase and the meat will be lighter (Warris and Brown, 1987). At pH 5.6, the muscle will be very exudative and light. PSE breast meat has a post mortem pH decline of .06 units/minute; whereas, normal breast meat has a decline of .03 units/minute (Pietrzak et al., 1997). The abnormal metabolism of pig and turkey muscle postmortem may be the result of similar defects in the ryanodine receptor. Significant differences were found in the SR of commercially raised turkeys compared to a genetically unimproved line (Wang et al., 1999). However, these differences have not been related to the occurrence of PSE.

B. Deep Pectoral Myopathy (DPM):

Myopathies are characterized as degenerative changes with replacement of muscle by fat and connective tissue. They involve focal or scattered fiber hyalinization, mineralization, and necrosis of breast and leg tissue (Merck Veterinary Manual, 7th edition). Deep pectoral myopathy was first reported by Dickinson et al. (1968). Degeneration, necrosis, and fibrosis of the deep pectoral muscle in heavily muscled turkeys characterize DPM. Following episodes of prolonged wing flapping when lame birds use their wings to assist ambulation, the muscle swells within it's dense, fascial

covering, becomes ischemic, and undergoes necrosis. The lesion can be produced artificially by stimulating the deep pectoral muscle to contract, and prevented by surgically opening the fascial sheath covering the muscle (Dickinson et al., 1968).

Compartment syndrome (Siller, 1985) is characterized by muscle swelling during activity that is due, primarily, to an increase in intracellular fluid. Selection to increase breast muscle mass did not result in a concomitant increase in facial growth, which could accommodate the increase in muscle mass. With increased activity, and subsequent increase in muscle swelling, there is a sustained increase in pressure around the muscle that leads to painful strangulation and necrosis. It is possible for the subfacial pressure to increase by one atmosphere due to a subsequent 20% increase in fluid volume expressed on the basis of initial weight (Martindale et al., 1979). This condition is not seen in lighter muscled birds. Siller (1985) hypothesized that training birds could correct the condition during the growing period. The premise is that birds, which are relatively inactive under normal growing conditions, will have an underdeveloped fascial covering; whereas, exercise may induce the fascia to keep pace with the developing muscle. However, this theory is unproven.

Siller and Wight (1978) presented a detailed description of the morphological features of field cases of DPM in turkeys. They described that, in the early stages, the muscle was swollen, edematous, and paler than normal. As the lesion progressed, the affected area would be confined to the middle and posterior thirds of the muscle. The central portion of the muscle would be the most affected with green coloration appearing on the surface and later spreading throughout the central portion of the

muscle. This pathology was similarly described by other researchers, (Henricks et al., 1979; Hollands et al., 1981; Sutherland, 1974; and Wight et al., 1981).

It has been discussed whether DPM is controlled by a specific genetic factor or whether it is merely due to significantly more breast musculature (Siller, 1985). Harper et al. (1981) described a genetic line where 80% of the males and 90% of the females were affected, supporting the idea that the condition was due to a polygenic trait with expression altered by modifiers. Hollands et al. (1981) showed that wild turkeys were not susceptible to the disorder; this finding indicated that the heavy musculature of domesticated birds might be a determinant of DPM. The theory that DPM was related to nutrition was disproved by Harper et al. (1972) when he conducted a study where vitamin E, selenium, and methionine were added to the diets of growing turkeys. The treated had no greater frequency of occurrence of DPM than the controls. In previous analysis of the vascular supply to the pectoralis muscles, Orr (1977) concluded that if a vascular lesion is the cause of DPM then this artery or its satellite vein would certainly be affected. Siller and Wight (1978) reported no vascular lesions in the normal anterior regions or atrophic posterior regions of the supracoracoideus muscle. However, the character of the arterial lesions in the diseased part of the muscle varied, depending on whether they were located in the necrotic lesion itself or immediately outside it. These findings led researchers to believe that DPM is a man-made disease resulting from intense genetic selection, and not the result of an inherent disposition of the species or the result of a nutritional deficiency.

Several investigators have attempted to induce the disorder in young birds, (Harper et al., 1981; Harper et al., 1983; Siller et al., 1979; and Wight et al., 1979).

Forced wing exercise can induce DPM at an occurrence of 69.4-74.2%. The frequency of occurrence increased as the age of the birds increased, with the earliest occurrence reported at 11 weeks of age when the wings are exercised. A series of experiments were conducted to determine if vascular occlusions were the cause of DPM (Wight et al., 1979). Birds were anesthetized, and one of three experiments were conducted individually or in combination: 1) occlude the subclavian artery, 2) application of electrical stimulus to the pectoral nerve, and 3) application of electrical stimulus to the muscle itself. Occlusion of the subclavian artery alone did not cause the development of DPM. However, when electrical stimulation was applied to the pectoral nerve, with subclavian artery occlusion, 11 of 14 birds showed signs of DPM. In addition, when occlusion of the subclavian artery was accompanied by direct stimulation of the supracoracoideus, a superficially green colored necrosis, similar to the acute stage of DPM, was observed. Stimulation of the pectoral nerve with no vascular occlusion did not cause DPM in lightweight birds, but 17 of 19 heavy-muscled birds were affected.

The capillary to fiber relationship in normal and ischemic *pectoralis thoracicus* and *biceps femoris* skeletal muscles was described by Sosnicki et al. (1991). He reported that ischemic muscles had a lower capillary density, fewer capillaries surrounding a single fiber, lower capillary to fiber ratio, and lower intercapillary distance. These differences were seen in comparisons of the oxidative *biceps femoris* to the glycolytic *pectoralis thoracicus*. These results may indicate that muscle from normal birds had higher oxidative capacity than muscle from birds with ischemic characteristics. However, this work raised two questions: 1) Was the relative-ischemia syndrome a result of alterations in the capillary and fiber cytoarchitecture? 2) Was the blood supply

to the ischemic muscle sufficient to fulfill the oxygen and nutrient needs for normal physiological activity? Sosnicki et al. (1991) concluded that, in addition to alterations in the capillary bed, low activity of birds did not stimulate vasodilatation, and thus caused a decrease in capillary blood flow. This decrease in blood flow restricted the intake of nutrients to the muscle and prohibited removal of waste products resulting from anaerobic metabolism. Blood pressure was found to increase in birds as a function of age (Thompson-Cowley, 1978). Males with DPM had pressures lower than unaffected birds of similar age. Within the DPM line, the males and females afflicted unilaterally had higher pressures than those affected bilaterally or those not affected.

Many investigators (Hollands et al., 1971; 1980; Thompson-Cowley et al., 1982; and Wilson et al., 1990) have evaluated methods for early detection of DPM. They used plasma levels of creatine kinase as the determining factor for development of DPM. The test birds were subjected to forced wing exercise and blood samples were taken periodically over the course of a week. Birds that developed the myopathies from forced wing exercise had greater creatine kinase levels 3-4 days post stimulation than the unaffected birds. Harper et al. (1983) used breast palpation as the method to determine presence or absence of DPM in the live bird. It involved palpation of the breast, to determine the presence of indentations caused by necrosis of the underlying supracoracoideus. The error in detection by palpation versus breast slashing is approximately 1%. Some difficulty may be found due to differences in conformation of the breasts of individual turkeys.

