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Divergent Selection for Four-Day Relative Breast Yield and the Effect of Thermal Manipulations on Growth Characteristics on Lines Selected for Different Processing Dates.

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Divergent Selection for Four-Day Relative Breast Yield and the Effect of Thermal Manipulations on Growth Characteristics on Lines Selected for Different Processing Dates.

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by

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ABSTRACT

Genetic selections currently utilized in the poultry industry have been highly successful in promoting broiler growth rate and yield. However, these selections may have come at the expense of broiler physiology, as evidenced by an increase in physiological disorders. To address this experimentation was undergone to develop a novel method of selection to promote growth with minimal physiological impact and to assess how a new embryonic technique affects growth in broiler lines exhibiting different growth rates.

In the first experiment, a random bred control (RAN) broiler line was divergently selected for four-day relative breast yield (BY4) resulting in the high (HBY4) and low (LBY4) BY4 lines. Selection resulted in clear divergence in BY4 of the HBY4 and LBY4 lines over four generations. This was made possible by the high heritabilities calculated for breast weight relative to live weight in both the HBY4 and LBY4 lines. Furthermore, BY4 selections were capable of impacting embryonic development.

In the second experiment, multiple hatches were obtained from selected generation three broiler breeders from the HBY4, LBY4 and unselected RAN lines. Hatches were used to examine correlated responses to selection during a traditional growout period. Results indicate that selection for BY4 was capable of promoting breast percentage at processing age with the HBY4 having a greater breast yield than the LBY4 at 42 and 56 days of age. Furthermore, this change occurred at minimal expense to broiler fitness and with no impact on broiler meat quality at processing age.

In the third experiment, the effects of thermal manipulation (TM) on broiler embryos from two broiler strains expressing differing rates of growth. TM was effective in promoting early growth in both strains compared to their control counterparts, but post Day 35 no difference in body weight was shown between TM and CTRL strains. However, at Day 42 differences in breast percentage between TM and CTRL strains approached significance with TM strains having a greater breast percentage than their CTRL counterparts at 60 days of age. The improved body composition was not accompanied by changes in meat quality parameters between CTRL and TM broilers. Results indicate that TM can be used to improve body composition without impacting meat quality.

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DEDICATION

This dissertation is dedicated to my ex fiancé Anne-Ashley Black. Her betrayal in a key time for me motivated me rather than destroying me. I wish her no ill will, and a truly melancholy life.

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List of Abbreviations

ADG – Average Daily Gain

BPBC – Broiler Primary Breeder Company

BY4 – Four-Day Relative Breast Yield

CO_2 – Carbon Dioxide

DFD – Dry, Firm, Dark

E# - Day of Embryonic Development

FCR – Feed Conversion Ratio

GGP – Great Grandparent

HBY4 – High Four-Day Relative Breast Yield

LBY4 – Low Four-Day Relative Breast Yield

PSE – Pale, Soft, Exhudative

RAN – Unselected Random Bred Control

RH – Relative Humidity

SDS – Sudden Death Syndrome

TM – Thermal Manipulation

WHC – Water Holding Capacity

INTRODUCTION

The purpose of this research was to develop and evaluate novel methods of selection intended to promote broiler growth, while minimalizing potential negative impacts on broiler physiology and meat quality. To that end this dissertation covers the development of the four-day relative breast yield (BY4) lines, the evaluation of correlated response(s) to BY4 both embryonically and posthatch, as well as the evaluation of thermal manipulation on lines exhibiting different growth rates. These topics were chosen for inclusion as both techniques represent novel methods of growth promotion and focus on impacting early growth leading to improved broiler performance throughout growout.

1. Literature Review

The purpose of this research is to develop and evaluate novel methods of selection intended to promote broiler growth, while minimalizing potential negative impacts on broiler physiology and meat quality. To that end this dissertation covers the development of the four-day relative breast yield (BY4) lines, the evaluation of correlated response(s) to divergent selection for BY4 both embryonically and posthatch, as well as the evaluation of thermal manipulation on lines exhibiting different growth rates. These topics were chosen for inclusion as both techniques represent novel methods of growth promotion and focus on impacting early growth leading to improved broiler performance throughout growout.

Industry

The poultry industry has applied several basic genetic principles to increase the pace of genetic advancements (Anthony, 1998). These principles include the use of highly heritable traits, quick generational turnover, large family size, and high selection pressure (Anthony, 1998). Increased production became necessary as the development of value added products resulted in increased demand for meat production. To meet these demands, poultry geneticists focused on creating a chicken that would grow quickly and efficiently. Growth rate was the first trait identified for selection, as not only is it economically important; it is also highly heritable and can be improved quickly via selection (Emmerson, 1997). Additional production traits also chosen for selection include breast yield and feed conversion ratio. These traits maximize the profitability of the broiler, by increasing the yield of the breast offering maximum return while decreasing the amount of input (feed) needed for growth.

Currently in the United States, three companies dominate the broiler poultry breeding industry. These companies, known as Broiler Primary Breeder Companies (BPBC), are Cobb-Vantress, Aviagen, and Hubbard. Depending on the company size and philosophy, a BPBC may maintain between six to 30 resource populations (Pollock, 1999). These companies operate in a pyramidal structure (Emmerson, 1997), allowing for a small number of pedigree individuals (0.4 million) to be multiplied into an eventual 400,000 million broilers (Pollock, 1999). Selection and any permanent genetic advances occur at the pedigree level.

Separate male and female lines comprise the pedigree structure. Male lines are selected for multiple highly heritable traits including age to market weight, body weight at a given age, yield and feed conversion (Anthony, 1998). Female lines are primarily selected for reproductive traits, such as egg production, fertility and hatchability, and secondarily selection for growth and confirmation (Anthony, 1998). The top individuals from the pedigree populations are selected for breeding the next pedigree generation while the remaining pedigree individuals are relegated to the Great Grandparent (GGP) population, as shown in figure 1. Little selection occurs past the pedigree level and broiler breeders below the pedigree level (GGP, GP, P) are tasked with reproducing to obtain the projected number of broilers for slaughter.

Growth Selections

Growth selections currently practiced by the poultry industry today rely mainly on postnatal growth, or an increase of muscle mass resulting from increased muscle fiber size through hypertrophy (Rehfeldt et al., 2004). Hooper et al. (1973) also found that selection for increased body weight results in similar changes in muscle mass. Muscle mass is dependent upon three characteristics: muscle fiber size and number and to a lesser degree the extracellular matrix (Brown and Stickland, 1994). While selection for growth rate and yield remain economically viable, it has been suggested that muscle fiber size may hit a biological limit, at which metabolic processes may be compromised (Mahon, 1999). Concurrent with selection for increased growth rate and yield there has been a rise in physiological disorders. In broilers, these disorders are often related to increased metabolism and rapid growth rate resulting in failure of body systems due to increased demand being placed upon these body systems (Julian, 2005). Common examples of these disorders include ascites, (Julian, 1987; Wideman et al., 1996, 1997; Pavlidis et al., 2007) sudden death syndrome (SDS) (Jackson et al., 1972; Volk et al., 1974; Julian, 1996) and tibial dyschondroplasia (Farquharson and Jefferies, 2000). For a review see Julian, 2005.

Two more recent muscle abnormalities are of particular interest to current research. White striping, described as white striations running parallel to muscle fibers in the pectoralis major and first described by Bauermeister and colleagues (2009) and wooden breast, described as a pale area of the pectoralis major with increased firmness in comparison to unaffected breast tissue (Sihvo et al., 2013). Both

conditions are seen in heavier weight broiler lines selected for increased breast percentage and are thought to arise from a growth rate exceeding physiological sustainability as evidenced by increased myodegeneration and muscle damage (Wilson, 1990; Mahon, 1999). These abnormalities have been observed separately or in unison in modern broilers (Mudalal et al., 2014).

Regardless of whether seen separately or together in breast tissue, both conditions are seen as defects and subsequently downgraded. Downgrading relegates the meat to further processing, which negates appearance issues but it has been shown that white striping (Petracci et al., 2013) and wooden breast both result in decreased meat quality (Mudalal et al., 2014). Notably meat expressing one or both of these defects exhibit decreased water retention, and poor cooking and processing yields further impacting broiler production profits (Mudalal et al., 2014). As both traits are viewed as arising due to increased growth rate and breast yield they provide further impetus into research exploring growth selections that rely on methods of muscle growth other than hypertrophy.

Hypertrophy is the process of muscle growth through an expansion in muscle fiber size. Muscle fiber hypertrophy occurs during the postnatal period and is made possible by the proliferation and incorporation of satellite cell nuclei (Rehfeldt et al., 2004). Satellite cell proliferation is of particular importance as fully differentiated muscle fibers do not divide, and muscle growth relies on a balance between precursor cell proliferation and differentiation into myocytes and muscle fiber (Brand-Saberi and Christ, 1999).

In general, for a muscle to grow, it must continually accumulate additional nuclei, which will support the accretion of more muscle protein (Halevy et al., 2006). Muscle growth is based on the concept of nuclear domain size (cytoplasmic volume per nucleus), in which a single myonucleus in a multinucleated myofiber governs the surrounding cytoplasm (Mozdziak et al., 1997). The additional nuclei needed for increases in muscle fiber diameter and length come from satellite cells (Goldspink, 2004). The use of satellite cells in adult muscle growth explains why satellite cells may also be referred to as “adult myoblasts”.

Hyperplasia is the growth of muscle via an increase in muscle cell number. Muscle fiber hyperplasia occurs almost entirely during gestation, but has been evidenced to occur after birth in some species

(rodents, swine, chickens) (Rehfeldt et al., 2004). Hyperplasia is crucial to postnatal growth, as there appears to be a physiological limit to the size of muscle fibers (Rehfeldt et al., 2004). Researchers have shown a negative correlation between postnatal muscle fiber hypertrophy and muscle fiber number (Rehfeldt et al., 2000). This relationship also forms the basis for lean growth, as lean growth depends on prenatal increases in muscle fiber number and the subsequent hypertrophy of the available muscle fibers (Rehfeldt et al., 2004). Lean growth is a desirable trait, as it is associated with improved meat quality. Increased muscle fiber number and moderate cell size have also shown a correlation between increased meat yield and quality (Rehfeldt et al., 2004).

In order to continue making selection progress in body weight and yield, novel methods of selection should be considered. To that end current research has focused on selection for relative breast yield at four-days posthatch and is viewed as a new opportunity to promote growth. As the time needed for a broiler to reach market weight decreases, the percentage of lifespan the bird spends in embryogenesis increased (Halevy et al., 2006). This prenatal period is also a time in which, almost all increases in muscle cell number will occur (Rehfeldt et al., 2004). It is hoped that through selection for BY4 this period may be impacted, thus utilizing this period of development to promote growth.

Muscle Development

Muscle development begins in the early stages of embryogenesis. It is during this prenatal period that muscle fiber formation occurs and is also the primary time for muscle growth via hyperplasia. The growth of these muscle fibers is most likely a maternal factor as the increase in fiber number occurs almost entirely prenatally (Rehfeldt et al., 2004). The potential for lean growth postnatally is dependent on the prenatal muscle fiber formation, as future growth in muscle via hypertrophy, or the increase in cell size, is limited by a combination of genetics, nutrition, and physiology (Rehfeldt et al., 2004). Therefore, understanding how muscle fiber formation occurs (myogenesis) is a crucial part of livestock development and growth selections.

Muscle fiber development occurs in two initial waves and then a third wave further along in prenatal development. This process is initiated by the presence of the proper mixture of promoter/depressor signaling. The first wave of myogenesis involves the formation of primary muscle fibers resulting from the

proliferation and differentiation of embryonic myoblasts (Harris et al., 1989). The second wave of myogenesis involves the proliferation and differentiation of fetal myoblasts, which fuse to form secondary muscle fibers. The sites of secondary muscle fiber formation are dependent on primary muscle fiber formation, as the primary muscle provides support for secondary fiber formation and attachment (Kelly and Zacks, 1969; Duxson and Usson, 1989). The third and final wave, which is relatively distinct from the other two waves, involves the proliferation, but not differentiation of satellite cells. These satellite cells, also called adult myoblasts are involved in muscle hypertrophy and regeneration (Mauro, 1961). All three myoblast classes play important roles in myogenesis.

Primary Muscle Fiber

As stated previously, primary muscle fibers provide a framework for secondary muscle fiber formation. The different fiber types can be identified by either histology or based on adenosine triphosphate activity, as in an example using swine (Stickland, 1995). Primary muscle fibers will react as slow twitch fibers, with secondary fibers being fast twitch (Stickland, 1995). Around these primary muscle fibers, upwards of 20 secondary muscle fibers will form (Wigmore and Stickland, 1983). In research done using swine (Wigmore and Stickland, 1983; Dwyer and Stickland, 1991), primary fiber number was shown to be relatively fixed in an animal, thus more indicative of that animals genotype for meat production (Stickland, 1995). The secondary fiber still plays a significant role in meat production as it appears to be more responsive to nutrition, but primary muscle fiber is the determining factor.

Secondary Muscle Fiber

Secondary fibers form on the surface of primary fibers (Kelly and Zacks, 1969; Duxson and Usson, 1989). The number of primary fibers formed is relatively unaffected by environmental factors, such as underfeeding. Research has established that primary fiber number is difficult to manipulate through changes in intrauterine environment (Russel and Oteruelo, 1981; Du et al., 2009). Secondary fibers, unlike primary fibers, are susceptible to many environmental factors that permanently affect secondary fibers (Dwyer et al., 1994).

In swine, variation in piglet birth weights in litters has been attributed to utero undernutrition (Wigmore and Stickland, 1983; Handel and Stickland, 1987). This variation is a product of decreased secondary muscle fiber number, caused by malnutrition. In a study done examining maternal feed intake and litter birth weights in swine, primary muscle fiber number was not affected, but secondary muscle fiber was affected. The study demonstrated that a 30 day increased feed prior to muscle fiber hyperplasia increased secondary fiber number 9 to 13% (Dwyer et al., 1994).

Myoblasts

Myoblasts, also known as muscle fibers, originate from myogenic precursor cells, which come from the mesodermal layer (Rehfeldt et al., 2004). Specifically, myoblasts are formed by mitotic division of mesodermic somites (Lawrence and Fowler, 2002). They are derived from the dermomyotome, located in the dorsal somite between the neural tube and somatopleural mesoderm, but beneath the ectoderm (Christ and Brand-Saberi, 2002). The somite forms in halves: the medial half forming as part of Henson's node, and the lateral half originating from a region in the primitive streak (Selleck and Stern, 1991).

The first fibers appear in the rostral somite, in an area referred to as the myotome (Holtzer et al., 1957; Stockdale, 1992). Research has shown that muscle development is attributable to several myogenic populations: embryonic, fetal and adult myoblasts (Cossu and Molinaro, 1987; Stockdale, 1992). It is from the embryonic myoblasts that the primary muscle fibers, which form a base for future muscle growth, of the limb bud of birds and mammals, derive (Stockdale, 1992).

Embryonic Myoblasts

Embryonic myoblasts numbers peak at Day 5 of embryonic development (E5) (Stockdale, 1992). Within this period there are three embryonic subtypes based on myosin heavy chain (MHC) (Miller and Stockdale, 1986). The two primary types express (1) a fast MHC isoform and a slow MHC1 and (2) slow MHC1 and slow MHC3 (Page et al., 1992). These embryonic myoblasts will make up the primary muscle fibers of the limb bud that first form in dorsal and ventral muscle masses (Stockdale, 1992). This process is accomplished by the fusion of myoblasts together, resulting in the formation of myotubes (Lawrence

and Fowler, 2002). These myotubes form myofibrils through the synthesis of myosin and actin (Lawrence and Fowler, 2002). Primary fibers will go on to provide the support structure for secondary muscle fibers (Wigmore and Evans, 2002).

Fetal Myoblasts

The fetal period of avian development is noted for the change in the primary myoblast type isolated in muscle-forming regions of the limb; changing from embryonic myoblasts to fetal myoblasts (Stockdale, 1992). Fetal myoblasts express several sarcomeric proteins and are capable of forming long, mutli-nucleated fibers (Stockdale, 1992). Fibers formed from fetal myoblasts express MHC classes different than those from embryonic myoblasts (Cerny and Bandman, 1986; Vivarelli et al., 1988). Fetal myoblasts also differ from embryonic myoblasts in desmin expression and surface antigens (Kaufman et al., 1991).

As secondary fibers increase in fetal muscle, fetal myoblasts become the primary myoblast isolated (Stockdale, 1992). As fetal myoblasts are the primary myoblast collected during secondary fiber formation, they are seen as the progenitor of secondary muscle fibers. Secondary myotubes arise at sites of innervation in primary myotubes (Stockdale, 1992). Once embryonic differentiation is completed no further muscle cell division occurs and future muscle growth is dependent on adult myoblasts (Goldspink, 2004).

Adult Myoblasts

Adult myoblasts were originally termed satellite cells based on their location near muscle fibers (Mauro, 1961). Adult myoblasts can be isolated in the sublaminar membrane surrounding myofibers around E16 to E18 days of development (Feldman and Stockdale, 1992; Hartley et al., 1992). Adult myoblasts differ from fetal myoblast in several ways including, but not limited to size, MHC expression and type of regenerated fibers they form in muscle (Cossu et al., 1985; Hoh and Hughes, 1988, 1991; Feldman and Stockdale, 1990, 1992; Hartley et al., 1991, 1992; Yao et al., 1992). Adult myoblasts are also the only myoblast isolated at the time of hatch (Feldman and Stockdale, 1992; Hartley et al., 1992). They are vital to postnatal growth, as they are the only source of new fiber formation and fiber growth.

Thermal Manipulations

Genetic selection for improved growth rate, meat yield and feed efficiency has reduced the number of days to processing. This coupled with advances in incubation, have led to the incubation period being an increasingly larger proportion of the broiler life cycle (Havenstein et al., 2003; Wolanksi et al., 2004). Therefore, several studies have focused on utilizing the embryonic growth period as a way to promote performance gains in postnatal growth. Before delving into how differing incubation factors may be used to promote postnatal growth, it is important to understand the basic mechanics of avian incubation.

Incubation

Chicken eggs incubate for just over 21 days. During this time, the embryo is provided all necessary nutrition in ovo and develops under the care of the hen. The hen is responsible for the turning of the eggs, as well as all environmental conditions present in the nest, such as temperature and airflow. These factors are influenced by the nest building capability of the hen, as well as the attentiveness of the hen (Huggins, 1941; Freeman and Vince, 1974).

As the poultry industry developed and grew, poultry production moved away from backyard flocks toward an industrial level of production. At this point the brooding hen was replaced by still-air incubators, followed by forced draught incubators (Molenaar et al., 2010). As the industry grew, incubation technology progressed to large multi-stage incubators, in which eggs of multiple setting dates were incubated. In the late nineties, multi-stage technology was replaced by single-stage incubators utilizing an all-in, all-out policy. This shift began when research showed that multi-stage incubators failed to meet many requirements of the embryo and thus negatively impacted hatchability (Hill, 2000). Single-stage incubators are now the industry standard, as they allow for the control of multiple environmental conditions such as temperature, relative humidity and carbon dioxide (CO_2) concentration (French, 1997; Bennett, 2010). These conditions are among some of the most important factors to consider during incubation.

Incubation Temperature

Multiple sources agree that the temperature is the number one factor to regulate in avian incubation (Freeman and Vince, 1974; Decuypere and Michels, 1992; Meijerhof, 2000, 2009). In nature, the embryo is capable of managing its body temperature by redistributing its blood flow depending on various temperatures affecting the eggshell. During artificial incubation, the embryo is largely unable to manage its temperature by redistributing blood flow as it is surrounded by air of the same temperature (Molenaar et al., 2010). Therefore, incubators must be set at 37.8 °C, which has been shown to maximize chick quality and hatchability (Barott, 1937). This temperature has been industry practice for some time as close regulation minimizes possible negative effects temperature may have on development, hatchability, chick quality and postnatal growth (Krausova and Peterka, 2007).

Egg temperatures being influenced by incubator environment have been shown to be 1.0°C to 1.5°C greater than incubation temperature (Leksrisompong et al., 2007). Given that measuring embryo temperature is difficult and often destructive, embryo temperature is usually determined by taking the egg shell temperature, which deviates no more than 0.1°C to 0.2°C from actual embryo temperature (Meijerhof and Van Beek, 1993; French, 1997). In addition to incubation temperature, embryonic heat production is a major influence on embryo temperature.

Embryonic Heat Production

Factors influencing embryonic heat production are age of the breeder flock, egg size and incubation stage (Tona et al., 2004; Lourens et al., 2006; Hamidu et al., 2007; Meijerhof, 2009). As breeder flocks age, the amount of heat production in eggs from the flock increases (O'Dea et al., 2004, Lourens et al., 2006). Egg size is also influential, as larger eggs and thus larger embryos result in increased heat production. The most important factor in heat production is incubation stage (Meijerhof, 2009). Starting around E9, embryonic heat production begins to exceed heat loss and incubator temperature has to be gradually decreased to regulate eggshell temperature (Romijn and Lokhorst, 1960; Lourens et al., 2005, 2006; Yahav et al., 2009).

Relative Humidity and CO2 Concentration

Relative humidity (RH) is a significant factor in heat transfer from the incubator to the embryo. If heat is transferring more efficiently, then it helps to minimize variation in the incubator microenvironment. Humid air is more effective for heat transfer than dry air, and the poultry industry capitalizes on RH to create a more uniform environment (Molenaar et al., 2010). A RH of 50% promotes maximum hatchability (Robertson, 1961), but a RH ranging from 40 to 70% still may be considered optimal (Lundy, 1969). In a study conducted to determine the effects of relative humidity on body weight, hatchability and associated traits, a RH of 53% resulted in the highest hatchability, and a RH of 63% resulted in increased late term mortality (E18 to E21) (Bruzual et al., 2000). A significant effect was shown in hatching body weight, but this difference was not present when birds were pulled from the machine. Researchers postulated that the increase in hatching weight was additional water weight and that RH affects hatchability, but not chick weight.

CO2 concentration increases during incubation due to the increased respiration throughout embryonic development. During early incubation embryos can survive CO2 concentrations up to 5% (Taylor & Kreutziger, 1965). During late incubation, a CO2 concentration of 5.6% encourages chicks to pip the air cell and begin hatching (Romijn and Roos, 1938; Visschedijk, 1968). These findings demonstrate that CO2 concentration is a major factor in hatchability, but how CO2 concentration affects chick quality is still unclear.

Enhancing Postnatal Development

Poultry consumption has greatly increased worldwide and is currently the world's most widely consumed source of animal protein. Obtaining the top spot in animal proteins was made by possible by advances in genetics, nutrition, environment, and rearing. The genetic gains utilized selections that occurred in the postnatal period. While postnatal growth is the primary contributor to a broiler's growth curve, the incubation period represents a significant portion of a broilers life. By controlling different environmental variables during incubation, postnatal growth may be positively or negatively impacted by the prenatal environment. This was the initial theory behind the introduction of thermal manipulations (TM) during incubation.

Thermotolerance

A negative byproduct of increased growth rate is that many broilers exhibit poor heat tolerance. Birds are homeotherms and must maintain their body temperature in a narrow window. Increasing or failing to meet this window may result in a potentially fatal cascade of thermoregulatory events. It was theorized that by increasing incubation temperature to a certain degree and manipulating the time period of increased incubation temperature, a long-lasting epigenetic change via an alteration in the hypothalamic threshold would occur (Uni and Yahav, 2010). Improved thermotolerance would reduce heat-related morbidity and mortality.

While the optimum combination of temperature, and time duration has yet to be determined, it has been shown that a short TM is all that is necessary to improve thermotolerance (Holland et al., 1997). While TMs of greater duration may negatively affect embryo morphology (Kaplan et al., 1978). The goal is to find the right period in which to influence the development of the hypothalamus-hypophysis-thyroid axis (HHTA), and the hypothalamus-hypophysis-adrenal axis (HHAA) (Uni and Yahav, 2010). TMs were tested on E8 and E12 based on the development, maturation and function of HHTA and HHAA (Uni and Yahav, 2010). Multiple studies have been conducted at various ages and temperatures throughout this period (E8 to E18) (Yahav et al., 2004a;b). TMs during mid (E8 to E10) and late embryogenesis (E16 to E18) failed to improve long term thermotolerance (Collin et al., 2007; Tona et al., 2008). Testing of TMs at 39.5º C and 65% relative humidity from Day 7 to Day 16 of embryogenesis for 12 hours per day, showed improved thermotolerance without negatively impacting the growth performance of caged broilers (Loyau et al., 2013). Improvement in thermotolerance was demonstrated by lower body temperature prior to slaughter (Piestun et al., 2008; 2009).

Growth Rate

Postnatal muscle growth typically involves the formation of additional nuclei to increase the buildup of muscle protein (Halevy et al., 2006). This is for two reasons: (1) differentiated muscle cells do not undergo cell division and (2) nuclear domain size, where a myonucleus in a myofiber governs a specific area of cytoplasm (Mozdziak et al., 1997). TMs from E16-E18 are believed to promote growth as they are targeting a critical period in satellite cell population expansion (Hartley et al., 1992). TMs for

three hours during this time at either 38.5°C or 39.5°C increased breast yield and body weight up to 42 days of age (Halevy et al., 2006; Collin et al., 2007).

To verify that this promoter effect held true throughout even an extended grow-out period, Piestun et al. (2013) conducted a study. Among the variables examined were body weight, feed intake, feed conversion ratio (FCR), breast muscle yield and abdominal fat (Piestun et al., 2013). Piestun et al. (2013) utilized a TM of 39.5°C and a 65% RH for 12 h/d from E7 to E16, incubation past this point and hatching occurred under standard incubation conditions.

The results of this study may prove to be influential. The TM populations had a decreased hatch window time but showed a three percent decrease in hatchability (86% < 89%) (Piestun et al., 2013). In males, there was no significant difference in body weight at hatch or during the growth period between TM and control population males, but feed intake was lower in the TM males (Piestun et al., 2013). TM females exhibited a lower weight at hatch and growout, but had a 0.1 improvement in FCR due to having a lower feed intake (Piestun et al., 2013). TM males also had a lower feed intake and a 0.08 decrease in FCR (Piestun et al., 2013). Decreased feed intake and improved FCR are of high economic value to the poultry industry. Additionally, researchers observed a significant increase in percent breast yield in TM populations regardless of sex, decreased fat pad in TM males compared to control males and no significant effect on fat pad in females (Piestun et al., 2013).

The increased breast yield is likely an effect of muscle cell hypertrophy, due to increased satellite cell proliferation. This theory is supported by a previous study that showed higher levels of hypertrophy in broilers subjected to TMs in ovo (Piestun et al., 2011). These benefits are of major economic benefit. A 1% increase in breast yield amounts to at least 39 additional grams of breast yield, worth $1.07 billion (Piestun et al., 2013). While a 0.1 improvement in FCR in an 8.6 billion broiler population would save roughly 225 grams of feed per bird, thus $553 million in savings (Piestun et al., 2013). Selections to improve hatchability at 39.5°C would allow TMs to be used efficiently, resulting in major economic benefit to the industry.

Muscle Fiber Number

Muscle mass is a combination of muscle fiber number and muscle fiber size (Stickland and Dwyer, 1996). Of these two, muscle fiber number is the primary determinant of muscle mass (Miller et al., 1975). The number of fibers varies less than muscle fiber size, as factors such as exercise and nutrition may affect muscle fiber size, but will not influence fiber number (Stickland et al., 1975; Goldspink and Ward, 1979). Fiber number is unaffected as it determined prior to hatch and is thought to be indicative of an animal's future performance. In addition to being a decisive factor in post-natal skeletal muscle growth, fiber number has a significant effect on the animal's meat quality. Research focused on increasing hyperplasia, may uncover ways to improve both yield and quality.

Swine studies show that the muscle growth potential of an individual is largely determined by total fiber number, which is essentially fixed at birth (Stickland and Handel, 1986; Rehfeldt et al., 1987, 2000). This limit to growth potential is due to an inverse relationship between muscle fiber number and the growth of muscle fibers. Hypertrophy occurs faster when muscle fiber number is lower, than when fiber number is higher (Rehfeldt et al., 2000). This relationship has been observed in multiple species (Staun, 1972; Osterc, 1974; Locniskar et al., 1980; Fiedler et al., 1997; Larzul et al., 1997). While fiber number is a primary factor in muscle mass, hypertrophy can be promoted by selection (Rehfeldt et al., 2004) and through TM due to decreased body temperatures exhibited by TM broilers (Piestun et al., 2008; 2009), and thus lower maintenance energy requirements.

Growth Selections and Fiber Characteristics

Poultry selection has targeted total breast yield (pectoralis and supracoracoideus) as these muscles are of the highest economic importance to poultry producers (Velleman and McFarland, 2014). While selection has mainly targeted muscle growth via hyperplasia, selection for growth has resulted in faster-growing strains that exhibit higher numbers of muscle fibers than other strains of the same species (Ezekwe and Martin, 1975; Miller et al., 1975). In addition faster-growing lines, for example, geese selected for meat yield, show increased muscle fiber size compared to birds selected for egg production (Klosowska et al., 1993). This trend holds true for other avian species, i.e. meat-type chicken fiber size in comparison to layer chickens.

Growth Characteristics

Considerable effort has been put into studying the effect of muscle fiber size and number on growth and other correlated responses. In swine, piglet weight and its relationship to growth and muscle fiber characteristics have been of particular interest. Previous research has shown that smaller piglets form a lower number of muscle fibers during prenatal development as compared to heavier littermates (Wigmore and Stickland, 1983; Handel and Stickland, 1987; Gondret et al., 2006). Low weight (LW) piglets exhibit higher percentages of internal organs, bones and skin, and a smaller percentage of muscle tissue when compared to heavy weight (HW) piglets (Rehfeldt and Kuhn, 2006). Extending this trend into poultry means LW chicks would produce proportionally more offal and yield proportionally less meat.

Lower numbers of muscle fibers results in decreased performance in various economic traits associated with animal production such as yield or fat percentage. LW pigs have lower lean meat content, higher fat percentage, and an increased FCR as compared to the average or heavier pigs (Rehfeldt and Kuhn, 2006). Faster-growing strains of pigs have an improved FCR with a decreased fat percentage when compared to slower-growing strains (Campbell and Taverner, 1988). Low muscle fiber number has been cited as the cause, since it limits postnatal growth and resulting lean growth (Rehfeldt and Kuhn, 2006). With nutritional energy no longer necessary for increased lean growth, it is funneled into fat accretion (Rehfeldt et al., 2008). These small piglets will require more time to reach market weight than larger littermates (Wolter et al., 2002; Le Cozler et al., 2004).

This trend has also been shown in poultry research comparing high and normal breast yield broilers (HBY and NBY) to leghorn chickens. Both the HBY and NBY broilers showed a greater muscle fiber number than the leghorn chickens (Scheuermann et al., 2004). Furthermore, muscle fiber number had positive phenotypic correlations with body weight, breast weight and percentage breast relative to live weight, 0.58, 0.58 and 0.69, respectively (Scheuermann et al., 2004). Thus emphasizing the contribution of muscle fiber number to broiler growth and its potential commercial importance (Scheuermann et al., 2004). Therefore, decreased muscle fiber number hurts producers by increasing the duration and cost of production. While increased muscle fiber number may decrease the duration and cost of production.

Meat Quality

Growth selection in swine and other livestock species have resulted in improvements in average-daily-gain (ADG), FCR, and lean meat content. Unfortunately, these improvements have adversely affected stress response and meat quality (Fiedler et al., 2004). In order to combat the downturn in meat quality while continuing to promote growth, it is important to consider variables that are positively related to both growth and meat quality (Lengerken et al., 1994; Ender et al., 2000). Muscle fiber number is a trait that has been described previously in regards to morphological and physiological properties that affect muscle quality (Swatland, 1982,1992; Wicke et al., 1998; Fiedler et al., 1999). Furthermore, the phenotypic relationship between muscle fiber number, muscle growth, stress and meat quality have been noted in several studies (Fiedler et al., 1993, 2003; Lengerken et al., 1994; Karlsson et al., 1999; Suzuki et al., 2003).

LW piglets have a lower number of muscle fibers when compared to heavier littermates (Wigmore and Stickland, 1983; Rehfeldt and Kuhn, 2006). At market weight, pigs identified as LW piglets, exhibit poor carcass quality; increased fat deposition, and decreased lean meat content (Hegarty and Allen, 1978; Powell and Aberle, 1980,1981; Kuhn et al., 2002; Bee, 2004; Gondret et al., 2006; Rehfeldt and Kuhn, 2006). Meat quality is also negatively impacted by low birth weight as both tenderness and water holding capacity are negatively affected (Gondret et al., 2005; 2006, Rehfeldt and Kuhn, 2006).

LW pigs are thought to exhibit poor meat quality due to increased fiber hypertrophy and abnormal fiber hypertrophy (giant fibers), which are correlated with reduced pork quality (Fiedler et al., 2004). Two generations of selection for increased muscle fiber size, resulted in a clear increase in muscle fiber size, decreased fiber number and resulted in decreased meat quality in swine (Fiedler, 1988).

Commercial poultry strains selected for growth have also shown increased meat quality issues, notably deep pectoral myopathy (green muscle) (Wilson et al., 1990; Sosnicki and Wilson, 1991), white stripping (Bauermeister et al., 2009; Kuttappan et al., 2012), and Pale, Soft, Exudative (PSE) and Dry, Firm, Dark (DFD). PSE is characterized by pale, soft almost gel-like meat, low pH with a poor WHC. DFD in contrast is dark in color, firm feel and increased pH (Allen et al., 1997, 1998; Wulf et al., 2002). Pectoralis tissue expressing white stripping has been shown to possess variable fiber size, mononuclear

cell proliferation, fibrosis, increased fat content and reduced protein content (Kuttapan et al., 2013). Traits similar to those seen in swine exhibiting low fiber number and reduced meat quality. These issues become more severe as poultry develop, as evidenced by turkey research showing decreased endomysial and perimysial (sources of blood flow and innervation, respectively) spacing at 8 weeks of age, leading to muscle degeneration and fragmentation by 20 weeks of age (Velleman et al., 2003).

Selecting for Muscle Growth and Meat Quality

In a study conducted by Fiedler et al. (2004) the concept of utilizing fiber characteristics was examined. The study was a simulated selection study utilizing roughly 2,000 swine on which data was collected for three years. From this data, a heritability of 0.22 was calculated for body weight and 0.2 for fiber number (Fiedler et al., 2004). Selection for live weight alone with 10 or 50% selection intensity provided a gain of 3.69 or 1.73 kg, respectively. These selections had a slight negative effect on muscle quality; given multiple selections over time a significant negative effect may be expected. A variant of the live weight selection criteria that selected for increased live weight but also including the additional characteristics of fiber number, white fiber and giant fiber frequency, still promoted growth while improving meat quality (1.8, 1.05); (Fiedler et al., 2004). The inclusion of other meat quality characteristics into a selection criterion along with growth selections showed similar results (Fiedler et al., 2004).

While growth is still a major selection factor in the animal industry, the importance of muscle quality has increased. If meat quality criteria can be added to growth selections, an opportunity exists to improve meat quality at minimal cost to growth. Furthermore, since increased muscle fiber number is associated with improvements in ADG and FCR, there is potential to decrease costs of production.

Muscle fiber number is a major determinant of body weight, skeletal muscle growth and muscle quality. Finding ways to promote fiber number and fiber characteristics may prove to be beneficial to not only the poultry industry but also the animal industry as a whole. Continued work designed to manipulate fiber number offers the opportunity to reduce both the cost and duration of animal production while continuing to promote growth and meat quality. Proper introduction of these techniques into the poultry industry should result in improved FCR, increased growth and maintenance of optimal meat quality.

Improvements in these categories will allow the poultry industry to continue low cost, efficient production and maintain its place as a primary supplier of animal protein worldwide.

Works Cited

Allen, C. D., S. M. Russell, and D. L. Fletcher. 1997. The relationship of broiler breast meat color and pH to shelf-life and odor development. Poult. Sci. 76:1042-1046.

Allen, C. D., D. L. Fletcher, J. K. Northcutt, and S. M. Russell. 1998. The relationship of broiler breast color to meat quality and shelf life. Poult. Sci. 77:361-366.

Anthony, N. B. 1998. A review of genetic practices in poultry: efforts to improve meat quality. J. Muscle Foods. 9:25-33.

Bauermeister, L. J., A. U. Morey, E. T. Moran, M. Singh, C. M. Owens, and S. R. McKee. 2009. Occurrence of white striping in chicken breast fillets in relation to broiler size. Poult. Sci 88, no. Suppl 1:33.

Barott, H. G. 1937. Effects of temperature, humidity, and other factors on hatch of eggs and on energy metabolism of chick embryos. USDA Tech. Bulletin. No. 553.