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Chapter 1

Influence of Selection for Breast Muscle Mass on pH and Metabolism of Supracoracoideus Muscle from Male and Female Turkey

Introduction:

Increased consumer demand for poultry has stimulated changes in selection practices used in the turkey production industry. Originally, lines intended to produce male breeding stock were selected for muscle mass, muscle quality, and growth characteristics. Female lines were selected for reproductive efficiency. In order to produce larger muscled birds that can be used in further processing situations, male and female lines were selected similarly for muscle and growth traits resulting in dramatic increases in muscle mass and growth rates. These increases have resulted in lowering production costs and increasing the utility of turkeys. However, selection for growth rate and muscle mass has predisposed birds to muscle disorders, such as deep pectoral myopathies, and anomalies of postmortem muscle metabolism, such as pale, soft, and exudative muscle.

Mckee et al. (1998) reported that PSE is associated with rapid postmortem metabolism. As post-mortem pH approaches 6.0, breast muscle exudate will increase and the meat will be lighter (Warris and Brown, 1987). At pH 5.6, the muscle will be very exudative and light. PSE breast meat has a post mortem pH decline of .06 units/minute; whereas, normal breast meat has a decline of .03 units/minute (Pietrzak et al., 1997). Enfalt et al. (1993) reported, in pigs, that the pH decline during the first 50 minutes was nonlinear for PSE muscle and that the initial pH was well below the pH of normal or dark, firm, and dry (DFD) muscle. The lower initial pH was attributed to elevated levels of lactate before slaughter.

Preslaughter handling and techniques used during slaughter dramatically affect ante-mortem muscle metabolism and, subsequently, the occurrence of PSE in poultry

breast meat. Van Hoof (1979) found that birds slaughtered without transportation reached a lower ultimate pH and had greater expressible fluid than birds that were slaughtered immediately after transportation or those slaughtered 24 hours after transportation. It appears that during transportation muscle glycogen stores are depleted; thus, substrate available for muscle metabolism is decreased resulting in smaller pH declines after slaughter. Ma and Addis (1973) found that birds allowed to struggle during slaughter exhibited a faster rate of glycolysis and that the decline in pH was rapid enough to denature muscle proteins. Muscle from heat stressed and birds allowed to struggle freely during exsanguination had lower pH values and more expressible fluid than cold stressed or anesthetized birds (Froning et al., 1978). Birds slaughtered using a modified kosher technique produced breast muscle that exhibited faster rates of post-mortem pH decline and higher L^* values than birds that were electrically stunned before exsanguination (Kenney et al., 1996). Holding birds for 6 or 12 hours prior to slaughter had no effect on pH declines or muscle quality.

The effects of stress on *in vivo* muscle function and postmortem muscle quality may be explained by breast muscle fiber type composition. Mean lactate removal is 39-50% greater in red than white muscles (Jeul et al., 1991). The pectoralis muscle of newly hatched broiler chicks is composed primarily of anaerobic white fibers (Ashmore and Doerr, 1971). Turkey pectoralis was found to contain 90-100% anaerobic fibers (Wiskus et al., 1976). Beginning with the first appearance of myosin (ED 3), developing embryos express stage-specific myosin isoforms (Mouly et al., 1987). Wan Horn and Crow (1989) reported that an embryonic form is expressed from ED 3 through ED 5, and a fetal isoform is expressed from ED 5 through ED 10. Beginning at ED 13, a

neonatal isoform is expressed along with the fetal isoform, and the neonatal is predominate by one week post-hatch (Hofmann et al., 1988). An adult isoform first appears around two weeks post-hatch and is predominate by 26 weeks post-hatch in the broiler. All muscles undergo the same transitions of embryonic, fetal, neonatal (Crow and Stockdale, 1986). This process is 90% complete in the pectoralis major (PM) by 2 weeks, but is only 65% complete in mixed muscles of the leg. The PM will completely change from neonatal to the adult isoform by 26 weeks of age; whereas the muscles of the leg retain the neonatal isoform along with the adult. However, there is no information on the timing of the transition or effects of the different myosin heavy chain isoforms on muscle quality in turkeys. Differences in moisture, protein, and fat content of breast muscle from different crosses of broilers have been reported Xiong et al. (1993).

Gender can affect stress susceptibility and muscle metabolism. Wood and Richards (1975) reported that female broilers were more susceptible to heat and cold stress than males. Lactate dehydrogenase activity of female pigs was 4-10% higher than in male pigs (Peterson et al., 1997). However, there is little information on the effects of gender and line on metabolic activity of turkey breast muscle, or changes in pH that occurs in muscle during activity. Therefore, it was the purpose of this study to evaluate the effects of gender and selection for muscle mass on intramuscular pH following electrical stimulation, and to relate the affect of these variables to anaerobic potential of the muscle and myosin isoform composition.

Materials and Methods:

Birds:

Sixty-seven white turkeys, males and females of two meat lines and one female line, were obtained from British United Turkeys of America (Lewisburg, WV). The lightweight male line (LM) was selected to produce carcasses for the whole bird market, when crossed with an acceptable female line. Selection emphasized meat yield, muscle shape, and moderate frame size. The heavyweight meat line (HM) has had pressure placed on carcass and growth traits (Average Daily Gain (ADG), meat yield, feed conversion, frame to support a heavier weight). It is used in commercial applications intended for further processed products. The female line (FL) was selected for reproductive performance and was used in commercial crosses. Two groups of birds, $n = 31$ and $n = 36$, were obtained at 14 weeks of age and transported from Lewisburg, WV, to the West Virginia University Poultry Facility in Morgantown, WV, where they were separated and penned according to line and gender. The birds were maintained on a 14% protein diet until 18 weeks of age. Three birds died during the first trial, two during the second, and one bird was miss identified and results were not used for analysis.

Stimulation of Supracoracoideus Muscle:

Experimental procedures and handling of birds was approved by the West Virginia University Animal Care and Use Committee (ACUC). At 18 weeks of age, birds were individually transported (approximately 0.5 miles) from the poultry facility to the WVU Food Animal Research Facility via a small coupe. Each bird was weighed and placed on the surgical table. An assistant held the bird under one arm and secured the

head with their other. A suitable mask was placed over the bird's head, and gas was administered with a Forregger 705 Anesthesia machine (Kansas City, MO). Isoflorane gas (Abbott Laboratories, North Chicago, IL) was used at a concentration of 2-4% in air with a flow rate of 1-2 Liters/Min. Surgical anesthesia was determined by lack of response to pinching of the pericloacal skin. Once surgical anesthesia was obtained, a three-inch incision was made at the base of the neck, opening the anterior thoracic cavity on the side to be stimulated. The pectoral nerve was exposed through the interclavicular space as described by Siller et al. (1979). Two hook-shaped, silver-wire electrodes were placed in contact with the pectoral nerve (Wight et al. 1979). A 3.4mm stainless steel pH electrode (IQ Scientific, San Diego, CA) was inserted into each breast muscle, 25 mm lateral to the point of the keel. The probe was inserted until it made contact with the lateral surface of the keel bone and was withdrawn approximately 12.5 mm, which positioned the point of the probe in the center of the supracoracoideus muscle. The supracoracoideus muscle was then stimulated to contract with a 344 Harvard Apparatus Stimulator (Millis, Mass) by applying an intermittent 1-volt stimulus of 10 ms duration at a frequency of 2-4 pulses per second. Between birds, two points (pH 7.0 and 4.0) were used for calibration of pH meters. Measurements of pH were taken every 30 seconds for 4 minutes of stimulation with an IQ200 pH meter (IQ Scientific, San Diego, CA). Stimulus was then removed and pH was monitored every minute for 10 minutes of recovery. Following the recovery period, birds were sacrificed using Beuthanasia (Schering-Plough Animal Health Corp. Kenilworth, NJ) (150mg/Kg).