Bee, G. 2004. Effect of early gestation feeding, birth weight, and gender of progeny on muscle fiber characteristics of pigs at slaughter. J. Anim. Sci. 82:826-836.

Bennett, B. 2010. The advantages of single stage versus multi stage incubation. Intl. Hatchery Practice 24:7-9.

Brand-Saberi, B., and B. Christ. 1999. Genetic and epigenetic control of muscle development in vertebrates. Cell Tissue Res. 296:199-212.

Brown, S. C., and N. C. Stickland. 1994. Muscle at birth in mice selected for large and small body size. J. Anat. 184:371-380.

Bruzual, J .J., S. D. Peak, J. Brake, and E. D. Peebles. 2000. Effects of relative humidity during incubation on hatchability and body weight of broiler chicks from young breeder flocks. Poult. Sci. 79:827-830.

Campbell, R. G., and M. R. Taverner. 1988. Genotype and sex effects on the relationship between energy intake and protein deposition in growing pigs. J. Anim. Sci. 66:676-686.

Cerny, L. C., and E. R., Bandman. 1986. Contractile activity is required for the expression of neonatal myosin heavy chain in embryonic chick pectoral muscle cultures. J. Cell. Biol. 103:2153-2161.

Christ, B., and B. Brand-Saberi. 2002. Limb muscle development. Int. J. Dev. Biol. 46:905-914.

Collin, A., C. Berri, S. Tesseraud, F. Requena, S. Cassy, S. Crochet, M. J. Duclos, N. Rideau, K. Tona, J. Buyse, V. Bruggerman, E. Decuypere, M. Picard, and S. Yahav. 2007. Effects of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poult. Sci. 86:795-800.

Cossu, G., P. Cicinelli, C. Fieri, M. Coletta, and M. Molinaro. 1985. Emergence of TPA-resistant ‘satellite’ cells during muscle hitogenesis of human limb. Exp. Cell. Res. 160:403-411.

Cossu, G., and M. Molinaro. 1987. Cell heterogeneity in the myogenic lineage. Current Topics in Dev. Biol. 23:185-208.

Decuypere, E., and H. Michels. 1992. Incubation temperature as a management tool: a review. World’s Poult. Sci. J. 48:28-38.

Du, M., J. Tong, J. Zhao, K. R. Underwood, M. Zhu, S. P. Ford, and P. W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci 88: Epub.

Duxson, M. J., and Y. Usson. 1989. Cellular insertion of primary and secondary myotubes in embryonic rat muscles. Development 107:243-251.

Dwyer, C. M., and N. C. Stickland. 1991. Sources of variation in myofiber number within and between litters of pigs. Anim. Prod. 52:527–533.

Dwyer, C. M., N. C. Stickland, and J. M. Fletcher. 1994. The influence of maternal nutrition on muscle fiber number development in the porcine fetus and on subsequent postnatal growth. J. Anim. Sci. 72:911-917.

Emmerson, D. A. 1997. Commercial approaches to genetic selection for growth and feed conversion in domestic poultry. Poult. Sci. 76:1121-1125.

Ender, K., M. Wicke, I. Fiedler, J. Wegner and K. Nürnberg. 2000. Biological bases of muscle meat quality. Daily report "Quality of feed and animal primary products," 17-18. Nov, Halle (Saale), pp. 2-9.

Ezekwe, M. O., and R. J. Martin. 1975. Cellular characteristics of skeletal muscles in selected strains of pigs and mice and the unselected controls. Growth 39:95-106.

Farquharson, C., and D. Jefferies. 2000. Chondrocytes and longitudinal bone growth: the development of tibial dyschondroplasia. Poult. Sci. 79:994-1004.

Feldman, J. L., and F. E. Stockdale. 1990. Skeletal muscle satellite cell diversity: Satellite cells form fibers of different types in cell culture. Dev. Biol. 143:320-334.

Feldman, J. L., and F. E. Stockdale. 1992. Temporal appearance of satellite cells during myogenesis. Dev. Biol. 153:217-226.

Fiedler, I. 1988. Leistungsfruherkerming von fleischatstz und fleischbeschaffenheit durch muskelfasermerkmate. Tag-ber. Akad. Landwirtsch. Wiss. Berlin. 268:187-196.

Fiedler, I., G. Dietl, C. Rehfeldt, J. Wegner, and K. Ender. 2004. Muscle fibre traits as additional selection criteria for muscle growth and meat quality in pigs – results of a simulated selection. J. Anim. Breed. Genet. 121:331-344.

Fiedler, I., K. Ender, M. Wicke, and G. V. Lengerken. 1993. Zusammenhänge zwischen der mikrostruktur des muskelgewebes bei schweinen der landrasse und ihrer stressempfindlichkeit. Arch. Tierz. 36:525-538.

Fiedler, I., K. Ender, M. Wicke, S. Maak, and G. V. Lengerken. 1999. Structural and functional characteristics of muscle fibres in pigs with different malignant hyperthermia susceptibility (MHS) and different meat quality. Meat Sci. 53:9-15.

Fiedler, I., K. Nürnberg, T. Harge, G. Nürnberg, and K. Ender. 2003. Phenotypic variations of muscle fiber and intramuscular traits in Longissimus muscle of F2 population Duroc x Berlin Miniature Pig and relationships to meat quality. Meat Sci. 63:131-139.

Fiedler, I., C. Rehfeldt, G. Dietl, and K. Ender. 1997. Phenotypic and genetic parameters of muscle fiber number and size. J. Anim. Sci. 75:S165.

Freeman, B. M., and M. A. Vince. 1974. Incubation requirements. In: Freeman, B. M., M. A. Vince (Ed) *Development of the avian embryo*. Chapman and Hall, London, UK.

French, N. A. 1997. Modeling incubation temperature:The effects of incubator design, embryonic development, and egg size. Poult. Sci. 76:124-133.

Goldspink, G. 2004. “Local and systemic regulation of muscle growth” *Muscle Development of Livestock Animals*. Eds. M.F.W. tePas, M.E. Everts and H.P. Haagsman. CAB International.

Goldspink, G., and P. Ward. 1979. Changes in rodent fiber types during post-natal growth, undernutrition and exercise. J. Physiol. 296:453-469.

Gondret, F., L. Lefaucher, H. Juin, I. Louveau, and B. Lebret. 2006. Low birth weight is associated with enlarged muscle fiber area and impaired meat tenderness of the longissimus muscle in pigs. J. Anim. Sci. 84:93-103.

Gondret, F., L. Lefaucher, L. Louveau, B. Lebret, X. Pichodo, and Y. Le Cozler. 2005. Influence of piglet birth weight on postnatal growth performance, tissue lipogenic capacity and muscle histological traits at market weight. Livestock Prod. Sci. 93:137-146.

Halevy, O., S. Yahav, and I. Rozenbaum. 2006. Enhancement of meat production by environmental manipulations in embryo and young broilers. World's Poult. Sci. J. 62:485-497.

Hamidu, J. A., G. M. Fasenko, J. J. R. Feddes, E. E. O'Dea, C. A. Ouellette, M. J. Wineland, and V.J. Christensen. 2007. The effect of broiler breeder genetic strain and parent flock age on eggshell conductance and embryonic metabolism. Poult. Sci. 86:2420-2432.

Handel, S. E., and N. C. Stickland. 1987. Muscle cellularity and birth weight. Anim. Prod. 44:311-317.

Harris, A.J., M.J. Duxson, R.B. Fitzsimmons, and F. Rieger. 1989. Myonuclear birthdates distinguishes the origins of primary and secondary myotubes in embryonic skeletal muscles. Development 107:771-784.

Hartley, R. S., E. Bandman, and Z. Yablonka-Reuveni. 1991. Myoblasts from fetal and adult skeletal muscle regulate myosin expression differently. Dev. Biol. 148:249-260.

Hartley, R. S., E. Bandman, and Z. Yablonka-Reuveni. 1992. Skeletal muscle satellite cells appear during late chicken embryogenesis. Dev. Biol. 153:206-216.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1509-1518.

Hegarty, P. V., and C. E. Allen. 1978. Effect of pre-natal runting on the post-natal development of skeletal muscles in swine and rats. J. Anim. Sci. 46:1634-1640.

Hill, D. 2000. Embryo temperatures in multi-stage incubation. Avian and Poult. Biol. Rev. 8:168.

Hoh, J. F. Y., and S. Hughes. 1988. Myogenic and neurogenic regulation of myosin gene expression in cat jaw-closing muscles regenerating in fast and slow limb muscle beds. J. Muscle Res. Cell. Motil. 9:59-72.

Hoh, J. F. Y. and S. Hughes. 1991. Expression of superfast myosin in aneural regenerates of cat jaw muscle. Muscle Nerve 14:316-325.

Holland, S., M. Nichelmann, and J. Höchel. 1997. Development in heat loss mechanisms in avian embryos. Verh. Dtsch. Zool. Ges. 90:105.

Holtzer, H., J. M. Marshall, and H. Finck. 1957. An analysis of myogenesis by the use of fluorescent antimyosin. J. Biophys. Biochem. Cytol. 3:705-729.

Hooper, A. C., I. Bryne, and J. C. McCarthy. 1973. The effects of selection for body weight on muscle structure in mice. J. Anat. 115:146.

Huggins, A. R. 1941. Egg temperatures of wild birds under natural conditions. Ecology 2:148-157.

Jackson, C. A. V., D. J. Kingston, and L. A. Hemsley. 1972. A total mortality study of nine batches of broiler chickens. Austr. Vet. J. 48:481-487.

Julian, R. J. 1987. The effect of increased sodium in the drinking water on right ventricular hypertrophy, right ventricular failure and ascites in broiler chickens. Avian Path. 16:61-71.

Julian, R.J. 1996. “Cardiovascular disease” *Poultry Diseases*. Eds. F. T. W. Jordan and M. Pattison. W. B. Saunders Company Ltd., London, pp. 343-374.

Julian, R. J. 2005. Production and growth related disorders and other metabolic diseases of poultry–a review. Vet. J. 16:350-369.

Kaplan, S., G. L. Kolesari, and J. P. Bahr. 1978. Temperature dynamics of the fertile chicken eggs. Am. J. Physiol. 234:183-187.

Karlsson, A. H., R. E. Klont, and X. Fernandez. 1999. Skeletal muscle fibres as factors for pork quality. Livestock Prod. Sci. 60:255-270.

Kaufman, S. J., M. George-Weinstein, and R. F. Foster. 1991. In vitro development of precursor cells in the myogenic lineage. Dev. Biol. 146:228-238.

Kelly, A. M., and S. I. Zacks. 1969. The histogenesis of rat intercostal muscles. J. Cell. Biol. 42:135-153.

Klosowska, D. B., A. Rosinski, and G. Elminowska-Wenda. 1993. Microstructural characteristics of the pectoralis muscle of white Italian geese. Pages 144-148 in: Proceedings of the XI European Symposium on the Quality of Poultry Meat. Vol 1. Tours, France.

Krausova, T., and M. Peterka. 2007. Teratogenic and lethal effects of 2-24 h hyperthermia episodes on chick embryos. J. Therm. Biol. 32:193-203.

Kuhn, G., C. Rehfeldt, M. Hartung, and K. Ender. 2002. Heavy newborn piglets develop a high carcass quality. Fleischwirtschaft. 82:128-129.

Kuttappan, V. A., V. B. Brewer, J. K. Apple, P. W. Waldroup, and C. M. Owens. 2012. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult. Sci. 91:2677-2685.

Kuttappan, V. A., H. L. Shivaprasad, D. P. Shaw, B. A. Valentine, B. M. Hargis, F. D. Clark, S. R. McKee, and C. M. Owens. 2013. Pathological changes associated with white striping in broiler breast muscles." Poult. Sci. 92:331-338.

Larzul, C., Lefaucheur, L., Ecolan, P., Gogue, J., Talmant, A., Sellier, P., and G. Monin. 1997. Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in large white pigs. J. Anim. Sci. 75:3126-3137.

Lawrence, T. L. J., and V. R. Fowler. 2002. Growth of farm animals. CAB INTL. LONDON.

Le Cozler, Y., X. Pichodo, H. Roy, C. Guyomarc’h, H. Pellois, N. Quiniou, I. Louveau, B. Lebret, L. Lefaucher, and F. Gondret. 2004. Influence du poids individual et de la taille de la portée á la naissance sur la survie du porcelet, ses performances de croissance et d’abattage et la qualité de la viande. J. Rech. Porcin. 36:443-450.

Leksrisompong, N., H. Romero-Sanchez, P. W. Plumstead, K. E. Brannan, and J. Brake. 2007. Broiler incubation. 1. Effect of elevated temperature during late incubation on body weight and organs of chicks. Poult. Sci. 86:2685-2691.

Lengerken, G. V., S. Maak, M. Wicke, I. Fiedler, and K. Ender. 1994. Suitability of structural and functional traits of skeletal muscle for genetic improvement of meat quality in pigs. Arch. Tierz. 37:133-143.

Locniskar, F., A. Holcman, and F. Zagozen. 1980. Muscle fiber investigations in poultry. Zb. Biotehniske Fa. Univ. v. Ljubliani Kmetistvo. 35:7-24.

Lourens, A., R. Molenaar, H. Van den Brand , M. J. W. Heetkamp, R. Miejerhof, and B. Kemp. 2006. Effect of egg size on heat production and the transition of energy from egg to hatchling. Poult. Sci. 85:770-776.

Lourens, A., H. Van den Brand, R. Meijerhof, and B. Kemp. 2005. Effect of eggshell temperature during incubation on embryo development, hatchability, and posthatch development. Poult. Sci. 84:914-920.

Loyau, T., C. Berri, L. Bedrani, S. Métayer-Coustard, C. Praud, M. J. Duclos, S. Tesseruad, N. Rideau, N. Everaert, S. Yahav, S. Mignon-Grasteua and A. Collin. 2013. Thermal manipulations of the embryo modifies the physiology and body composition of broiler chickens reared in floor pens without affecting breast meat processing quality. J. Anim. Sci. 91:3674-3685.

Lundy, H. 1969. A review of the effects of temperature, humidity, and gaseous exchange environment in the incubator on the hatchability of the hen's eggs. Pp. 143-176 in *The Fertility and Hatchability of the Hen's Egg*. T.C. Carter and B.M. Freeman, ed. Oliver and Boyd, Edinburgh, UK.

Mahon, M. 1999. Muscle abnormalities-morphological aspects in: Richardson, R.I. and Mead, G.C. (Eds) Poult. Meat Sci., Poult. Sci. Symposium Series, pp 19-64. Wallingford, CAB INTL.

Mauro, A. 1961. Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Cytol. 9:493-495.

Meijerhof, R. 2000. Embryo temperature as a tool in the incubation process. Incubation and Fertility Research Group [WPSA Working Group 6 (Reproduction)], St. Edmand's Hall, Oxford, UK.

Meijerhof, R. 2009. Incubation principles: What does the embryo expect from us? Proc. Of the 20th Australian Poult. Sci. Symp. Sydney, Australia. P 106-110.

Meijerhof, R., and G. Van Beek. 1993. Mathematical modelling of temperature and moisture loss of hatching eggs. J. Theor. Biol. 165:27-41.

Miller, L. R., V. A. Garwood, and M. D. Judge. 1975. Factors affecting porcine muscle fiber type, diameter and number. J. Anim. Sci. 41:66-77.

Miller, J. B., and F. E. Stockdale. 1986. Developmental origins of skeletal muscle fibers: clonal analysis of myogenic cell lineages based on fast and slow myosin heavy chain expression. Proc. Natl. Acad. Sci. USA. 83:3860-3864.

Molenaar, R., I. A. M. Reijrink, R. Meijerhof, and H. Van De brand. 2010. Meeting embryonic requirements of broilers throughout incubation: A review. Brazilian J. Poult. Sci. 12:137-148.

Mozdziak, P. E., E. Schlutz, and R. G. Cassens. 1997. Myonuclear accretion is a major determinant of avian skeletal muscle growth. Am. J. Phys. 272:C565-C571.

Mudalal, S., M. Lorenzi, F. Soglia, C. Cavani, and M. Petracci. 2014. Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. Animal. 1-7.

O'Dea, E. E., G. M. Fasenko, J. J. R. Feddes, F. E. Robinson, J. C. Segura, C. A. Ouellette, and J. H. Van Middelkoop. 2004. Investigating the eggshell conductance and embryonic metabolism of modern and unselected domestic avian genetic strains at two flock ages. Poult. Sci. 83:2059-2070.

Osterc, J. 1974. Diameter and number of muscle fibers in musculus longissimus dorsi in connection with production properties of some cattle breeds. PhD Thesis. University of Ljubljana, Slovenia.

Page, S., J. B. Miller, E. J. Hager, and F. E. Stockdale. 1992. Developmentally regulated expression of three slow isoforms of myosin heavy chain: Diversity among the first fibers to form in avian muscle. Dev. Biol. 154:118-128.

Pavlidis, H. O., J. M. Balog, L. K. Stamps, J. D. Hughes, W. E. Huff, and N. B. Anthony. 2007. Divergent selection for ascites incidence in chickens. Poult. Sci. 86:2517-2529.

Petracci, M., S. Mudalal, A. Bonfiglio, and C. Cavani. 2013. Occurrence of white striping and its impact on breast meat quality in broiler chickens. Poult. Sci. 92:1670-1675.

Piestun, Y., S. Druyan, J. Brake, and S. Yahav. 2013. Thermal manipulations during broiler incubation alter performance of broilers to 70 days of age. Poult. Sci. 92:1155-1163.

Piestun, Y., O. Halevy, D. Shinder, M. Ruzal, S. Druyan, and S. Yahav. 2011. Thermal manipulations during broiler embryogenesis improves post hatch performance under hot conditions. Therm. Biol. 36:469-474.

Piestun, Y., O. Halevy, and S. Yahav. 2009. Thermal manipulations of broiler embryos - The effect on thermoregulation and development during embryogenesis. Poult. Sci. 88:2677-2688.

Piestun, Y., D. Shinder, M. Ruzal, O. Halevy, J. Brake, and S. Yahav. 2008. Thermal manipulations during broiler embryogenesis: Effect on the acquisition of thermotolerance. Poult. Sci. 87:1516-1525.

Pollock, D. L. 1999. A geneticist's perspective from within a primary breeder company. Poult. Sci. 78:414-418.

Powell, S. E., and E. D. Aberle. 1980. Effects of birth-weight on growth and carcass composition of swine. J. Anim. Sci. 50:860-868.

Powell, S. E., and E. D. Aberle. 1981. Skeletal muscle and adipose tissue cellularity in runt and normal birth weight swine. J. Anim. Sci. 52:748-756.

Rehfeldt, C., I. Fiedler, and J. Wegner. 1987. Veränderungen der mikrostruktur des muskelgewebes bei Labormäusen, Rindern und Schweinen während des Wachstums. Z. Mikrosk. Anat. Forsch. 101:669-680.

Rehfeldt, C., I. Fiedler, G. Dietl, and K. Ender. 2000. Myogenesis and postnatal skeletal muscle cellgrowth as influenced by selection. Livestock Prod. Sci. 66:177-188.

Rehfeldt, C., I. Fiedler, and N. C. Stickland. 2004. "Number and size of muscle fibers in relation to meat production" *Muscle Development of Livestock Animals*. Eds. M.F.W. tePas, M.E. Everts and H.P. Haagsman. CAB International.

Rehfeldt, C., and G. Kuhn. 2006. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J. Anim. Sci. 84S:E113-E123.

Rehfeldt, C., A. Tuchscherer, M. Hartung, and G. Kuhn. 2008. A second look at the influence of birth weight on carcass and meat quality in pigs. Meat Sci. 78:170-175.

Robertson, I.S. 1961. Studies in the effect of humidity on the hatchability of hen's eggs. I. The determination of optimum humidity for incubation. J. Agric. Sci. 57:185-194.

Romijn, C., and W. Lokhorst. 1960. Foetal heat production in the fowl. J. Physiol. 150:239-249.

Romijn, C., and J. Roos. 1938. The air space of the hen's egg and its changes during the period of incubation. J. Physiol. 94:365-379.

Russell, R. G., and F. T. Oteruelo. 1981. An ultrastructural study of the differentiation of skeletal muscle in the bovine fetus. Anat. Embryol. 162:403-417.

Scheuermann, G. N., S. F. Bilgili, S. Tuzun, and D. R. Mulvaney. 2004. Comparison of chicken genotypes: myofiber number in pectoralis muscle and myostatin ontogeny. Poult. Sci. 83:1404-1412.

Selleck, M. A., and C. D. Stern. 1991. Fate mapping and cell lineage analysis of Hensen's node in the chick embryo. Development. 112:615-626.

Sihvo, H. K., K. Immonen, K. and E. Puolanne. 2013. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. Vet. Path. Online, 0300985813497488.

Sosnicki, A. A., and B. W. Wilson. 1991. Pathology of turkey skeletal muscle: implications for the poultry industry. Food Struct. 10:317-326.

Staun, H. 1972. The nutritional and genetic influence on number and size of muscle fibers and their response to carcass quality in pigs. World Rev. Anim. Prod. 3:18-26.

Stickland, N. C. 1995. Muscle Growth. Meat Focus Intl. 4:241-245.

Stickland, N. C., and C. M. Dwyer. 1996. The pre-natal influence on post-natal growth. Mol. Physiol. Growth. 60:135-150.

Stickland, N. C., and S. E. Handel. 1986. The numbers and types of muscle fibers in large and small breeds of pigs. J. Anat. 174:181-189.

Stickland, N. C., E. M. Widdowson, and G. Goldspink. 1975. Effects of severe energy and protein deficiencies on the fibres and nuclei in skeletal muscles of pigs. Br. J. Nutr. 34:421-428.

Stockdale, F. E. 1992. Myogenic cell lineages. Dev. Biol. 154:284-298.

Suzuki, K., T. Shibata, H. Kadowaki, and T. Toyoshima. 2003. Meat quality comparison of Berkshire, Duroc and crossbred pigs sired by Berkshire and Duroc. Meat Sci. 64:35-42.

Swatland, H. J. 1982. The challenges of improving meat quality. Can. J. Anim. Sci. 62:15-24.

Swatland, H. J. 1992. Growth physiology and post-mortem meat quality. In: E. Poulanne, D. I. Demeyer (Eds), "*Pork Quality: Genetic and Metabolic Factors*." CAB Intl., Wallingford, UK, pp. 115-139.

Taylor, L. W., and G. O. Kreutziger. 1965. The gaseous environment of the chick embryo in relation to its development and hatchability. 2. Effects of carbon dioxide and oxygen levels during the period of the fifth through the eight days of incubation. Poult. Sci. 44:98-106.

Tona, K., O. Onagbesan, B. De Ketalaere, E. Decuypere, and V. Bruggeman. 2004. Effects of age of the broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight, and chick posthatch growth to forty-two days. J. Appl. Poult. Res. 13:10-18.

Tona, K., O. Onagbesan, V. Bruggeman, A. Collin, C. Berri, M. J. Duclos, S. Tesseraud, J. Buyse, E. Decuypere, and S. Yahav. 2008. Effect of heat conditioning at d 16 to 18 of incubation or during early broiler rearing on embryo physiology, post-hatch growth performance and heat tolerance. Arch. Geflugelkd. 72:75-83.

Uni, Z., and Yahav, S. 2010. Managing prenatal development of broiler chickens to improve productivity and thermotolerance. In: P. L. Greenwood, (Ed). *Managing the Prenatal Environment to Enhance Livestock Productivity*. pp. 71-90. Springer, Netherlands.

Velleman, S. G., J. W. Anderson, C. S. Coy, and K. E. Nestor. 2003. Effect of selection for growth rate on muscle damage during turkey breast muscle development. Poult. Sci. 82:1069-1074.

Velleman, S. G., and McFarland, D.C. 2014. Skeletal muscle. In: C. G. Scanes, (Ed). *Sturkie's Avian Physiology* (Sixth Edition). pp. 379-402. Academic Press, San Diego.

Visschedijk, A. H. J. 1968. The air space and embryonic respiration. 3. The balance between oxygen and carbon dioxide in the air space of the incubation chicken egg and its role in stimulating pipping. Brit. Poult. Sci. 9:197-210.

Vivarelli, E., W. E. Brown, R. G. Whalen, and G. Cossu. 1988. The expression of slow myosin during mammalian somitogenesis and limb bud differentiation. J. Cell. Biol. 107:2191-2197.

Volk, M., M. Herceg, B. Marzan, M. Kralj, S. Meknic, and V. Tadic. 1974. Fatal syncope in broilers: occurrence, clinical symptoms, lesions and pathogenesis. Vet. Archives. 44:14-23.

Wicke, M., S. Maak, and G. V. Lengerken. 1998. Structural and functional traits of the skeletal muscle for the improvement of pork quality. Pol. J. Food Nutr. Sci. 47/48(S):S21-S31.

Wideman, R. F., Y. K. Kirby, C. D. Tackett, N. E. Marson, C. J. Tressler, and R. W. McNew. 1996. Independent and simultaneous unilateral occlusion of the pulmonary artery and extra-pulmonary primary bronchus in broilers. Poult. Sci. 75:1417-1427.

Wideman, R. F., Y. K. Kirby, R. L. Owen, and H. French. 1997. Chronic unilateral occlusion of an extra-pulmonary primary bronchus induces pulmonary hypertension syndrome (ascites) in male and female broilers. Poult. Sci. 76:400-404.

Wigmore, P. M., and D. J. Evans. 2002. Molecular and cellular mechanisms involved in the generation of fiber diversity during myogenesis. Intl. Rev. Cyto. 216:175-232.

Wigmore, P. M. C., and N. C. Stickland. 1983. Muscle development in large and small pig fetuses. J. Anat. 137:235-245.

Wilson, B. W. 1990. Developmental and maturational aspects of inherited avian myopathies. Proceedings of the Society for Experimental Biology and Medicine. 194:87-96.

Wilson, B. W., P. S. Nieberg, and R. J. Buhr. 1990. Turkey muscle growth and focal myopathy. Poult. Sci. 69:1553-1562.

Wolanski, N. J. E. J. Luiten, R. Meijerhof, and A. L. J. Vereijken. 2004. Yolk utilization and chick length as parameters for embryo development. Avian Poult. Biol. Rev. 15:233-234.

Wolter, B. F., M. Ellis, B. P. Corrigan and J. M. DeDecker. 2002. The effect of birth weight and feeding of supplemental milk replacer to piglets during lactation on pre-weaning and post-weaning growth performance and carcass characteristics. J. Anim. Sci. 80:301-308.

Wulf, D. M., R. S. Emnett, J. M. Leheska, and S. J. Moeller. 2002. Relationships among glycolytic potential, dark cutting beef, and cooked beef palatability. J. Anim. Sci. 80:1895-1903.

Yahav, S., Collin, A., Shinder, D., & Picard, M. 2004a. Thermal manipulations during broiler chick embryogenesis: Effects of timing and temperature. Poult. Sci. 83:1959-1963.

Yahav, S., Rath, R. S., & Shinder, D. 2004b. The effect of thermal manipulations during embryogenesis of broiler chicks (*Gallus domesticus*) on hatchability, body weight and thermoregulation after hatch. J. Therm. Biol. *29*:245-250.

Yahav, S., D. Shinder, M. Ruzal, M. Giloh, and Y. Piestun. 2009. Controlling body temperature – The opportunities for highly productive domestic fowl. In: Cisneros, A.B., Goins, B.L. (Eds), *Body Temperature Regulation*. NovaScience Publishers. New York.

Yao, Y., M. Nakamura, J. I. Miyazaki, M. Kirinoki, and T. Hirabayashi. 1992. Expression pattern of skeletal muscle troponin T isoforms is fixed in cell lineage. Dev. Biol. 151:531-540.

2. Divergent selection for relative breast yield at four-days posthatch and the effect on embryonic and early posthatch development

Abstract

Genetic selections for growth promotion in poultry have been highly successful in improving growth, yield and feed conversion in the modern broiler. These selections have focused on the use of hypertrophy, the increase of muscle fiber size to promote growth. Muscle growth however is not limited to hypertrophy, but is largely attributable to both hypertrophy and hyperplasia. Hyperplasia being the increase of muscle fiber number, hyperplasia along with hypertrophy are the primary determinants of muscle size with a smaller contribution coming from satellite cells and the extracellular matrix. As muscle fiber size has been theorized to reach an eventual physiological limit, it was determined to develop of novel method of selection focusing on hyperplasia. Divergent selection for four-day relative breast yield (BY4) was chosen as it is believed to occur at point at which muscle cell number per gram is maximized and satellite cell activity is higher than later in life.

Pilot testing of two commercial pedigree populations showed large variation in relative breast yield at four days and heritabilities greater than 0.3, these results provided the confidence to proceed with divergent selection. Using a random bred control (RAN) population divergent selection was undergone for four-day relative breast yield. The two broiler lines divergently selected for BY4 are noted as the high and low BY4 lines, respectively (HBY4 and LBY4). Heritability estimates for selection of four-day breast percentage in the upward and downward directions were 0.63 and 0.44 respectively. Divergent selection resulted in clear divergence in BY4 and shows promise in utilizing BY4 to promote broiler growth and body composition.

2.1 Introduction

The use of genetic selection in the animal industry, particularly the poultry industry has resulted in significant livestock improvement. Improved performance has proven necessary as feed costs, which make up a significant portion of production costs, and product demand, continue to increase. Therefore, selection that focuses on improving the economic efficiency of livestock, while minimalizing feed intake (Luiting, 1990) and increasing the overall growth of the animal is imperative.

Poultry breeding programs focus on promoting growth, feed efficiency, and increased yield (Rauw et al., 1998). Growth-promoting selections are possible because growth traits exhibit moderate to high heritabilities (Emmerson, 1997) and often correlate with increased feed efficiency. Poultry selections have been so successful that in a study comparing a 1957 broiler population to a 1991 broiler strain, modern broiler weights were 3.2 times greater than the 1957 broiler when both were fed a modern diet (Havenstein et al., 1994). Growth and yield selections have resulted in a broiler reaching market weight at an earlier age. This change is important, as the industry is primarily an age-for-weight market with slaughter being performed at a particular weight as opposed to at a given age (Emmerson, 1997).

Growth selections currently practiced by the industry rely mainly on postnatal growth, or an increase of muscle mass resulting from increased muscle fiber size or hypertrophy (Rehfeldt et al., 2004). Hypertrophy is only one component contributing to muscle mass, as hyperplasia (muscle fiber number) and to a smaller extent extracellular matrix also contribute (Brown and Stickland, 1994). It has been suggested that the intense focus and duration of selection for muscle fiber size may soon lead to biological limits for muscle fiber size (Mahon, 1999).

In order to continue making selection progress in body weight and yield, novel methods of selection should be considered. To that end current research has focused on selection for relative breast yield at four-days (BY4) posthatch and is viewed as a new opportunity to promote growth. As the time needed for a broiler to reach market weight decreases, the percentage of lifespan the bird spends in embryogenesis increased (Halevy et al., 2006). This prenatal period is also a time in which, almost all increases in muscle cell number will occur (Rehfeldt et al., 2004).

Hyperplasia is the growth of muscle via an increase in muscle cell number. Muscle fiber hyperplasia occurs almost entirely during gestation, but has been evidenced to occur briefly after birth in some species (rodents, swine, chickens) (Rehfeldt et al., 2004). Researchers have shown an inverse correlation between postnatal muscle fiber hypertrophy and muscle fiber number (Rehfeldt et al., 2000). Growth may be maximized by utilizing a combination of fiber size and fiber number.

It is important to understand that myogenesis i.e. muscle fiber formation and the subsequent hypertrophy of muscle fibers is dependent on three waves of development. The first wave stems from the

proliferation and differentiation of embryonic myoblasts into primary muscle fibers. Primary muscle fiber formation is crucial to muscle formation, as they provide a framework for secondary fiber attachment. The secondary wave consists of the proliferation and differentiation of fetal myoblasts into secondary muscle fibers. The third and final wave of myogenesis, the proliferation of satellite cells, is of particular interest to this research, as satellite cells are required for muscle hypertrophy and regeneration. Satellite cells are the only myoblasts isolated at the time of hatch (Feldman and Stockdale, 1992; Hartley et al., 1992).

It has been hypothesized that selection for BY4 will promote growth as it targets an age at which muscle cell number per gram is maximized (0 to 4 days posthatch) and a point at which satellite cell activity is much greater then later in life (Halevy et al., 2006). Therefore, it may be assumed that the relative breast yield of Day 4 chicks relies primarily on the muscle fiber number, as opposed to fiber size. Therefore, selection should result in increased fiber number at hatch, with an increase in available satellite cells thus promoting postnatal hypertrophy. In addition, broilers selected for BY4 should exhibit improved growth characteristics and improved meat quality.

Characteristics associated with lower meat quality, such as decreased tenderness and poor water holding capacity are observed at slaughter in animals with low birth weights (Gondret et al., 2005; 2006; Rehfeldt and Kuhn, 2006). In swine animals with low birth weights form less skeletal muscle fibers, than larger littermates (Wigmore and Stickland, 1983; Handel and Stickland, 1987; Gondret et al., 2006). Low fiber number may restrict postnatal growth and result in increased fat deposition (Rehfeldt and Kuhn, 2006), which negatively affects meat quality. Furthermore, low fiber number has been related to extreme fiber hypertrophy, often resulting in giant fiber formation, known to correlate with reduced meat quality (Fiedler et al., 2004). Therefore, selection resulting in increased fiber number should promote improved growth yield and meat quality.

2.2 Materials and Methods

Pilot Test

Before undergoing full-scale implementation, two pilot studies were conducted with support from Cobb-Vantress. The two populations chosen for study were known to express differing growth rates and selection criteria. These studies were utilized to determine the variation present in percentage breast

yield at Day 4, as well as to calculate the heritability of percentage breast at the same age. Three hatches of fully pedigreed line A day-old chicks were obtained from a Cobb hatchery and transported to the University of Arkansas poultry research farm. Line A was reared under typical broiler management conditions up to four days of age with 23 hours of light and 1 hour of dark. Birds were provided feed and water *ad libitum* with a starter ration that was formulated to meet or exceed NRC (1994) minimum standards. Feed was withdrawn from line A 12 hours prior to euthanasia and processing. Birds were then placed on trays, covered and refrigerated at 1°C for a minimum of three hours to promote the onset of rigor mortis. Line B consisted of four hatches of fully pedigreed day-old chicks obtained from the same Cobb hatchery and transported to the University of Arkansas poultry research farm. Line B was reared and processed according to the protocol used for line A. After refrigeration, body weights were recorded, and researchers dissected birds from line A, and B. Sex was determined by gonadal examination. Weights were obtained for the bone-in-breast, breast, tenders, keel and yolk sac. The weight values were then used to calculate the part yields relative to body weight. The two studies revealed large variation in breast yield and body weight in both populations and provided high heritabilities thus giving confidence to proceed with selection.

Initial Line Formation

The base population for this study was developed in 1997 by crossing 7 male and 6 female lines at the University of Arkansas (RAN) (Harford et al., 2014). The population is randomly mated with the exception of sibling and half-sibling matings. The population consists of 24 sire families with each family consisting of 1 sire and 3 dams. In 2012, divergent selection for BY4 was begun using a sibling selection protocol similar to that previously described for ascites (Pavlidis et al., 2007) and muscle color (Harford et al., 2014). Figure 2.1 provides a schematic for the development of the High Breast Yield (HBY4) and Low Breast Yield (LBY4) lines from the RAN. To establish the research lines, four hatches were reared using standard industry practices. Pedigree replacements derived from hatches 1 and 2. Bird from hatch 1 and 2 were provided *ad libitum* access to a starter ration that was formulated to meet or exceed NRC (1994) minimum standards up to 3 weeks of age. Post 3 weeks of age, birds were sex separated, sample

weights obtained and placed on a feed and photoperiod restriction program to regulate growth and maximize adult reproductive efficiency. Water was provided *ad libitum* throughout the rearing process.

Hatches 3 and 4 were reared under typical broiler management conditions up to four days of age with 23 hours of light and 1 hour of dark. Birds were provided feed and water *ad libitum* with a starter ration that was formulated to meet or exceed NRC (1994) minimum standards. At four days of age, birds were prepared for processing. Feed was removed 12 hours prior to euthanasia, and all remaining crop contents surgically removed to minimize the confounding effects of crop contents on body weight. Birds were then placed on trays, covered and refrigerated at 1°C for a minimum of three hours to promote the onset of rigor mortis. All birds were necropsied for the purpose of obtaining component weights. Incisions were made; skin retracted, and cuts were then made along the ribs and clavicle to allow extraction of the intact keel. Upon removal of the keel, the yolk sac was removed and weighed. Sexing also occurred at this point through gonadal examination. The breast and tenders removed from the keel and then weighed (0.001 g). Relative values were calculated based on the part weight expressed as a percentage of live weight.