Muscle Samples:

The superficial pectoralis and supracoracoideus muscles were dissected from each bird and weighed. The sum of these weights was expressed as a percent of liveweight. The point of insertion of the pH probe was determined and a cross section of the deep pectoralis (supracoracoideus) muscle was taken so that the area that contained the probe was available for enzymatic analysis and myosin isoform quantification. Muscle samples were snap frozen in liquid nitrogen and kept on dry ice until they could be transported to the lab for storage at -70°C .

Enzyme Analysis:

Lactate Dehydrogenase (LDH). One to two grams of muscle tissue were diluted in sample buffer (1:10) containing .1M potassium phosphate, and the muscle and buffer were homogenized with a Polytron Homogenizer (Brinkmann Instruments, Inc. Westbury, NY). Between samples, the homogenizer was cleaned by running the blade in 100% ethanol and rinsing with distilled water. The homogenate was then centrifuged in an IEC B-20A centrifuge (Needham Heights, Mass) at 500 X G for 20 minutes at 5°C . One milliliter of supernatant was removed and diluted to a final ratio of 1:15000, and the pellet was discarded. The assay was conducted with a LDH kit from Sigma Diagnostic (St. Louis, MO). One vial of NADH/potassium phosphate buffer was used to assay four samples in triplicate. A 2.85 mL volume of phosphate buffer, provided in the kit, was pipetted into each vial of NADH. A 180 μL volume of NADH/potassium phosphate buffer was pipetted into each well of a 96 well microtiter plate (plate). Five microliters of homogenate was added to each well, and this mixture was allowed 20 minutes for native pyruvate to be metabolized at room temperature. Ten microliters of sodium

pyruvate, provided in kit, were added to each well. Plates were immediately read at 340nm every 15 seconds for 10 minutes using negative kinetics in a Uvmax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). Negative kinetics was used because the disappearance of NADH was measured.

Glyceraldehyde Phosphate Dehydrogenase (GAPDH). One to two grams of muscle tissue was diluted in sample buffer (1:10) containing 50 mM of 2-amino-2-methyl-1,3-propanediol (AMPD) and homogenized with a Polytron Homogenizer (Brinkmann Instruments, Inc. Westbury, NY). Samples were centrifuged in an IEC B-20A centrifuge (Needham Heights, Mass) at 500 X G for 20 minutes at 5⁰ C. Supernatant was removed and diluted with sample buffer to 1:7500 for use in the assay; the pellet was discarded. Reaction buffer contained 50 mM AMPD, 1 mM NaHAsO₄, 2 mM mercaptoethanol, and 2 mM NAD⁺ (Passonneau and Lowry, 1993). Ten microliters of homogenate were added to each well of a 96 well microtiter plate with 180 µL of reaction buffer. The plate was allowed to equilibrate for 10 min before addition of 100µL of 2mM glyceraldehyde phosphate (GAP) solution to each well to initiate the reaction. Plates were immediately read at 340nm every 15 seconds for 10 minutes using positive kinetics in a Uvmax microplate spectrophotometer (Molecular Devices, Sunnyvale CA). Positive kinetics was used to measure the synthesis of NADH.

Myosin Isoform Quantification by Sodium Dodecyl Sulfate, Polyacrylamide Gel Electrophoresis (SDS-PAGE):

Migration order of the myosin heavy chain isoforms is represented in Figure 1. One to two grams of muscle tissue were diluted 1:40 in sample buffer containing 8 M urea, 2 M thiourea, 0.05 M trizma base, 0.075 M dithiothreitol (DTT), 3% (w/v) SDS, pH 6.8 (Fritz et al., 1989). Samples were homogenized using a Polytron PT 1200C

homogenizer (Brinkmann Instruments, Inc., Westbury, NY); the homogenate was heated for three minutes at 100°C and homogenized a second time. Following the second homogenization, samples were centrifuged (14000 X G) for three minutes in a Hermle Z230M centrifuge (National Labnet Co., Woodbridge, NJ); supernatant was removed and diluted with sample buffer so that the final muscle dilution was 1:1000. Sample (6 µL of sample or approximately 0.21 µg of myosin) was loaded into alternating wells and sample buffer was loaded in empty wells to prevent band diffusion. To generate a standard curve, four gels, each with seven concentrations (0.75 µL, 1.5 µL, 3.0 µL, 6.0 µL, 12 µL, 24 µL, and 48 µL) of a myosin standard (Sigma Chemical Co., St. Louis, MO; 0.1 mg/ml), were run separately. Electrophoresis was initially conducted at a constant current of 11 mA per gel until the tracking dye reached the separating gel; the current was then increased to 18 mA per gel for 22 hours.

Protocol for preparation of gels was the same as that used by Blough et al. (1996). All gels were run in a Hoefer SC600 Standard Dual Cooler Vertical Unit (Hoefer Scientific Instruments, San Francisco, CA) at 18°C. Gels were 15 cm wide, 15 cm long, and 0.75 cm thick. The stacking gel consisted of 4% (w/v) acrylamide, with an acrylamide:N,N'-methylene-bis(acrylamide)(bis) ratio of 20:1, 12% glycerol, 125 mM tris (pH 6.8) and 0.1% SDS. The separating gel consisted of 9% (w/v) acrylamide, with an acrylamide:bis ratio of 200:1, 12% glycerol, 0.75 M tris (pH 8.8) and 0.1% (w/v) SDS. Before pouring, 200 µL of a 10% (w/v) ammonium persulfate solution and 15 µL of TEMED were added for polymerization. The separating gel was allowed 1 hour to polymerize at room temperature before pouring the stacking gel, which was given 30 minutes to complete polymerization. The lower electrode buffer in the electrophoresis

unit consisted of 25 mM tris, 192 mM glycine, and 0.1% SDS at pH 8.3. The upper electrode buffer is identical except for the addition of 800 μ L of 2-mercaptoethanol per liter.

To prepare gels for staining, proteins were fixed in 10% trichloroacetic acid for 1 hour, transferred to a cross-linking solution (3% glutaraldehyde and 50 mM potassium phosphate, pH 7.0) for 30 minutes, washed in 50% methanol and 10% glacial acetic acid, and stained overnight in 0.05% Coomassie blue R-250 in 50% methanol/10% glacial acetic acid. Gels were destained by soaking in 10% methanol and 7.5% glacial acetic acid. A Chem-wipe was placed in the pyrex dish during gel destaining to facilitate the destaining process. Following destaining, gels were placed on an illuminator (Northern Light Precision illuminator Model B90, Imaging Research Inc., St. Catharines, Ontario, Canada) at $\lambda=500$ nm. The gel images were recorded with a video camera (CCD 72, Dage-MTI, Michigan City, IN) for use in the Optimas image analysis system (Optimus Inc., Edmonds, WA), where they were scanned. Individual bands of adult and neonatal myosin were magnified 200% and the perimeter around each band was marked. The gray values (GV) of marked areas were determined for adult and neonatal myosin. The gel background was subtracted by calculating the mean GV from six positions on the gel and subtracting that value from myosin standards and experimental samples. Adult and neonatal myosin was quantified by comparing GV of experimental samples to GV of standard curve.