Sire family selection was used to diverge the RAN line into the HBY4 and LBY4 lines. Mean percentage breast yield was calculated for the original 24 sire families. The 8 sire families with the greatest percentage breast yield became the HBY4 line, while the 8 sire families with the lowest percentage breast yield became the LBY4 line.

Current Selection Techniques

The current selection method and propagation of the breast growth lines, implemented with the addition of DMU and EBV's, starting with Generation 2, is illustrated in Figure 2.2. Matings were performed by artificial insemination and pedigree information collected. Each subsequent generation consisted of 4 pedigreed hatches. Hatches 1 and 2 were grown under standard broiler breeder conditions as previously mentioned. Birds were allowed *ad libitum* access to water and a broiler starter and grower feed formulated to meet or exceed NRC (1994) minimum standards. At 3 weeks of age, birds were sex separated and placed on a feed and photoperiod restriction program to enhance reproductive performance. Water was provided *ad libitum* during the course of feed restriction.

Birds from hatches 3 and 4 were necropsied as described for the initial line formation. Data obtained were used to calculate the dam's estimated breeding values (EBVs) via DMU (Madsen and Jensen, 2000). Based on these EBVs hatch 1 and 2 progeny from the top 36 dam families were maintained, while progeny from the bottom 36 dam families were euthanized. Selected breeders were caged between 14 and 16 weeks of age. Sire families were created by random assignment of 3 females to each male with the avoidance of sibling and half-sibling matings.

Embryology

To assess how BY4 selections have impacted embryological development three one-week egg collections were incubated. Fertile hatching eggs (N ≈ 180 per line) from the HBY4, LBY4 and RAN lines were obtained. Multiple collections were utilized to provide an increased sample size and allow for sampling to be done at multiple ages. Eggs were incubated under standard conditions and randomly distributed throughout the incubator trays in order to minimize potential variation attributable to differences in incubator microenvironment. Collection one was used to assess growth from developmental age 21 to developmental age 24, developmental age being counted from the start of incubation. Collections two and three were used for samplings from developmental age 15 to age 20.

Eggs (N = 30 HBY4, LBY4; N = 20 RAN) were randomly sampled daily from developmental age 15 to 20. At Day 18 of embryonic development (E18) eggs not previously selected for sampling were transferred into hatching trays. For developmental ages 21 to 24, 20 chicks were selected for sampling from each of the three lines. The procedures used for collections two and three were identical to the procedures used in collections one. Characteristics measured include body, yolk, leg, gastrointestinal tract and bone-in-breast weight. These values are represented as both the absolute value and relative value to the embryo weight.

Data Analysis

Heritability estimates and genetic correlations were calculated for body weight, breast and percentage breast at four-days of age using data from all four generations. The calculations were performed using a derivative free multivariate analysis by restricted maximum likelihood along with the

DMU package (Madsen and Jensen, 2000). These calculations included the use of a fixed effect, which involved the combination of generation, hatch and sex for the HBY4 and LBY4 lines. Generational changes in percentage breast yield and additional processing traits were analyzed with line as the main effect by generation and with generation as the main effect by line using the general linear model procedure of SAS 9.3 (SAS Institute Inc., 2001, Cary, NC). Means separations were carried out using Duncan's multiple range test using SAS 9.3 (SAS Institute Inc., 2001, Cary, NC).

Data obtained from the embryological study was analyzed with line as the main effect by developmental age and examined separately from data collected during the growout phase of the trial. Trait means separation was performed via the general linear model procedure (GLM) along with Duncan's multiple range test using SAS 9.3 (SAS Institute Inc., 2001, Cary, NC). An alpha level of .05 was determined to assign significance.

2.3 Results

Pilot Testing

Body weight heritability was found to be moderately to highly heritable in the pedigree populations (Table 2.1). Breast weight heritability was also found to be moderately to highly heritable, with four-day breast percentage being moderately heritable (Table 2.1).

Genetic Parameters for Generations 1 to 4

Heritability estimates for four-day body weight were found to be high in both research lines, greater than 0.4 (Table 2.2). Breast weight heritability estimates were found to be high for both the LBY4 (0.52) and HBY4 (0.63). Heritability estimates for four-day relative breast yield were found to be high for both the HBY4 and LBY4 lines, greater than 0.4 (Table 2.2). Lines expressed clear divergence throughout selection, the line means diverged by 0.33 from Generation 1 to 2 and continued to diverge in Generations 3 and 4 while the average divergence from Generation 1 to 4 was 0.19 (Fig 2.3). By Generation 4, the HBY4 line had achieved a percentage breast of 2.95, with the LBY4 at 2.21% (Fig 2.4).

Four-Day Part Response to Divergent Selection

In addition to percentage breast, data was collected on body, keel, breast, tender, and yolk weight over four generations. Body weight decreased in all three lines post Generation 1, with body weights increasing in the HBY4 from Generations 2 to 4 and the LBY4 from 2 to 3 (Table 2.3). The HBY4 had a greater body weight than the LBY4 from Generation 2 onwards. The HBY4 also had a greater body weight than the RAN that exhibited an intermediate body weight exceeding the LBY4 (Table 2.3). The HBY4 keel weight increased from Generation 2 to 4 (Table 2.3). LBY4 keel weight did not increase from Generation 2 to 3 but increased from Generation 3 to Generation 4 (Table 2.3). Keel was not different from Generations 1 and 4 in the RAN, but was greater than the LBY4 in Generation 4 (Table 2.3). Breast weight was greatest in the HBY4 in Generation 4 and lowest in the LBY4 in Generations 2 and 4 (Table 2.3). The phenotypic correlations between traits are shown in Table 2.4.

Prehatch Development of BY4 Lines

At Day 15 and 18 of embryo development, the HBY4 line had a greater egg weight than the RAN and LBY4 lines (Fig 2.5). However, body weight showed no difference at any of the prehatch sampling dates (Fig 2.6). The HBY4 line had a greater supply organ weight than the LBY4, at days 15 and 17 of embryo development (Fig 2.7). The HBY4 also had a greater supply organ weight than the RAN at Day 15 (Fig 2.7). RAN supply organ weight was greater than LBY4 supply organ weight at Day 15 (Fig 2.7). Past this age, there was no difference in supply organ weight between the RAN and LBY4. Yolk weight was greater in the HBY4 than the LBY4 at days 19 and 20, but did not differ between the RAN and HBY4 or the LBY and RAN at any age prehatch (Fig 2.8).

Up to Day 18 there were no differences in bone-in-breast weight, but at Day 19 the HBY4 began exhibiting increased bone-in-breast weight, being greater than the LBY4 at days 19 and 20 (Fig 2.9). No difference in bone-in-breast weight was found prehatch between the RAN and HBY4 lines (Fig 2.9). The RAN exhibited greater bone-in-breast weight than the LBY4 at Day 19, but this difference was not seen at any other age prehatch (Fig 2.9). Absolute leg weight did not differ between the BY4 lines at any age prehatch (Fig 2.10).

HBY4 supply organ percentage was greater than the LBY at Day 17, no other differences in supply organ percentage were found between BY4 lines at any other age prehatch (Fig 2.11). Bone-in-breast percentage followed the trend of bone-in-breast weight, where beginning at Day 19 and continuing to Day 20 the HBY4 line had a greater bone-in-breast percentage than the LBY4 (Fig 2.12). HBY4 bone-in-breast percentage failed to differ from the RAN line at any age prehatch (Fig 2.12), while the RAN bone-in-breast percentage was greater than the LBY4 at days 16 and 20 (Fig 2.12). Leg weight percentage relative to live weight was greater in the HBY4 than the RAN and LBY4 lines at 20 days of age (Fig 2.13).

Early Posthatch Development of the BY4 Lines

At developmental age 21, i.e. hatch, body weight was lower in the RAN line than either the HBY4 and LBY4 lines, which did not differ (Fig 2.6). There were no differences in body weight at Day 22, but from Day 23 to 24, the HBY4 has a greater body weight than the LBY4 line (Fig 2.6). The HBY4 also had a greater body weight than the RAN line at Day 23 (Fig 2.6). The RAN and LBY4 did not differ in body weight at either 23 or 24 days (Fig 2.6). The HBY4 and RAN line did not differ in supply organ weight from developmental Day 21 to 24, while the HBY4 supply organ weight was greater than the LBY4 line from 23 to 24 days (Fig 2.7). The RAN line displayed a greater supply organ weight than the LBY4 at days 22 and 24 (Fig 2.7). HBY4 line yolk weight was greater than the RAN at days 21, 22 and 23 (Fig 2.8). The LBY4 also expressed greater yolk weight than the RAN at days 22 and 23 (Fig 2.8). There were no differences in yolk weight between the HBY4 and LBY4 at any of the early posthatch sampling dates (Fig 2.8). HBY4 bone-in-breast weight was greater at hatch than in the RAN and greater than both the RAN and LBY4 from days 22 to 24 (Fig 2.9). No difference in bone-in-breast weight was found between the LBY4 and RAN from days 21 to 24 (Fig 2.9). At hatch no difference in absolute leg weight was shown, but by Day 22 the HBY4 line had a greater leg weight than the RAN and greater than the LBY4 at days 23 and 24 (Fig 2.10). The RAN and LBY4 did not differ in leg weight from days 21 to 24 (Fig 2.10).

At hatch the RAN line had a greater supply organ percentage than the LBY4 line, and continued to have a higher supply organ percentage throughout the early posthatch period, excluding Day 23 where no difference in supply organ percentage was shown between any of the BY4 lines (Fig 2.11). No

consistent difference was shown in supply organ percentage between the RAN and HBY4 lines (Fig 2.11). At Day 22, the HBY4 line had a greater percentage bone-in-breast than the LBY4 but did not differ from the RAN line (Fig 2.12). From Day 23 on, the HYB4 had a greater percentage bone-in-breast than either the RAN or LBY4 (Fig 2.12). Neither the RAN or LBY4 lines were different from each other in percentage bone-in-breast from days 21 to 24 (Fig 2.12). Percentage leg was greater in the RAN than the HBY4 and LBY4 at hatch (Fig 2.13). By Day 24 leg percentage had flipped, with the HBY4 having a greater leg percentage than the RAN and LBY4 (Fig 2.13). No consistent differences were observed for either percentage thigh or drum during this part of the study (data not presented).

2.4 Discussion

Pilot Testing

The moderate to high heritabilities calculated for body weight and breast weight in the pedigree populations corresponded with prior research. The body weight heritability was consistent with estimated body weight heritabilities of seven-day old quail ranging from 0.26 to 0.37 (Sefton and Siegel, 1974), and 0.43 to 0.52 at four and six weeks in broilers, respectively (Kuhlers and McDaniel, 1996). Breast weight heritability was consistent with Le Bihan-Duval et al. (1999), which calculated a heritability of 0.51 in six-week old broilers. The calculated heritabilities for four-day percentage breast were lower than the estimated percentage breast heritability ranging from 0.51 to 0.55 at six weeks in broilers (Le Bihan-Duval et al., 1999;2001). The heritabilities were consistent with swine research, which estimated heritability as high as 0.28 for muscle fiber number (Larzul et al., 1997; Fiedler et al., 2004). While four-day percentage breast does not measure muscle fiber number directly, research has shown that fiber number does not increase after hatch in chickens (Smith, 1963) and sampling occurs at a point shown to maximize muscle cell number per gram (Halevy et al., 2006). Implying that BY4 is capable of substituting for direct selection for fiber number, an idea similar to that of Smith, 1963. The use of simulated selection in swine showed promise, as selection based on fiber number resulted in improved growth, and promoted swine muscle quality by a resulting decline in abnormal fibers shown to correlate with poor meat quality (Fiedler et al., 2004).

Genetic Parameters for Generations 1 to 4

The estimated four-day body weight heritability is consistent with research estimating body heritability between 0.43 to 0.52 in four and six-week old broilers, respectively (Kuhlers and McDaniel, 1996). The high heritabilities found for breast weight in the LBY4 and HBY4, are consistent with the estimated breast weight heritability of 0.51 found in broilers at six-weeks of age (Le Bihan-Duval, et al., 1999).

Heritability estimates for four-day percentage breast yield were found to be higher than the estimated heritabilities for muscle fiber number calculated by Larzul et al. (1997), and Fiedler et al. (2004), and the heritabilities calculated during pilot testing. The results are consistent with heritabilities calculated for breast yield percentage ranging from 0.43 in turkey toms at 14 weeks (Case et al., 2012) to 0.55 at 6 weeks in broilers (Le Bihan-Duval et al., 2001). Results suggest several possibilities. First, selection for four-day percentage breast yield may be targeting fiber number, with a higher heritability in avian species than swine. Second, selection may be affecting both fiber number and satellite cell development. Or third, early selection may be shifting the age at which hypertrophy begins, as evidenced by the similar heritability of breast percentage at six weeks in broilers (Le Bihan-Duval et al., 1999;2001).

Regardless of the exact mode of action, both research lines showed the desired movement in percentage breast yield in Generation 2, the first generation post divergence. The HBY4 line increased in breast percentage from Generation 1 to 3 with a slight decreased from Generation 3 to 4. The LBY4 obtained the lowest breast percentage in Generations 2 and 4.

Four-Day Part Response to Divergent Selection

The drop in BY4 line body weights from Generation 1 to 2, were due to a change in seasonal conditions, as well as a decrease in breeder age. The HBY4 expressing a greater body weight than the LBY4 from Generation 2 onward and the RAN line serving as an intermediate with a greater body weight than the LBY4 was the expected outcome of divergent selection for BY4. Research has shown that selection for muscle fiber number promotes growth (Fiedler et al., 2004); therefore it is reasonable to assume that divergent BY4 selection would both promote and obstruct growth. Divergent selection for

BY4 resulted in concurrent changes in keel weight. While it might be assumed the divergent selection for BY4 would result in an increased keel weight in the HBY4 and a decreased keel weight in the LBY4, both BY4 lines exhibited increases in keel weight across generations. In the selected populations changes in keel weight, can be explained by research showing that body weight is a result of a union between skeletal structure, condition and fleshing (Tierce and Nordskog, 1985). Keel weight was shown to have a higher correlation with body weight than breast percentage in the BY4 lines. Furthermore, body weight improved in both the HBY4 and LBY4 lines post Generation 2. Therefore it stands to reason that as divergent BY4 selection improved fleshing in the HBY4 and both the LBY4 and HBY4 exhibited increases in body weight that increases in keel weight would occur regardless of the direction of selection for BY4. Divergent BY4 selection led to clear divergence in breast and tender weight with the HBY4, RAN, LBY4 ranking 1st, 2nd and 3rd in these categories respectively. This is to be expected given the correlations between breast percentage, tender weight and breast weight. The changes in breast and tender weight reflect the desired impact of divergent selection for BY4 from the original RAN populations.

BY4 Development Days 15 to 20

Egg weight was different at embryonic developmental ages 15 and 18, but these were the only dates where differences were detected. However, it appears the HBY4 may have had a greater egg weight, and lack of difference in egg and embryo weight may be due to sampling error. No differences in embryo weight were detected, however differences were detected in embryo composition as evidenced by differences in supply organ percentage at 17 days and bone-in-breast percentages at 16, 19, and 20 days. The difference in supply organ percentage between the HBY4 and LBY4 at Day 17, may be demonstrative of the HBY4 line preparing for rapid early growth, by having an improved supply system needed to promote growth and muscle development (Siegel, 2002). The lack of difference in supply organ percentage past this point is presumably due to the emergence of differences in bone-in-breast percentage between the HBY4 and LBY4, made possible by the increased supply organ percentage at Day 17.

Embryonically it has been shown that days 16-18 are a key period in satellite cell development (Hartley et al., 1992). As the HBY4 line showed no difference in bone-in-breast prior to Day 19, it is

reasonable to suggest that increased satellite cell proliferation also contributed to an increase in bone-in-breast weights. Furthermore the HBY4 leg percentage was not negatively impacted by increased bone-in-breast, which would be expected as resources are finite and increased resource devotion to breast yield should have drawn resources needed for concurrent leg development (Siegel et al., 2009), suggesting that increased satellite cell proliferation increased the resource pool available for growth to the HBY4 embryo. The HBY4 lines supply organ weight and percentages also support the idea that increased satellite cell proliferation resulted in improved bone-in-breast yields, as traditionally selection for increased growth results in decreased supply organ percentage (Deeb and Lamont, 2002).

Early Posthatch Development of BY4 Lines

Muscle fiber number has been shown to be fixed by the time of hatch (Smith, 1963), but research has also demonstrated that the maximum number of muscle cells per gram of tissue can be found up to three days of age dropping significantly at five days of age (Halevy et al, 2006). This development is almost perfectly mimicked by the BY4 lines. No differences in body weight between lines were shown at developmental age 22, but at Day 23 the HBY4 body weight was greater than the LBY4 and RAN lines. The HBY4 continued to outperform the LBY4 at 24 days, and the RAN line became the intermediate. Furthermore, at hatch there was no difference in percentage bone- in-breast, but by Day 23, the HBY4 line began exhibiting a greater bone-in-breast percentage than the other two lines. The change in bone-in-breast suggesting that BY4 selection altered the muscle fiber number of the HBY4 and LBY4 lines. The increased fiber number resulting in a greater body weight in the HBY4 when compared to the LBY4 and a greater bone-in-breast percentage than either the RAN or LBY4 lines.

The brief time period posthatch is also the point of maximum satellite cell activity (Halevy et al., 2000, 2003) and declines rapidly afterwards (Moss et al., 1964; Halevy et al., 2000, 2003; Velleman et al., 2010). While four days posthatch does not represent the peak period for satellite cells, it is a point near maximum satellite cell activity and before gains in muscle fiber size have begun to occur rapidly (Halevy et al., 2006). Increased satellite cell proliferation is suggested both by the bone-in-breast percentage exhibited by the HBY4 line and the leg percentage shown in the HBY4 line. Traditionally increased breast growth has come without proportional increases in leg muscle (Nestor et al., 1985; Lilburn, 1994; Yalcin

et al., 2001). While leg percentage did not increase to the degree of bone-in-breast, the HBY4 line still exhibited a greater leg percentage than the other two lines at Day 24. Again, suggesting that selection could have resulted in increased fiber number and or increased satellite cell proliferation providing the necessary resources to allow for growth in both the breast and legs of the HBY4 chick.