To confirm the presence and migration order of adult and neonatal myosin, Western Blot analysis was preformed. Initially, two identical gels were run, one for quantification of proteins and a second for Western Blot analysis. A 0.45 μ m

nitrocellulose membrane (Hoefer Pharmacia Biotech Inc., San Francisco, CA) was placed on one side of the gel, and the gel and membrane were sandwiched between two pieces of filter paper and two fibrous pads. The sandwich was secured in a holder and placed in a Bio-Rad Trans-Blot Unit (Bio-Rad Laboratories, Hercules, CA). Proteins were transferred in 10 mM 3-cyclohexylamino-1-propane sulfonic acid (CAPS) (Sigma Chemical Co., St. Louis, MO), 5% methanol, for 16 hours at 4°C and a constant current of 50 mA (Cho et al., 1994). For visualization of proteins, a ProtoBlot Western Blot AP System (Promega Co., Madison, WI) was used. Primary antibodies for adult and neonatal myosin were obtained from Everett Bandman at the University of California-Davis.

Experimental Design:

A split-split plot design was used for assessing the effects of line and gender on pH decline during and after stimulation. Stimulation/Recovery (Period 1 or 2) was the block, day was the whole plot, bird was the subplot, and stimulation or nonstimulation was the sub-subplot. There were six line X gender combinations, and supracoracoideus was assigned to stimulation or nonstimulation. Statistical analysis of the data was performed using statistical analysis system software (SAS, 1989). A regression coefficient was calculated for the slope of pH decline during stimulation and recovery, for each bird, using Microsoft Excel. Analysis of variance was used to evaluate the regression coefficient of pH declines during stimulation and recovery for the stimulated and nonstimulated muscles, initial pH, ultimate pH following stimulation and recovery for the stimulated and nonstimulated muscles, and breast weight as a

percent of body weight for the line X gender combinations. Differences were considered significant at $p < 0.05$ using least-square procedures.

For adult and neonatal myosin, a randomized-complete-block design was used, with gel as the block, for running and scanning gels. Samples were nested within gel, and each line X gender combination was represented on each gel. Statistical analysis of the data was performed using statistical analysis system (SAS, 1989). Analysis of variance was used to evaluate the effects of line and gender on the proportions of adult and neonatal myosin present in supracoracoideus muscle. Differences were considered significant at $p < 0.05$.

Results and Discussion:

Breast weight and myosin isoform composition:

The birds used in this study reflect three lines intended to address the needs of specific markets. Line HM, intended for use in further processing situations, was selected for a large frame size to support increased muscle mass. This selection was evident in that it had a greater body weight (14.2 Kg) ($p < 0.0001$) than lines FL (10.7 Kg) and LM (9.62) (table 1). Males reached a greater body weight at the end of the 18 week period than females (13.36 Kg Vs 9.68 Kg) ($p < 0.0001$). Swatland (1989) reported that lines intended for meat production were heavier in final body weight and breast muscle mass than birds selected for reproductive efficiency. Line HM had the greatest superficial pectoralis and supracoracoideus weights and thus total breast weight ($p < 0.0001$). The breast muscle mass of males was greater than hens ($p < 0.0001$).

Line HM was selected for frame size and muscle mass; whereas, line LM was selected for breast muscle mass and conformation while maintaining a moderate frame

size. As a percent body weight, the LM line had proportionally more breast muscle mass (12.95%) than did lines HM (11.29%) or FL (10.67%) ($p < 0.0001$). There was no difference in supracoracoideus weight as a percent body weight between lines or genders. Differences seen in breast muscle percentage were due to differences in the weight of the superficial pectoralis ($p < 0.0001$). Line LM had the greatest proportion of superficial pectoralis ($p < 0.0001$), as a percent of body weight. Females also had a greater percent breast weight than males (12.12 Vs 11.15) ($p < 0.0001$), which is in agreement with other studies (Dodge and Stadelman, 1959). Noticeable differences were seen in the breast muscle conformation of the three lines. Line HM had a flat pectoralis muscle that was longer and wider than the other two lines. Line FL had a flat pectoralis as well, however, it did not reach the dimensions of line HM. The pectoralis of line LM was similar in length and width to that of line FL, but exhibited more plumpness. The increased breast weight as a percent of body weight and plumpness of line LM may be due to differences in fiber diameter (Swatland, 1980).

The pectoralis of turkeys is composed of white, anaerobic fibers (Wiskus et al., 1976), that produce only fast MHC isoforms from embryonic day (ED) 8 and beyond (Miller et al., 1985). As muscle matures in poultry, stage-specific myosin isoforms are expressed (Mouly et al., 1987). In ovo, embryonic, fetal, and neonatal isoforms are sequentially expressed (Van Horn and Crow, 1989; Hofmann et al., 1988). The neonatal isoform is completely replaced with an adult form by 26 week post-hatch in the chicken. In our study, analysis of myosin composition revealed only fast myosin heavy chain isoforms in turkey supracoracoideus muscle. Based on densitometric arbitrary units, there was an average of 43% more of the adult myosin isoform than the neonatal

isoform in 18 week old turkey supracoracoideus muscle. Total myosin present in supracoracoideus muscle was affected by genetic selection ($p<0.01$) (Figure 2a). Lines HM and FL had approximately 18% more total myosin than line LM ($p<0.01$). This difference remained true for adult and neonatal myosin isoforms. Line HM had 9.5% and 16% ($p<0.05$) more of the adult isoform than lines FL and LM, respectively (Figure 2b). Line FL had 14% ($p<0.10$) and 35% ($p<0.0001$) more neonatal myosin than lines HM and LM (Figure 2c). Gender significantly affected total myosin present in supracoracoideus muscle ($p<0.05$). Hens exhibited 13% more total myosin than toms (figure 2a). Although not significantly different, hens displayed 6% more adult myosin than toms (Figure 2b). Hens had 25% more neonatal myosin than toms ($p<0.001$) (Figure 2c).

When the ratio of adult myosin to neonatal myosin was evaluated, there was a significant line by gender interaction ($p<0.05$). The ratio for line LM toms was greater than the other line by gender combinations ($p<0.1$). Line LM had the highest ratio (2.33:1) of adult to neonatal myosin. This LM ratio was higher than line FL (2.33:1 Vs 1.63:1) ($p<0.01$) but the difference was not significant for line HM (2.33:1 Vs 2.13:1). Interestingly, toms had a greater ratio of the adult to neonatal isoform than hens (2.30:1 Vs 1.78:1) ($p<0.05$). The higher ratio of the adult isoform to the neonatal may indicate that the supracoracoideus muscle of line LM, more specifically LM toms, is physiologically older, or more advanced than the other line by gender combinations.

Supracoracoideus pH and anaerobic capacity:

Lactic acid formed from glucose and glycogen metabolism is the major source of acid in skeletal muscle. Sahlin et al. (1978) reported that a decrease in muscle pH was

linearly related to an accumulation of lactate and pyruvate. Lactate produced during muscle activity can be removed in three ways: 1) metabolism to glycogen, 2) passive diffusion, and 3) via a lactate transporter (Jeul, 1996). While 27% of the lactate is converted to glycogen in the muscle, the remaining 73% is released into the blood stream to be converted to glycogen in the liver or other muscles (Bangsbo et al., 1991). The lactate/H⁺ cotransporter is responsible for half of the lactate efflux (Jeul, 1988). However, the total capacity to remove lactate and H⁺ is greater in red muscles than white (Jeul et al., 1991).

There is little information on the changes that occur in intracellular pH during activity of turkey breast muscle, and how genetic selection and gender affect it. In our study, genetic selection for breast muscle mass and gender did not effect rate of pH decline in turkey supracoracoideus muscle (Figure 3a). In addition, no difference was observed in pH declines of stimulated or nonstimulated muscles (0.04 units/min Vs 0.03 units/min) (Figure 3b). There was a greater pH decline during the four-minute stimulation period than during the ten minutes of recovery (0.06 units/min Vs 0.02 units/min) ($p < 0.0001$) (Figure 3c). The rapid pH decline observed during the 4 minute stimulation period was similar to the values reported for postmortem pH declines seen in PSE and normal breast meat (Pietrzak et al., 1997). It would be reasonable to think that if the live bird was susceptible to rapid pH declines during physical activity, then a similar decline could occur during perimortem struggle. As postmortem pH approaches 6.0, muscle exudate will increase and the meat will be lighter (Warris and Brown, 1987). At pH 5.6, muscle will be very exudative and light. There was no line by period interaction for initial pH, pH following the 4 minutes of stimulation, or the pH following

the 10 minutes of recovery (Table 2). Line LM had a lower average pH (6.68) ($p<0.0001$) than did lines HM (6.79) and FL (6.71), respectively (Figure 4a). Muscles that received stimulation had a lower average pH than did nonstimulated muscles (6.67 Vs 6.76) ($p<0.05$) (Figure 4b). There was a significant gender by stimulation interaction ($p<0.0001$). Stimulated muscle of hens had a lower average pH (6.62) than stimulated toms (6.72) or nonstimulated hens (6.79) and toms (6.74) ($p<0.0001$) (Figure 4c).

Differences in average pH were reflected in anaerobic capacity of turkey supracoracoideus muscle. Lactate Dehydrogenase (LDH) activity of turkey supracoracoideus muscle was significantly affected by line ($p<0.0001$). The highest LDH activity was observed in line LM ($293 \text{ mmol NADH min}^{-1} \mu\text{g}^{-1}$) ($p<0.05$) compared to lines FL ($236 \text{ mmol NADH min}^{-1} \mu\text{g}^{-1}$) or HM ($226 \text{ mmol NADH min}^{-1} \mu\text{g}^{-1}$) (Figure 5a). In pigs it has been reported that females have 4-10% higher LDH activity than males (Peterson et al., 1997). In this study, there was no significant effect of gender on LDH activity; however, females tended to have greater LDH activity than males ($255 \text{ mmol NADH min}^{-1} \mu\text{g}^{-1}$ Vs $250 \text{ mmol NADH min}^{-1} \mu\text{g}^{-1}$) (Figure 5a). Line affected the activity of the GAPDH enzyme in turkey supracoracoideus muscle (Figure 5b). The highest activity was observed in line LM ($0.445 \text{ mmol NADH min}^{-1} \mu\text{g}^{-1}$). There was no effect of gender on GAPDH activity.

Conclusion:

The high heritability of muscle and growth traits and short generation interval of poultry has allowed the industry to produce multiple genetic varieties of birds. Each genetic line is designed to be utilized to meet different consumer demands. In all cases, rapid growth rates and increased muscle mass are important selection criteria. The

increases in muscle mass have predisposed these birds to muscle disorders and anomalies of postmortem metabolism. However, little information is available on how selection for muscle mass has affected muscle function and composition. The present study has evaluated the effects of selection for muscle mass on pH decline during stimulation, anaerobic capacity, and myosin isoform composition of turkey supracoracoideus muscle.

This study has answered questions about the basic composition, physiology, and function of turkey supracoracoideus muscle. Turkey supracoracoideus muscle is composed of anaerobic, white, fibers that contain only the fast form of myosin. By 18 weeks of age the transition from the adult isoform to the neonatal isoform is 57% complete. The higher ratio of adult to neonatal myosin may indicate that line LM is a physiologically advanced, or older, bird.

Based on the results from this study, selection for increased muscle mass as a percent body weight has resulted in an increased adult myosin to neonatal myosin ratio, and an increased anaerobic capacity of turkey supracoracoideus muscle. The increased anaerobic capacity may predispose the muscles to a lower ultimate pH during physical activity. Line LM exhibited the highest activities for LDH and GAPDH, and the lowest average pH as result of stimulation of the supracoracoideus muscle. We expected line LM to have the highest anaerobic capacity because of the greater adult myosin to neonatal ratio, but this does not explain the differences in gender effects. Hens exhibited a lower ultimate pH and a tendency to have higher anaerobic capacity but they had a lower adult myosin to neonatal myosin ratio. The increased anaerobic capacity may be due to different binding affinities of the enzyme to the different myosin

isoforms (Morton et al., 1988), or simply to differences in enzyme concentrations. However, because of the gender differences there may be more of a hormonal influence.

In conclusion, this study revealed that selection for increased muscle mass resulted in increased anaerobic capacity and lower ultimate pH during physical activity. We suggest that to reduce incidence of muscle disorders related to handling stress, that caution should be taken in handling market age birds during catch, transport, and slaughter. We can not explain why line LM, with the greatest muscle mass, had the lowest total myosin, nor can we explain why the gender differences were noted for the adult myosin to neonatal ratio. Future work may address the different enzyme activities of the myosin heavy chain isoforms and their roles in muscle quality.

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Table 1. Body and breast weights of each line by gender combination along with breast weight as a percent of body weight

	Line					
	HM		LM		FL	
	Toms	Hens	Toms	Hens	Toms	Hens
Body Weight (Kg)	15.98 ^a	12.43 ^b	11.22 ^c	8.02 ^d	12.87 ^b	8.58 ^d
Whole Breast Wt. (g)	1546 ^a	1467 ^b	1364 ^c	1102 ^d	1239 ^{cd}	937 ^e
% of BW	10.85 ^a	11.74 ^b	12.17 ^b	13.72 ^c	10.42 ^a	10.91 ^a
S. Pectoralis Wt. (g)	1219 ^a	1056 ^b	1084 ^b	888 ^c	960 ^b	757 ^d
% of BW	8.50 ^a	8.45 ^a	9.67 ^b	11.05 ^c	8.08 ^a	8.82 ^a
D. Pectoralis Wt. (g)	328	334	279	213	273	234
% of BW	2.34	2.68	2.48	2.66	2.30	2.73

HM=heavyweight male line; LM = Light weight meat line; FL = Female line
Means with different superscripts within a line are different (p<0.05)

Table 2. Initial pH, 4 minute stimulation pH, and 10 minute recovery pH of each line by gender combination.