Typically the aforementioned growth boost would have resulted in a decreased ratio of supply to demand organs (Deeb and Lamont, 2002) in the HBY4 line. This was not seen in the current experiment; rather the LBY4 line had a lesser supply organ percentage compared to the RAN line, while the HBY4 line did not differ from the RAN line. Divergent selection may have resulted in additional resources being devoted to supply organs early on, thereby creating the support system the HBY4 line needed to outperform the LBY4 in body weight and bone-in-breast percentage, and the RAN in bone-in-breast percentage. Increased resources would allow for better resource allocation diminishing the imbalance between an increasingly greater body mass and a broilers support system (internal organs, vascular systems and skeletal structure) which has been cited as a primary cause of the reduction in broiler fitness (Katanbaf et al., 1988; Dunnington and Siegel, 1996; Julian, 2005).

In summary, selection for BY4 is feasible due to large breast yield variation and a high heritability for percentage breast at four days posthatch. The selected BY4 lines exhibited clear divergence in relative breast yield from the original population. Embryonic differences found between the three BY4 lines in supply organ and bone-in-breast percentages demonstrate that divergent selection for BY4 were capable of impacting embryonic development. It appears that the increased supply organ percentage at Day 17 of development of the HBY4 line allows it to gear up for increased early growth, as evidenced by the increased bone-in-breast percentage shown at days 19 and 20 compared to the LBY4 line. Furthermore, this growth does not come at the expense of leg growth as typically shown in traditional selection (Siegel et al., 2009). Whether this growth is the result of increased muscle fiber number, increased satellite cell proliferation, a combination of the two, or an as of yet undetermined cause is not known. While the exact mode of action driving the change in relative breast yield is unknown, results show promise in utilizing BY4 selections to promote broiler growth.

2.5 Figures and Tables

Table 2.1 Research line A and line B heritabilities and means[1] for four-day body weight and breast traits.

	Line A		Line B	
Trait	Heritability	Mean ± SEM	Heritability	Mean ± SEM
Body Weight (g)	0.18	77.70 ± .19	0.61	74.30 ± .19
Breast[2] (g)	0.32	2.57 ± .02	0.48	2.81 ± .02
% Breast	0.33	3.28 ± .01	0.38	3.74 ± .02

[1] All heritabilities and means calculated at four-days posthatch

[2] Pectoralis major only

Table 2.2. Heritabilities, and genetic correlations[1] for body weight and breast traits calculated from the HBY4 and LBY4 lines[2].

	Line Designation					
	HBY4 Line			LBY4 Line		
Trait	BW	BRST	%BRST	BW	BRST	%BRST
Body Weight	**0.41**	0.79	0.54	**0.45**	0.79	0.65
[3]Breast (g)	0.03	**0.63**	0.92	0.03	**0.52**	0.96
% Breast	0.06	0.01	**0.63**	0.04	0.01	**0.44**

[1] Heritabilities are on the diagonal line, genetic correlations are above the diagonal line, and genetic correlation SE are below the diagonal line.

[2] HBY4 and LBY4 are selected for high and low % breast, respectively

[3] Pectoralis Major only

Table 2.3 Four day chick weight measures (g) (means ± SEM) over four generations of divergent selection for BY4[1].

		Line Designation[2]		
Trait	Generation	HBY4	LBY4	RAN
Body Weight	1	66.68 ± 0.50a	66.68 ± 0.50a	66.68 ± 0.50a
	2	60.39 ± 0.31c,x	58.36 ± 0.38c,y	.
	3	64.20 ± 0.40b,x	59.83 ± 0.47b,y	.
	4	66.00 ± 0.37a,x	59.88 ± 0.38b,z	62.99 ± 0.55b,y
Yolk	1	0.49 ± 0.02a	0.49 ± 0.02a	0.49 ± 0.02a
	2	0.41 ± 0.01b	0.41 ± 0.02b	.
	3	0.31 ± 0.01d,x	0.26 ± 0.01d,y	.
	4	0.38 ± 0.01c,x	0.32 ± 0.01c,y	0.28 ± 0.01b,z
Keel[3]	1	1.25 ± 0.02c	1.25 ± 0.02a	1.25 ± 0.02b
	2	1.21 ± 0.02c,x	1.13 ± 0.02b,y	.
	3	1.34 ± 0.02b,x	1.17 ± 0.02b,y	.
	4	1.57 ± 0.03a,x	1.27 ± 0.02a,z	1.41 ± 0.03a,y
Tender	1	0.48 ± 0.01c	0.48 ± 0.01a	0.48 ± 0.01a
	2	0.44 ± 0.01d,x	0.38 ± 0.01c,y	.
	3	0.58 ± 0.01a,x	0.43 ± 0.01b,y	.
	4	0.53 ± 0.01b,x	0.37 ± 0.01c,z	0.43 ± 0.01b,y
Breast	1	1.61 ± 0.04b	1.61 ± 0.04a	1.61 ± 0.04a
	2	1.55 ± 0.02b,x	1.30 ± 0.03c,y	.
	3	2.02 ± 0.04a,x	1.47 ± 0.03b,y	.
	4	1.97 ± 0.03a,x	1.35 ± 0.03c,z	1.60 ± 0.04a,y

[a-d] Means for a trait across generations within a line are different (P < .05).

[x-z] Means for a trait within a generation but across lines are different (P < .05).

[1]BY4 (Four-day Relative Breast Yield)

[2]HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control

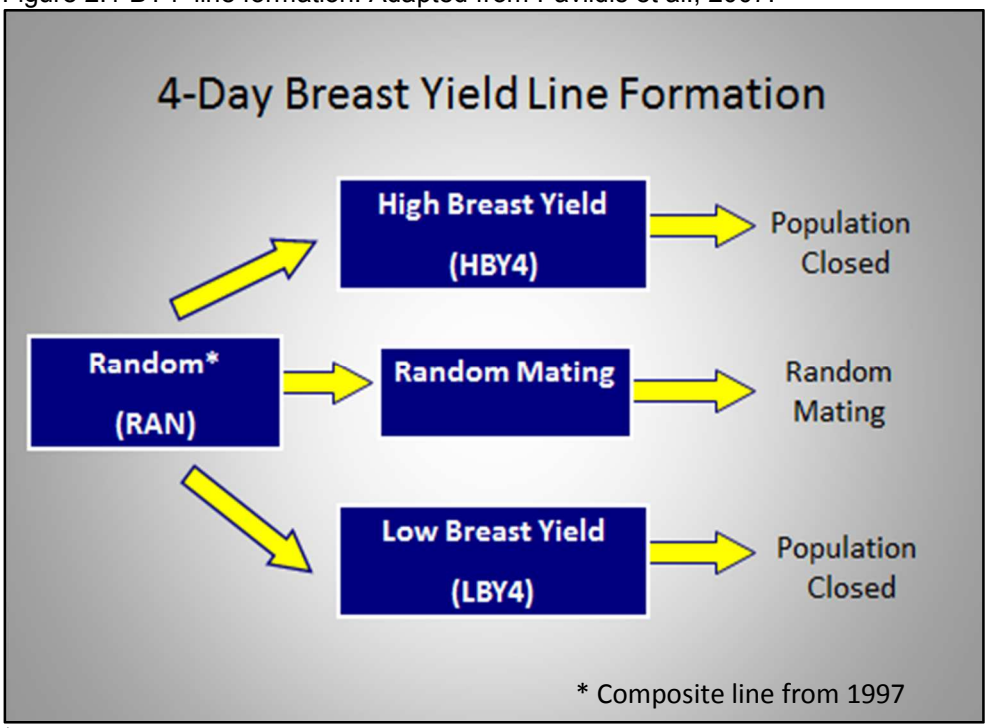
[3] Keel = Skeletal support system of the breast post breast removal

Table 2.4 Phenotypic correlations of processing trait means at four days of age from the BY4[1] lines.

Line	Trait	BWT	Breast	Tender	Keel	Yolk	TBRST	PBRST	PTBRST
HBY4[2]	BWT	1	0.84	0.75	0.52	0.15	0.85	0.67	0.68
	Breast	0.84	1	0.81	0.46	0.00	0.99	0.96	0.95
	Tender	0.75	0.81	1	0.40	-0.06	0.88	0.76	0.86
	Keel	0.52	0.46	0.40	1	0.07	0.46	0.39	0.38
	Yolk	0.15	0.00	-0.06	0.07	1	-0.01	-0.09	-0.10
	TBRST	0.85	0.99	0.88	0.46	-0.01	1	0.94	0.96
	PBRST[4]	0.67	0.96	0.76	0.39	-0.09	0.94	1	0.98
	PTBRST[4]	0.68	0.95	0.86	0.39	-0.10	0.96	0.98	1
LBY4[2]	BWT	1	0.83	0.74	0.58	0.09	0.82	0.66	0.67
	Breast	0.83	1	0.87	0.54	0.00	0.98	0.96	0.95
	Tender	0.74	0.87	1	0.48	-0.05	0.92	0.82	0.89
	Keel	0.58	0.54	0.48	1	0.05	0.53	0.45	0.45
	Yolk	0.09	0.00	-0.04	0.05	1	0	-0.03	-0.04
	TBRST	0.82	0.98	0.92	0.53	0	1	0.94	0.96
	PBRST[4]	0.66	0.96	0.82	0.45	-0.03	0.94	1	0.99
	PTBRST[4]	0.67	0.95	0.89	0.45	-0.04	0.96	0.99	1
RAN[3]	BWT	1	0.87	0.75	0.56	0.15	0.87	0.73	0.72
	Breast	0.87	1	0.84	0.57	0.08	0.99	0.96	0.95
	Tender	0.75	0.84	1	0.44	0.05	0.90	0.80	0.88
	Keel	0.56	0.57	0.44	1	0.02	0.56	0.52	0.50
	Yolk	0.15	0.08	0.05	0.02	1	0.08	0.02	0.02
	TBRST	0.87	0.99	0.90	0.56	0.08	1	0.95	0.96
	PBRST[4]	0.73	0.96	0.80	0.52	0.02	0.95	1	0.99
	PTBRST[4]	0.72	0.95	0.88	0.50	0.02	0.96	0.99	1

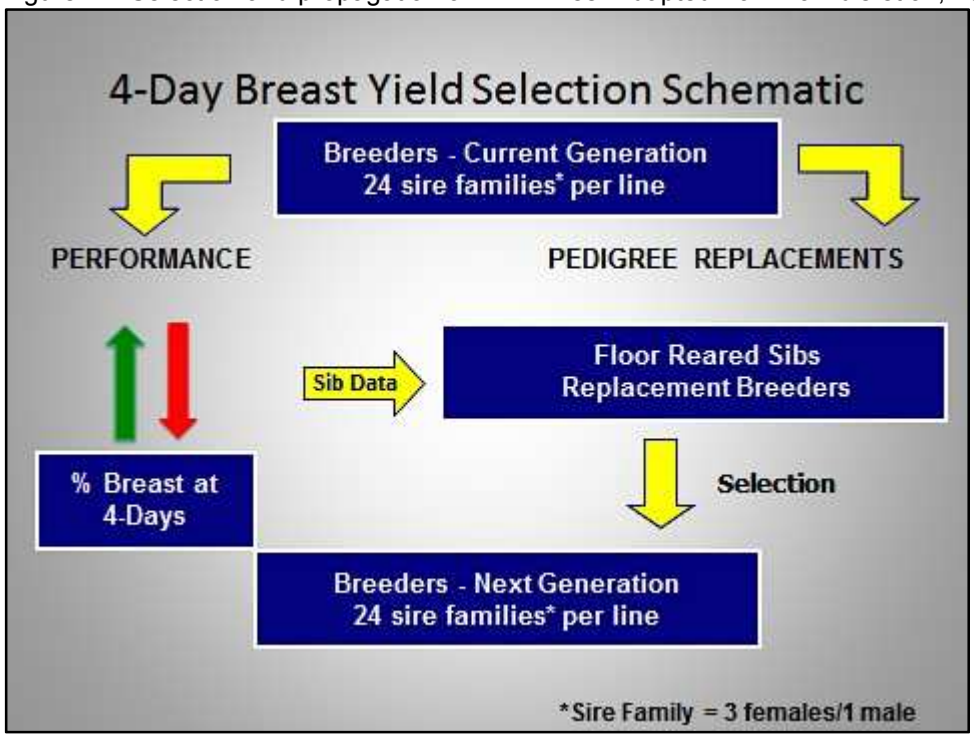
[1] BY4 (Four-day Relative Breast Yield)
[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively.
[3] RAN = unselected random bred control.
[4] Trait weight relative to live weight percentage

Figure 2.1 BY4[1] line formation. Adapted from Pavlidis et al., 2007.



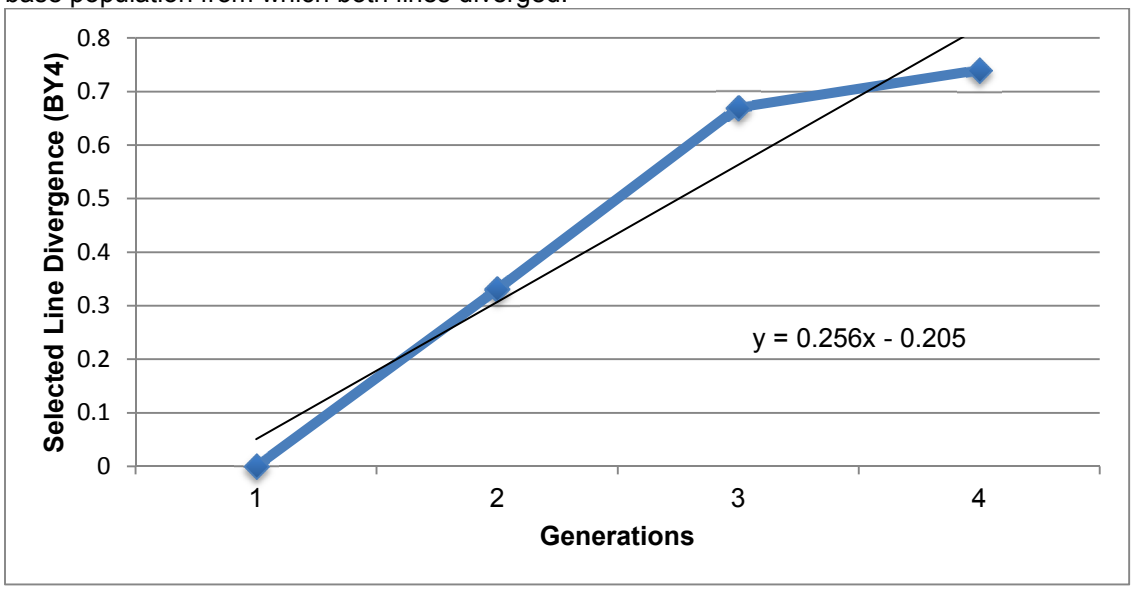
[1] BY4 (Four-Day Relative Breast Yield)

Figure 2.2 Selection and propagation of BY4[1] lines. Adapted from Pavlidis et al., 2007.



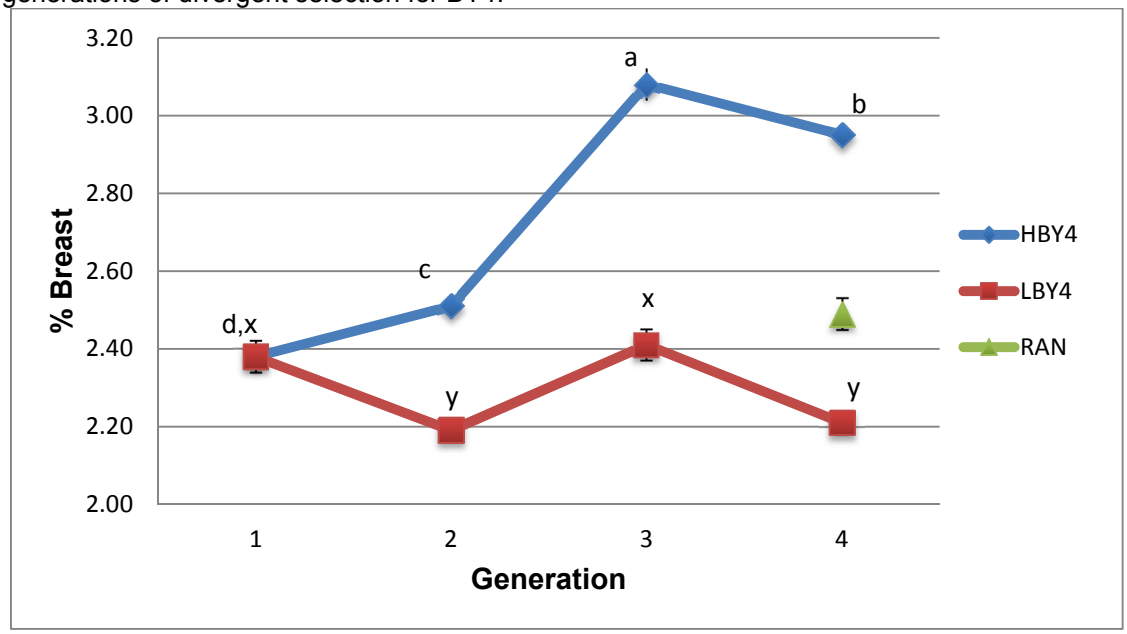
[1] BY4 (Four-Day Relative Breast Yield)

Figure 2.3 Generational divergence of lines divergently selected for BY4[1]. Generation one represents the base population from which both lines diverged.