	Line					
	HM		LM		FL	
	Toms	Hens	Toms	Hens	Toms	Hens
Initial pH	7.15	7.05	6.94	6.92	6.92	7.02
4 min stimulation	6.82	6.74	6.70	6.65	6.71	6.71
10 min recovery	6.52	6.49	6.46	6.42	6.48	6.42

HM=heavyweight male line; LM=lightweight male line; FL=female line

Figure 1. Migration order of the adult and neonatal myosin isoforms following SDS-PAGE.

Figure 2a. Effects of line on total myosin heavy chain, adult myosin heavy chain, and neonatal myosin heavy chain isoform content of turkey supracoracoideus muscle. HM=heavyweight male line; LM=lightweight male line; FL=female line. Means with different superscripts are different ($p<0.05$).

Figure 2b. Effects of gender on total myosin heavy chain, adult myosin heavy chain, and neonatal myosin heavy chain isoform content of turkey supracoracoideus muscle. HM=heavyweight meat line; LM=lightweight meat line; FL=female line. Means with different superscripts are different ($p<0.05$).

Figure 2c. Effects of line and gender on the ratio of adult myosin heavy chain to neonatal myosin heavy chain isoform content of turkey supracoracoideus muscle. HM=heavyweight male line; LM=lightweight male line; FL=female line. Means with different superscripts are different ($p<0.1$)

Figure 3a. Effects of line and gender on pH declines of turkey supracoracoideus muscle. HM=heavyweight male line; LM=lightweight male line; FL=female line.

Figure 3b. Effect of stimulation and nonstimulation on pH decline of turkey supracoracoideus muscle.

Figure 3c. Average pH decline of turkey supracoracoideus muscle.

Figure 4a. Effect of line and gender on average pH. HM=heavyweight male line; LM=lightweight male line; FL=female line. Means with different superscripts are different ($p<0.0001$).

Figure 4b. Effects of gender and level of stimulation on average pH. Means with different superscripts are different ($p<0.0001$).

Figure 5a. Effects of line and gender on Lactate Dehydrogenase activity of turkey supracoracoideus muscle. HM=heavyweight male line; LM=lightweight male line; FL=female line. Means with different superscripts are different ($p<0.0001$).

Figure 5b. Effects of line and gender on Glyceraldehyde Phosphate Dehydrogenase activity of turkey supracoracoideus muscle. HM=heavyweight male line; LM=lightweight male line; FL=female line. Means with different superscripts are different ($p<0.0001$).

APPENDICES

SODIUM DODECYL SULFATE – POLYACRILAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

Sample Preparation:

1. Sample buffer was same as that used by Fritz et al. (1989).
2. Calculate the amount of sample buffer to be added to tissue sample to arrive at an initial concentration of 40:1.

$$(\text{Sample weight}) \times 39 = \text{Volume of Buffer}$$

3. Homogenize sample in sample buffer.
4. Heat homogenized samples for 3 minutes in 100° C oven, and allow to cool to room temperature.
5. Homogenize sample and sample buffer.
6. Centrifuge sample at 500XG for 10 minutes.

Sample Dilution:

1. Take 400µL of sample and dilute with an additional 600µL of sample buffer to reach a dilution of 100:1.
2. Take 100µL of 100:1 sample and dilute with an additional 900µL of sample buffer to reach final dilution of 1000:1.
3. This solution can be stored in 1mL aliquots at –70° C.

Assembly of Gel Apparatus:

1. Two glass plates are assembled using .75mm spacers placed along each short side of the plate and clamped together in the casting stands with cams.

Resolving Gel:

1. Prepare 9% resolving gel by combining: 3.6mL acrylamide:bis (200:1) (50% stock); 5mL 3M tris, pH 8.8; 200µL 10% SDS; 2.4mL of 12% glycerol; 8.8mL dH₂O; and 200µL of ammonium persulfate (0.1mg/mL). Mix solution until glycerol is completely dissolved. (Blough et al., 1996)
2. Add 15µL of TEMED to gel solution and mix briefly.

3. Immediately pour solution into gel apparatus with a pipettor. Place pipette tip against spacer, and dispense slowly to prevent air bubbles in gel. Fill gel sandwich with gel solution until solution is 4cm from to of sandwich.
4. Cover top of gel to insure a flat surface for pouring stacking gel.
5. Allow to polymerize for 60 minutes.

Stacking Gel:

1. Prepare 4% stacking gel by combining: 1.6mL of 4% acrylamide:bis (20:1) (50% stock); 5mL 0.125M tris, pH 6.8, (0.5M tris stock); 200 μ L 0.1% SDS (10% stock); 2.4mL 12% glycerol; 10.8mL dH₂O; and 200 μ L ammonium persulfate (0.1mg/mL). Mix solution until glycerol is completely dissolved.
2. Add 15 μ L of TEMED to gel solution and mix briefly.
3. Invert gel sandwich onto a paper towel to remove water. Place a small amount of stacking gel solution in each sandwich to remove access water.
4. Immediately pour solution into gel apparatus with a pipettor. Place pipette tip against spacer, and dispense slowly to prevent air bubbles in gel. Fill sandwich to top with stacking gel solution.
5. Place .75mm comb into top of sandwich, and allow stacking to polymerize for 30 minutes.

Loading of Samples:

1. Carefully remove combs from gel sandwich to prevent tearing of well divisions.
2. Rinse wells with upper reservoir buffer to remove any acrylamide not polymerized and drain the wells by inverting sandwich onto a paper towel.
3. Refill each well with upper reservoir buffer.
4. Use a micropipettor to add 6 μ L of sample into alternating wells. Fill empty wells with sample buffer to prevent band diffusion.

Assembly of the Electrophoresis Unit:

1. Place cooling coil into lower chamber.
2. Make sure that the rubber gaskets are placed on the bottom of the upper reservoir.

3. Place upper reservoir over top of gel sandwich and secure with cams. Remove from casting stands.
4. Place gel sandwich into lower chamber, which is filled with lower electrode buffer. If necessary, adjust lower chamber level.
5. Fill upper reservoir with only enough buffer to cover electrode wire.
6. Attach safety lid so that the banana plugs plug into the female connectors. The red is the anode (+) and the black is the cathode (-).
7. Attach unit to the power supply and be sure that the positive lead is attached to the bottom chamber.
8. Apply 11mA constant current per gel until tracking dye reaches stack gel/separating gel interface. At this point, increase current to 18mA per gel. Gels are run for 22 hours at 18°C.

Staining and Destaining:

Note: All staining and destaining steps should be carried out under an exhaust hood.

1. Immediately after turning off power supply, remove gel from electrophoresis unit. Separate plates and place plate with gel attached into a Pyrex dish, which contains a 10% solution of trichloroacetic acid (TCA). Tilt plate in solution until gel is removed from plate. Agitate gel in TCA solution for 1 hour.
2. Transfer gel from TCA to fixing solution using a thin wire screen. Agitate in fixing solution for 30 minutes. **(Note: Be careful when transferring gel to prevent tearing)**
3. Transfer gel from fixing solution to washing solution for 1 hour.
4. Transfer gel from wash solution to staining solution, and agitate overnight. **(Cover Pyrex dish with cellophane to prevent evaporation)**
5. The next morning, transfer gel from staining solution to destaining solution. Agitate gel until desired contrast is achieved. (Note: a chemwipe can be placed in the Pyrex dish with gel to aid destaining).

Stock Solutions:

Resolving Gel

Add 30g acrylamide and 0.15g bis to about 50mL distilled water in a 200mL beaker. Stir until dissolved (warm if necessary). Dilute to final volume of 100mL and filter through a 1.2 μ m filter. Store in brown bottle at 4°C.

Acrylamide is a cumulative neurotoxin; therefore, avoid contact with skin by wearing gloves.

Stacking Gel

Add 10g acrylamide and 0.5g bis to about 80mL of distilled water in a 100mL beaker. Stir until dissolve (warm if necessary). Dilute to final volume of 100mL and filter through a 1.2 μ m filter. Store in brown bottle at 4°C.

3M tris buffer (pH 8.8)

Dissolve 36.35g tris in 50mL distilled water and adjust pH to 8.8 with 10N HCL. Dilute to 100mL. Filter and store in a brown bottle at 4°C.

0.5M tris buffer (pH 6.8)

Dissolve 6.06g tris in 50mL of distilled water and adjust pH to 6.8 with 10N HCL. Dilute to 100mL and filter. Store in brown bottle at 4°C.

10% SDS

Dissolve 10g SDS in approximately 80mL of distilled water and bring volume to 100mL. Filter through 1.2 μ m filter and store in brown bottle at 4°C.

10% Ammonium Persulfate

Dissolve 0.4g of ammonium persulfate in 4mL of distilled water. Prepare fresh daily.

Buffer for SDS-PAGE Sample

- A. Weigh 48g urea and 15.2g thiourea into 150mL glass beaker, add 40 ml of water. **(Be careful to avoid temperatures above 40°C – heating urea speeds up the formation of cyanate)**
- B. Stir gently on hot plate until solution is at room temperature
- C. Add 10g of a mixed bed resin, stir at room temperature for 15 minutes.

D. Filter mixture through filter paper into a 100mL graduated cylinder. Volume should be 80-90mL. Carefully rinse the resin with one or two 5mL aliquots of distilled water.

E. Add 0.605g trizma base and 3g SDS to urea-thiourea solution and stir until dissolved. F. Adjust pH to 7.5 carefully. Add 1.155g of solid DTT and stir until dissolved.

G. Adjust pH to 6.8 using 2M HCL. Add 2-4 mg of bromophenol blue, stir until dissolved.

H. Filter solution through a .45µm filter.

I. Store in 1mL aliquotes at -70°C until needed.

Upper Reservoir Buffer

Weigh 12.11g tris base, 11.26g glycine, and 1g SDS into 1000mL volumetric flask. Bring volume to 1000mL with disstilled water, and stir until dissolved. Adjust pH to 8.3 with 10N HCL.

Lower Electrode Buffer

Weigh 24.23g tris base, 22.52g glycine, and 2g SDS into 1000mL volumetric flask. Bring volume to 1000mL with distilled water, and stir until dissolved. Adjust pH to 8.3 with 10N HCL. Transfer solution to 4000mL graduated cylinder and bring volume to 4000mL. Check pH.

10% Trichloroacetic Acid Solution

Add 100g of trichloroacetic acid to 400 ml of distilled water in a 1000mL volumetric flask. Bring volume to 1000mL and stir until dissolved. Filter solution and store at room temperature until dissolved. Solution may be reused.

Fixing Solution

Weigh and add 7.55g potassium phosphate to 30mL of gluteraldehyde in a 1000mL volumetric flask. Bring volume to 1000mL and stir. (Make fresh daily).

Wash Solution

Add 500mL of methanol and 100ml of glacial acetic acid to a 1000mL volumetric flask. Bring volume to 1000mL and stir. Prepare fresh daily.

Staining Solution

Add 0.5g Coomassie Brilliant blue R-250 with 500mL methanol and 92mL glacial acetic acid, mix well. Bring volume to 1000mL with distilled water.

Destaining Solution

Add 100mL of methanol and 75mL of glacial acetic acid to a 1000mL volumetric flask. Bring volume to 1000mL and stir.

Equipment

1. SE 600 Dual Cooled Vertical Slab gel unit: Hoefer Scientific Instruments, San Francisco, CA.
2. Power Supply PS3000: Hoefer Scientific Instruments, San Francisco, CA.

Reference:

- Blough, E.R., E.R Rennie, F. Zhang, and P.J. Reiser, 1996. Enhanced electrophoretic separation and resolution of myosin heavy chains in mammalian and avian skeletal muscle. *Anal. Biochem.* 233:31-35.
- Fritz, J.D., D.R. Swartz, and M.L. Greaser, 1989. Factors affecting polyacrylamide gel electrophoresis and electroblotting of high-molecular-weight myofibrillar proteins. *Anal. Biochem.* 180:205-210.

Western Blot Procedure

Assembly of Gel/Membrane Sandwich:

1. Remove gel from electrophoresis unit and place in transfer buffer containing 10mM 3-cyclohexylamino-1-propane sulfonic acid (CAPS) and 5% methanol. Soak gel for 15 minutes. (Cho et al., 1994)
2. Disassemble gel/membrane holder, place 1 fiber pad (wetted with transfer solution) on gray panel.
3. Place a piece of filter paper, wetted with transfer solution on fiber pad.
4. Carefully place gel on top of filter paper.
5. Place wetted .45µm pore size Nitrocellulose membrane on gel. **(Membrane should be soaked in transfer solution for 30 minutes before use).** To exclude air bubbles from gel/membrane interface, roll surface with test tube.
6. Place wetted filter paper on membrane.
7. Place wetted fiber pad on filter paper.
8. Close and secure holder.

Transfer Procedure:

1. Place gel/membrane sandwich into transfer unit (gray panel of sandwich toward gray panel of transfer unit).
2. Insert cooling coil.
3. Fill tank with transfer buffer so that gel and membrane are covered.
4. Transfer for 16 hours at 4°C at constant current of 50mA.

Antibody Incubation and Staining:

Membrane Blocking

- a. Remove gel/membrane sandwich from transfer unit and disassemble holder. Place membrane in 200mL of blocking solution (TBST and 1% BSA) for 30 minutes (Blocking solution may be reused)

Primary Antibody Binding

- a. Transfer membrane from blocking solution to 10mL of solution containing TBST and 2mL of primary antibody. Incubate for 60 minutes.
- b. Wash membrane three times in TBST for 10 minutes each to remove unbound antibody.

Secondary Antibody Binding

- a. Transfer membrane to 10mL of TBST solution containing 2mL of secondary antibody, and incubate for 30 minutes.
- b. Following incubation, wash membrane 3 times in TBST for 10 minutes each to remove unbound secondary antibody.
- c. Wash membrane twice in TBS to remove Tween 20 from membrane surface.

Staining

- a. Prepare color development solution by adding 66 μ L of NBT to 10mL of alkaline phosphate buffer, mix and add 33 μ L of BCIP. Mix well. **(use within 1 hour)**
- b. Place membrane color development solution and incubate until bands reach desired intensity (30 minutes).
- c. To stop reaction, and prevent excessive background, wash membrane in distilled water for 10 minutes.