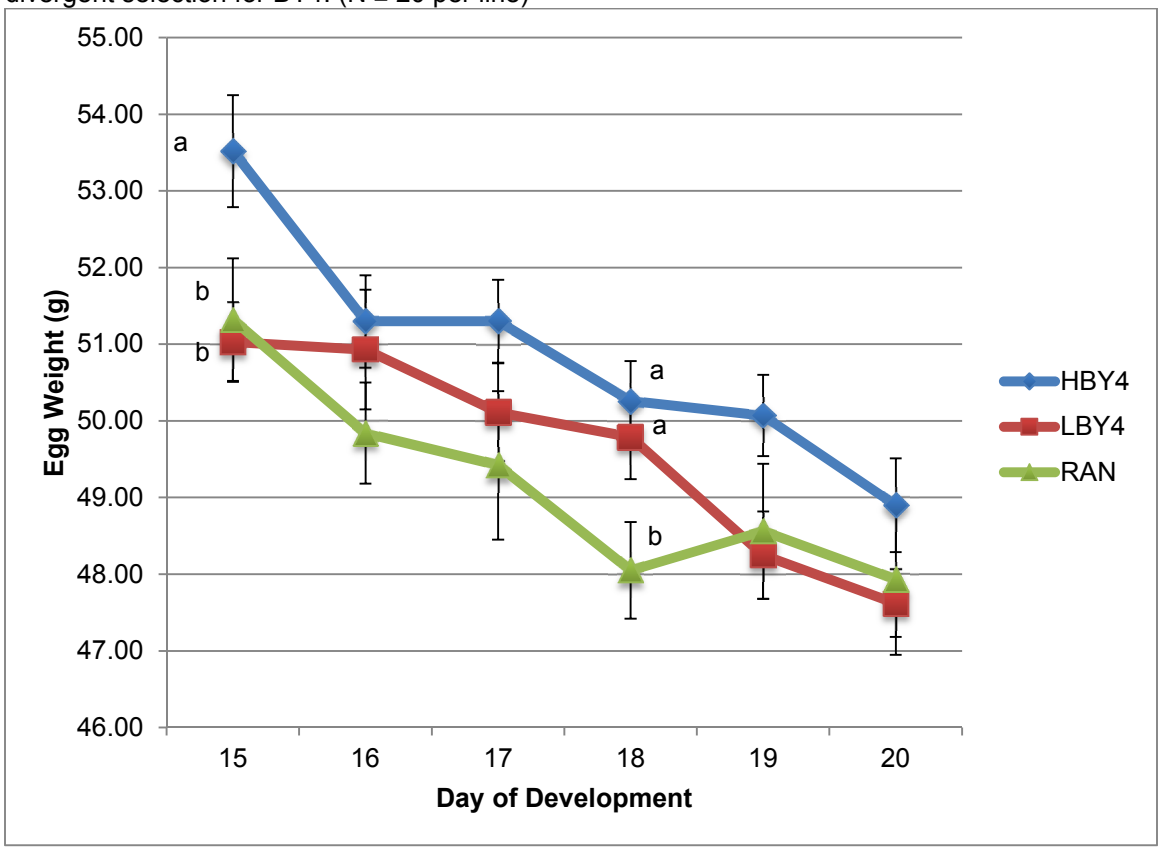
[1] BY4 (Four-Day Relative Breast Yield)

Figure 2.4 Generational percentage breast measures (means ± SEM) of the BY4[1] lines[2] over four generations of divergent selection for BY4.



[1] BY4 (Four-Day Relative Breast Yield)
[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control.
[a-c] Means for a trait across generations within the HBY line are different (P < .05).
[x-z] Means for a trait across generations within the LBY4 line are different (P < .05).

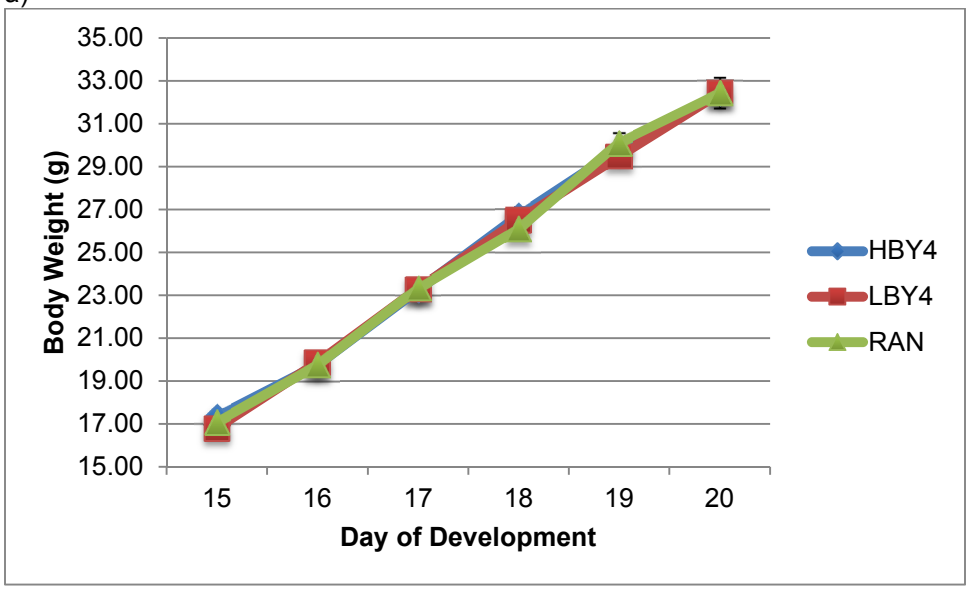
Figure 2.5 Weight measures (mean ± SEM) of eggs from the BY4[1] lines[2] after four generations of divergent selection for BY4. (N ≥ 20 per line)



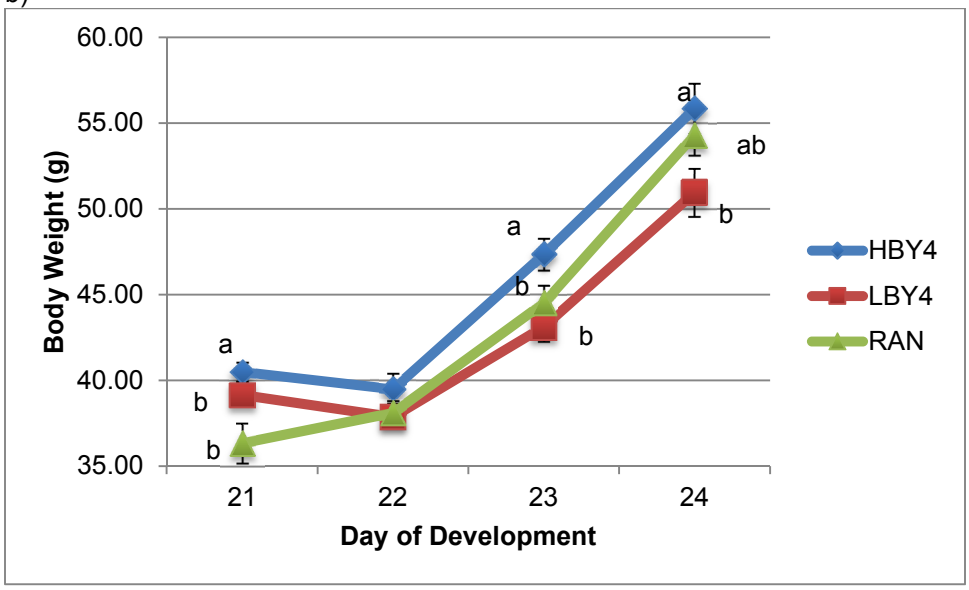
[1] BY4 (Four-Day Relative Breast Yield)
[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control.
[a-b] Means within an age but across lines are different (P < .05).

Figure 2.6 Body weight measures (mean ± SEM) collected from the BY4[1] lines[2] after four generations of divergent selection for BY4. (N ≥ 20 per line) Panel (a) includes 15 to 20 days, while (b) includes 21 to 24 days.

a)



b)



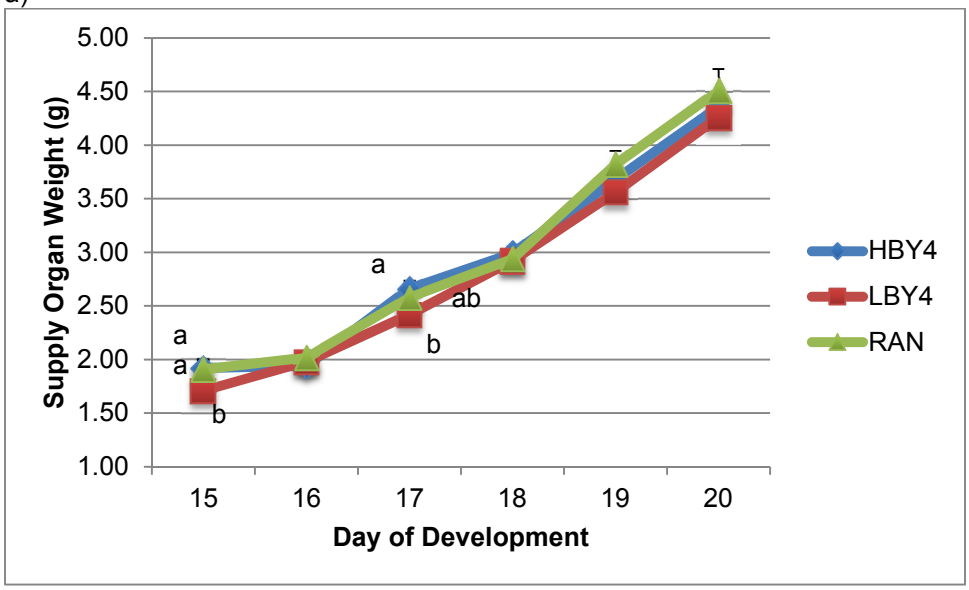
[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control.

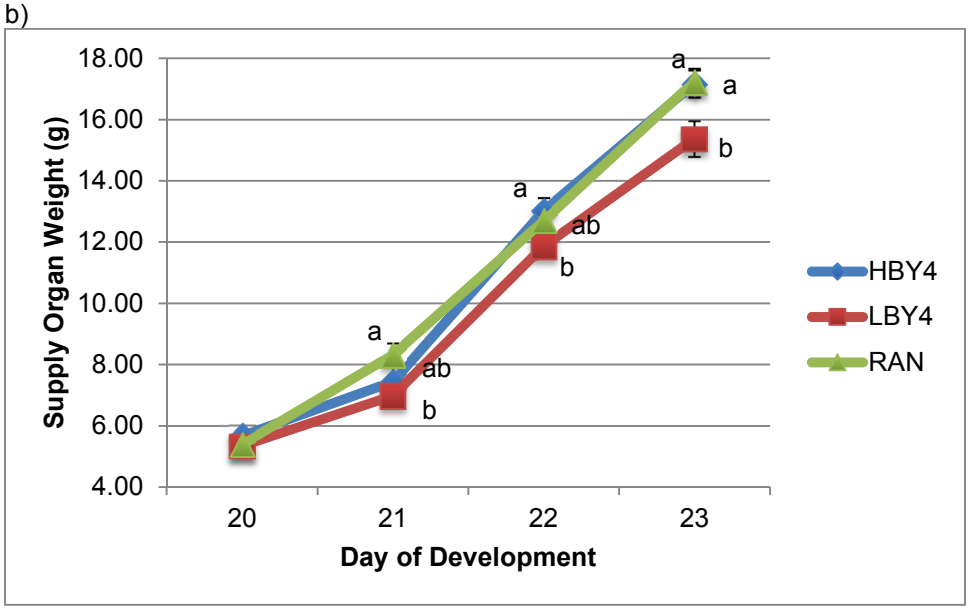
[a-b] Means within an age but across lines are different (P < .05).

Figure 2.7 Supply organ weight measures (mean ± SEM) collected from the BY4[1] lines[2] after four generations of divergent selection for BY4. (N ≥ 20 per line) Panel (a) includes 15 to 20 days, while (b) includes 21 to 24 days.

a)



b)



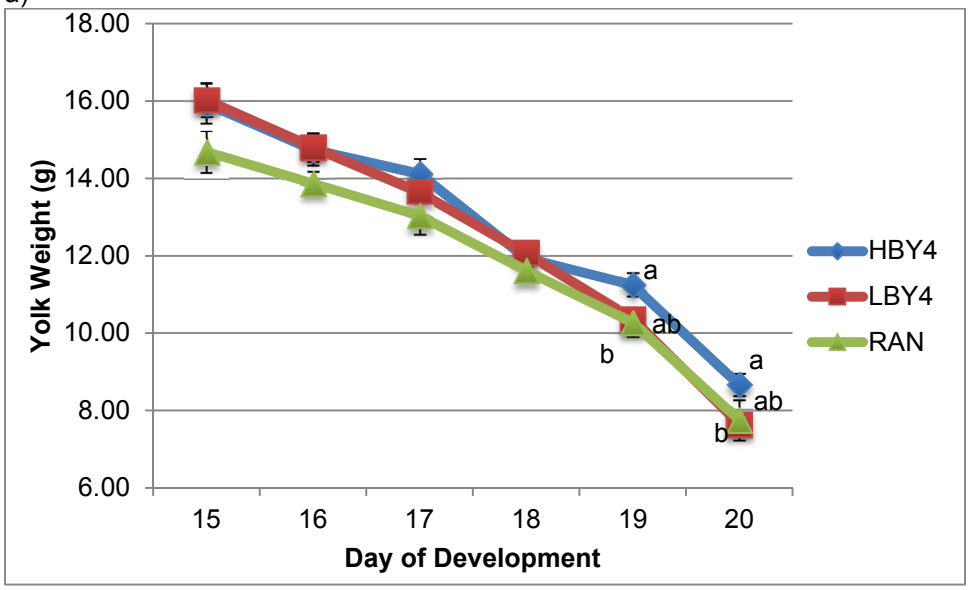
[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control.

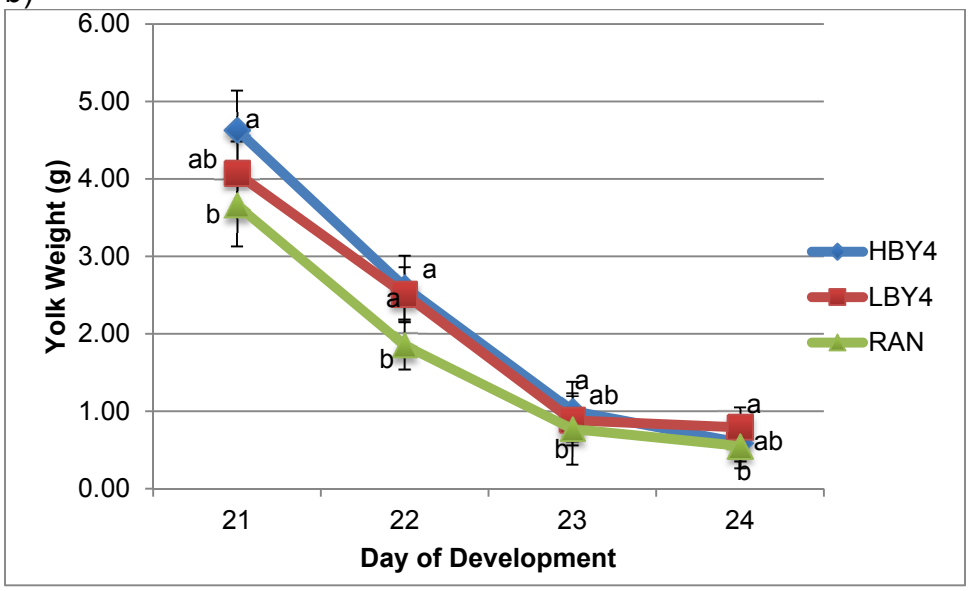
[a-b] Means within an age but across lines are different (P < .05).

Figure 2.8 Yolk weight measures (mean ± SEM) collected from the BY4[1] lines[2] after four generations of divergent selection for BY4. (N ≥ 20 per line) Panel (a) includes 15 to 20 days, while (b) includes 21 to 24 days.

a)



b)



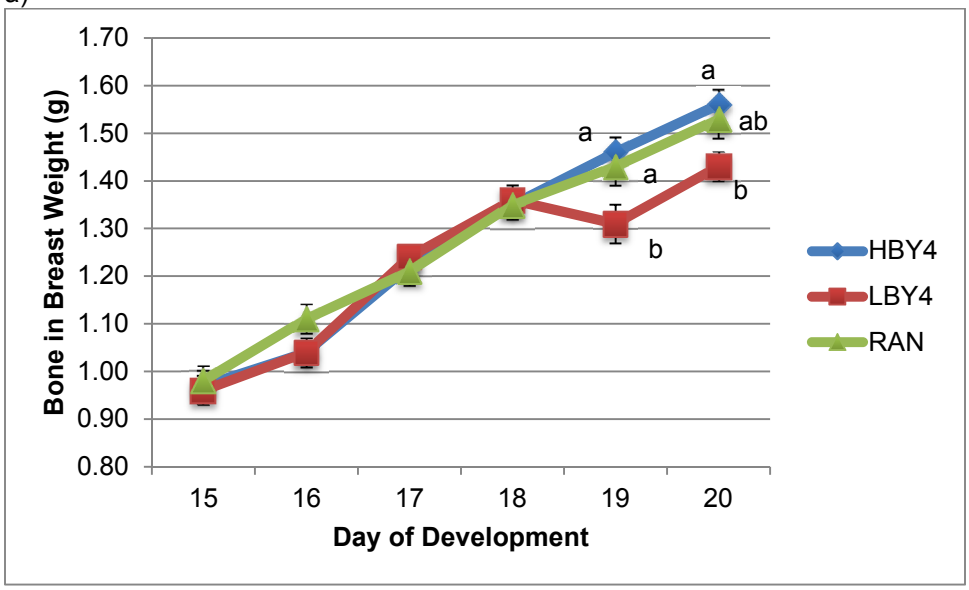
[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control.

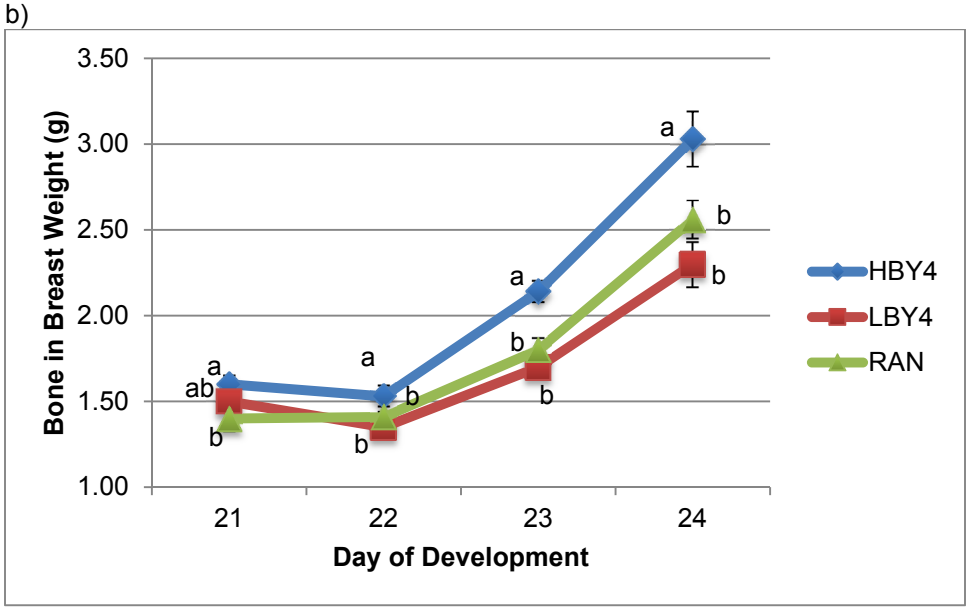
[a-b] Means within an age but across lines are different (P < .05).