Solutions:

Transfer Buffer

1. Weigh 4.42g CAPS and add to 100mL of methanol in a 2000mL graduated cylinder. Bring volume to 2000mL and mix. (Cho et al., 1994)

TBS

1. Weigh 3.152g of tris-HCL and place in a 1000mL volumetric flask containing 500mL of distilled water. Adjust pH to 7.5 with 10N HCL.
2. Weigh 3.5g of NaCl, add to tris solution. Bring volume to 1000mL.

TBST

1. Add 500 μ L of Tween 20 (provided in kit) to 1000mL of TBS solution

Blocking Solution

1. Weigh 4g of BSA and add to 400mL of TBST

Alkaline Phosphate Buffer

1. Weigh 1.57g tris-HCL and add to 80mL of distilled water in 200mL beaker. Adjust pH to 9.5 with 10N HCL.
2. Add 0.58g NaCl and 0.047g MgCl₂ to beaker, bring volume to 100mL, and stir until dissolved.

Equipment:

1. Bio-Rad Trans-Blot Unit, Bio-Rad Laboratories, Hercules, CA.
2. ProtoBlot Western Blot AP System, Promega Co., Madison, WI.

References:

Cho, M., S.M. Hughes, I. Karseh-Mizarchi, M. Travis, L.A. Leinwand, and H.M. Blau, 1994. Fast myosin expressed in secondary mammalian muscle fibers at the time of their inception. J.Cell Sci. 107:2361-2371.

Glyceraldehyde Phosphate Dehydrogenase Assay

Sample Preparation:

1. 100mL of stock homogenization buffer is diluted to 1000mL with distilled water and the pH is adjusted to 9.0 with 10N HCL.
2. Calculate the amount of homogenization buffer to be added to each tissue sample to arrive at an initial concentration of 10:1.

$$(\text{Sample weight}) \times 9 = \text{Volume of Buffer}$$

3. Homogenize the sample in sample buffer, and centrifuge homogenate at 500 X G of 10 minutes at 5°C.
4. Following centrifugation, remove 1mL of supernatant and bring volume to 50mL with homogenization buffer to make a 500:1 dilution. Take 1mL of 500:1 dilution and add 1mL of homogenization buffer to create a 1000:1 dilution. Take 1mL of the 1000:1 dilution and add 6.5mL to make final dilution of 7500:1.

Conduction of Assay:

1. Add 180μL of assay buffer that contains: 50mM 2-amino-2-methyl-1,3-propanediol (AMPD), 1mM NaHAsO₄, 2mM mercaptoethanol, and 2mM NAD⁺, to each well of a microplate.
2. Add 10μL of homogenate to each well and allow 10 minutes to metabolize native Glyceraldehyde Phosphate (GAP).
3. Add 100μL of 2mM GAP solution to each well to initiate reaction.
4. Read plate immediately at 340nm in a Uvmax microplate spectrophotometer.

Solutions:

1. Stock Homogenization Buffer

Weigh 52.55g of AMPD into a volumetric flask, bring to volume of 1000mL, stir until dissolved, and store at 4°C.

2. Assay Buffer

Weigh 525.5mg AMPD, 31.2mg NaHAsO₄, 100μL mercaptoethanol, and 2.268mg NAD⁺ into a 200mL beaker with 50mL of distilled water. Stir until dissolved. Make fresh daily.

3. Glyceraldehyde Phosphate Solution

Add 133 μ L of GAP to 20mL of homogenization buffer and stir.

Softmax Program:

1. In windows, under "Program Manager" click on "Applications"
2. Under "Applications" click on "Softmax"
3. Once Softmax is activated, click instrument under the setup menu and set kinetics to "Kinetic L1", run time to 10 minutes, and read interval to 15 seconds. Click OK.
4. Next, under the setup menu click on "template" and identify the wells that contain sample. (Note: If you do not identify the well the machine will not read the sample)
5. Under the setup menu click "analysis" and set analysis to "Vmax rate".
6. To read plate click on "read plate" under the "Control" menu.

Equipment:

1. Polytron Homogenizer, Brinkmann Instruments, Inc. Westbury, NY
2. IEC B-20A centrifuge, International Equipment Company, Needham Heights, Mass
3. Uvmax microplate spectrophotometer, Molecular Devices, Sunnyvale, CA

References:

Passonneau, J.V., and O.H. Lowry, 1993. In: Enzymatic Analysis: A Practical Guide. Humana Press, Totowa, NJ. Pg. 174-175.

Lactate Dehydrogenase Assay

Sample Preparation:

1. 100mL of stock 100mM potassium phosphate homogenization buffer is diluted to 1000mL with distilled water and the pH is adjusted to 7.5 with 10N HCL.
2. Calculate the amount of homogenization buffer to be added to each tissue sample to arrive at an initial concentration of 10:1.

$$(\text{Sample weight}) \times 9 = \text{Volume of Buffer}$$

3. Homogenize the sample in sample buffer, and centrifuge homogenate at 500 X G of 10 minutes at 5°C.
4. Following centrifugation, remove 1mL of supernatant and bring volume to 50mL with homogenization buffer to make a 500:1 dilution. Take 1mL of 500:1 dilution and add 1mL of homogenization buffer to create a 1000:1 dilution. Take 1mL of the 1000:1 dilution and add 15mL to make final dilution of 15000:1.

Conduction of Assay:

1. Remove one vial of NADH from kit and add 2.85mL of phosphate buffer (from kit). Cap vial and mix.
2. Add 180μL of NADH/phosphate buffer solution to each well of a microplate along with 10μL of homogenate.
3. Allow 20 minutes for native pyruvate to be metabolized.
4. Add 100μL of sodium pyruvate solution (provided in kit) to each well.
5. Read immediately at 340nm in a Uvmax microplate spectrophotometer set for negative kinetics. (Note: reading at negative kinetics will measure the disappearance of NADH).

Solutions:

1. Homogenization Buffer

Weigh 136.09g of potassium phosphate into volumetric flask, bring volume to 1000mL, and stir until dissolved. Store at 4°C.

Equipment:

1. Polytron Homogenizer, Brinkmann Instruments, Inc. Westbury, NY
2. IEC B-20A centrifuge, International Equipment Company, Needham Heights, Mass
3. Uvmax microplate spectrophotometer, Molecular Devices, Sunnyvale, CA
4. Lactate Dehydrogenase Kit, Sigma Diagnostic, St. Louis, MO

Regression Coefficients for pH Declines of Turkey Supracoracoideus Muscle

	Line					
	HM		LM		FL	
	Toms	Hens	Toms	Hens	Toms	Hens
Stim/Stim	-0.0713	-0.0759	-0.0763	-0.0628	-0.0497	-0.0907
Stim/Recov	-0.0241	-0.0294	-0.0282	-0.0165	-0.0164	-0.0247
Nonstim/Stim	-0.0809	-0.0630	-0.0282	-0.0521	-0.0431	-0.0667
Nonstim/Recov	-0.0286	-0.0181	-0.0244	-0.0233	-0.0209	-0.0262

HM=heavyweight male line; LM=lightweight male line; FL=female line

CURRICULUM VITAE'

John Kenneth Yost

Parents	Kenneth E. Yost Carolyn S. Yost
Birthplace	Fairmont, West Virginia
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Marriage	Jessica L. Harbert (June 12, 1999)
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