Figure 2.9 Bone-in-breast weight measures (mean ± SEM) collected from the BY4[1] lines[2] after four generations of divergent selection for BY4. (N ≥ 20 per line) Panel (a) includes 15 to 20 days, while (b) includes 21 to 24 days.

a)



b)



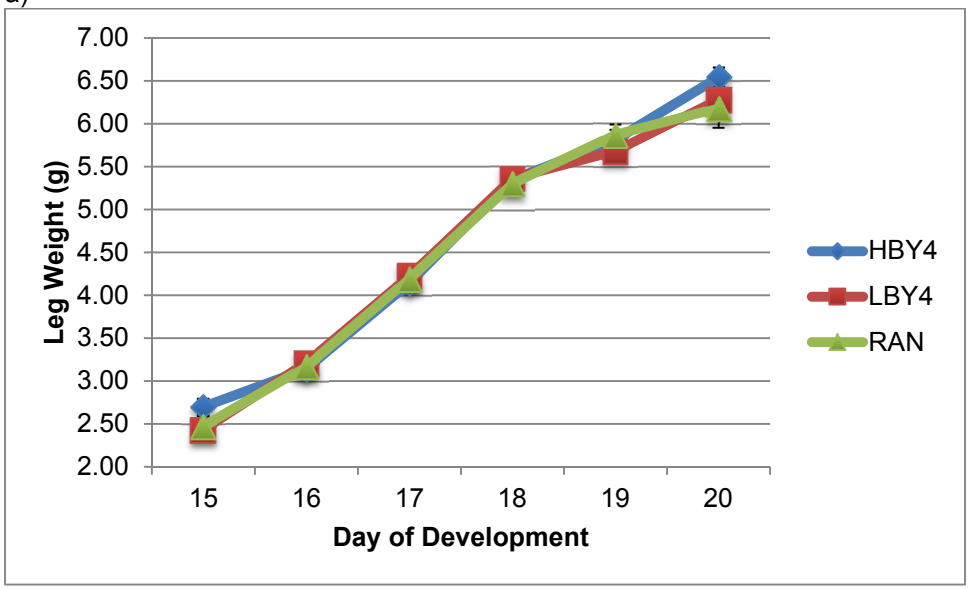
[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control.

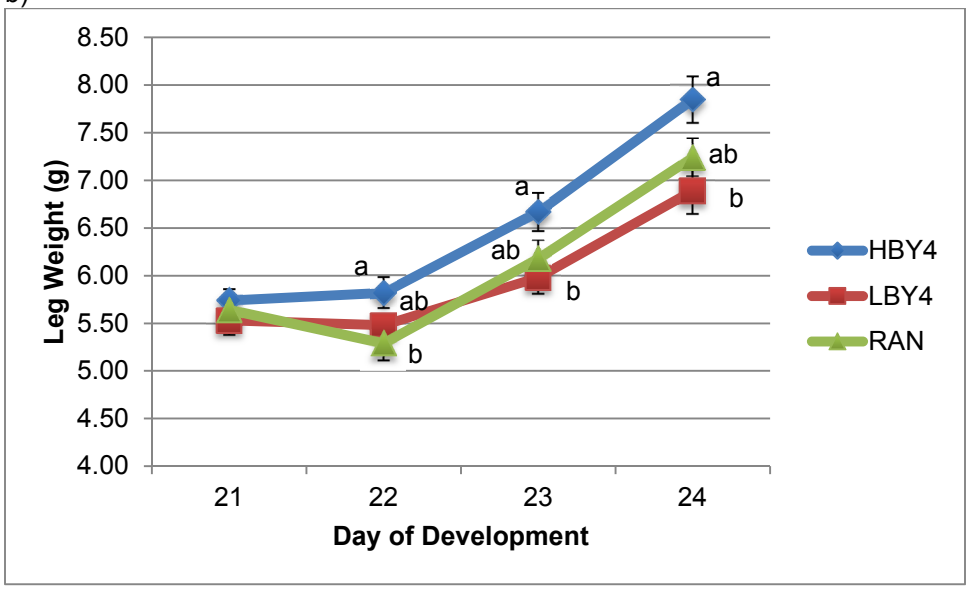
[a-b] Means within an age but across lines are different (P < .05).

Figure 2.10 Leg weight measures (mean ± SEM) collected from the BY4[1] lines[2] after four generations of divergent selection for BY4. (N ≥ 20 per line) Panel (a) includes 15 to 20 days, while (b) includes 21 to 24 days.

a)



b)



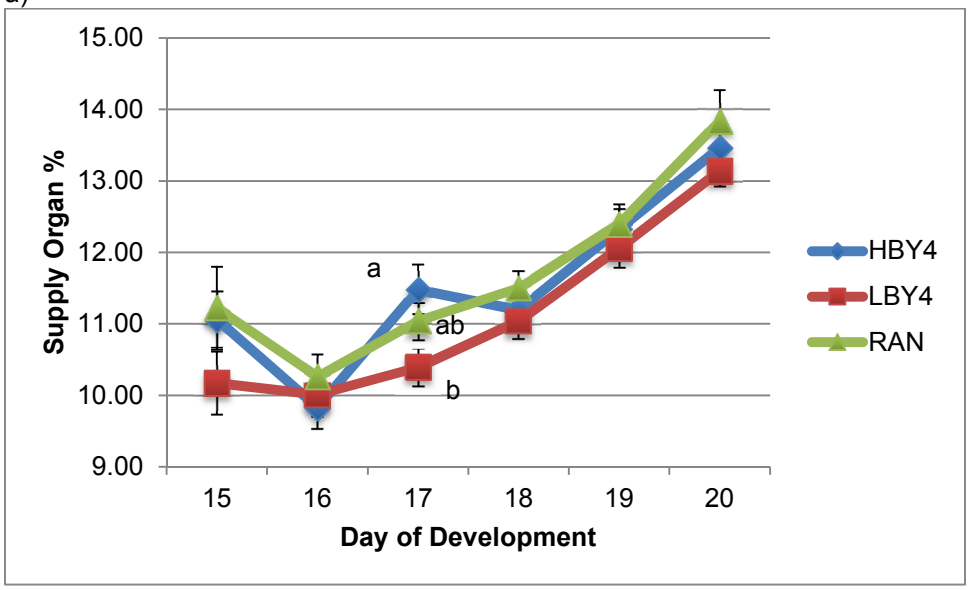
[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control.

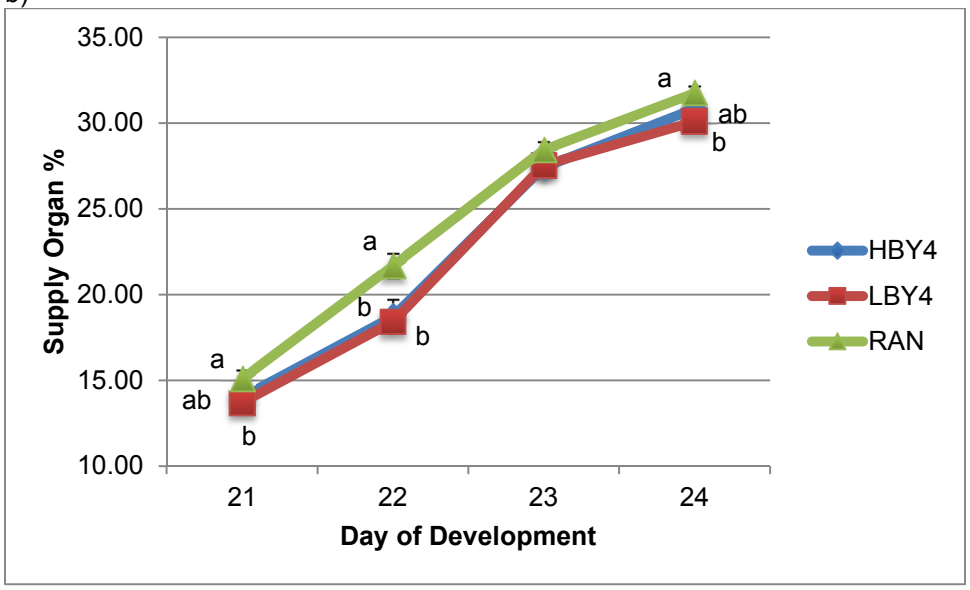
[a-b] Means within an age but across lines are different (P < .05).

Figure 2.11 Supply organ weight relative to live weight measures (mean ± SEM) collected from the BY4[1] lines[2] after four generations of divergent selection for BY4. (N ≥ 20 per line) Panel (a) includes 15 to 20 days, while (b) includes 21 to 24 days.

a)



b)



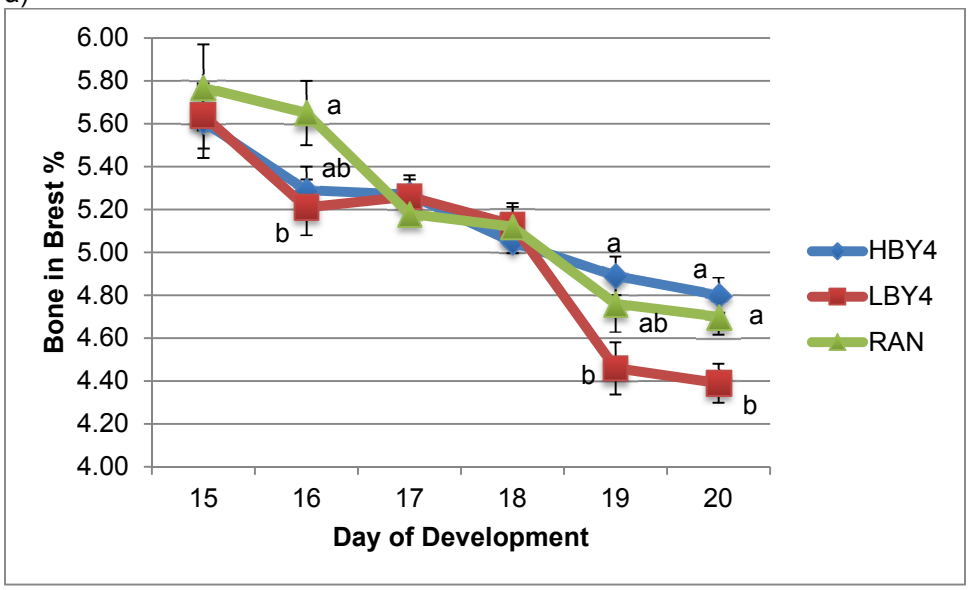
[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control.

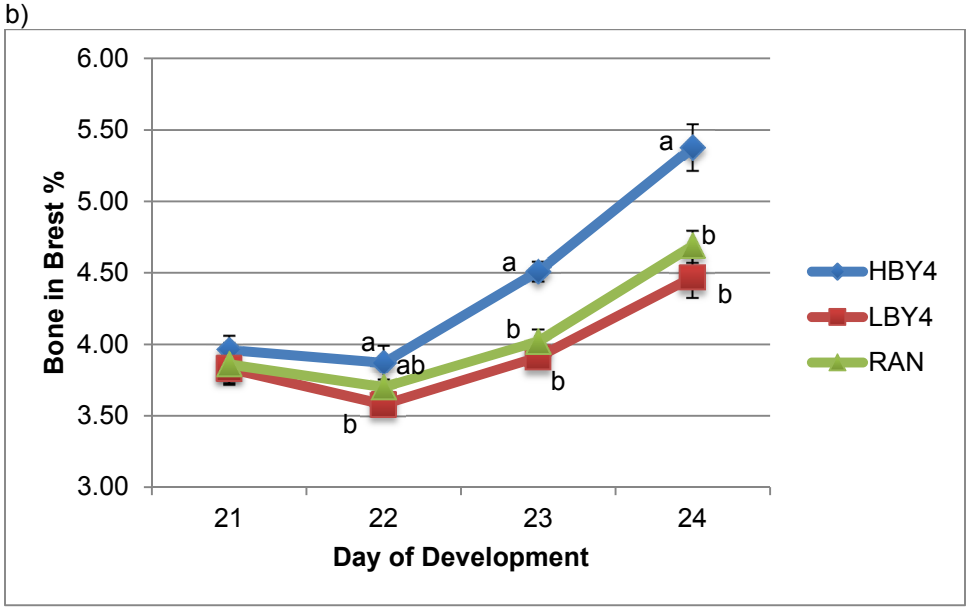
[a-b] Means within an age but across lines are different (P < .05).

Figure 2.12 Bone-in-breast weight relative to live weight measures (mean ± SEM) collected from the BY4[1] lines[2] after four generations of divergent selection for BY4. (N ≥ 20 per line) Panel (a) includes 15 to 20 days, while (b) includes 21 to 24 days.

a)



b)



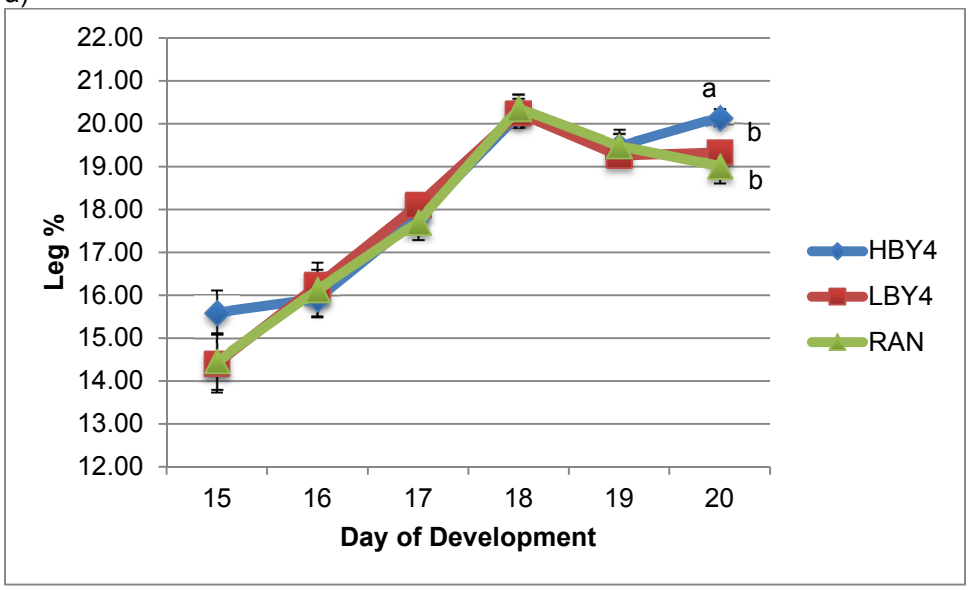
[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control.

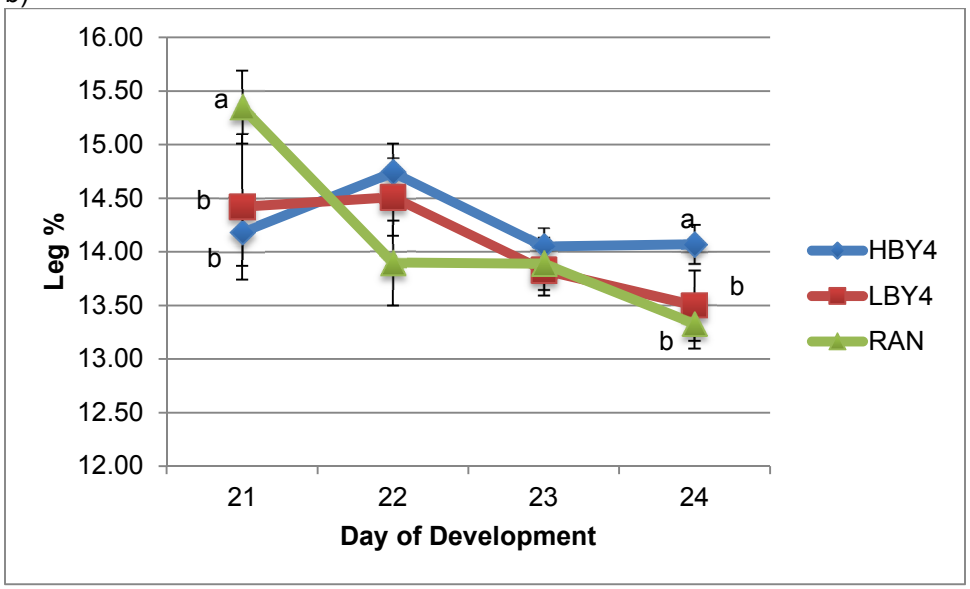
[a-b] Means within an age but across lines are different (P < .05).

Figure 2.13 Leg weight relative to live weight measures (mean ± SEM) collected from the BY4[1] lines[2] after four generations of divergent selection for BY4. (N ≥ 20 per line) Panel (a) includes 15 to 20 days, while (b) includes 21 to 24 days.

a)



b)



[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 are selected for high and low four-day % breast, respectively. RAN = unselected random bred control.

[a-b] Means within an age but across lines are different (P < .05).

2.6 Works Cited

Brown, S. C., and N. C. Stickland. 1994. Muscle at birth in mice selected for large and small body size. J. Anat. 184:371-380.

Case, L. A., B. J. Wood, and S. P. Miller. 2012. The investigation of ultrasound technology to measure breast muscle depth as a correlated trait to breast meat yield in turkey (Meleagris gallopavo). J. Anim. Sci. 90:3410-3417.

Deeb, N., and S. J. Lamont. 2002. Genetic architecture of growth and body composition in unique chicken populations. J. Heredity 93:107-188.

Dunnington, E. A., and P. B. Siegel. 1996. Long-term divergent selection for eight-week body weight in white Plymouth. Poult. Sci. 76:1121-1125.

Emmerson, D. A. 1997. Commercial approaches to genetic selection for growth and feed conversion in domestic poultry. Poult. Sci. 76:1121-1125.

Feldman, J. L., and F. E. Stockdale. 1992. Temporal appearance of satellite cells during myogenesis. Dev. Biol. 153:217-226.

Fiedler, I., G. Dietl, C. Rehfeldt, J. Wegner, and K. Ender. 2004. Muscle fibre traits as additional selection criteria for muscle growth and meat quality in pigs – results of a simulated selection. J. Anim. Breed. Genet. 121:331-344.

Gondret, F., L. Lefaucher, H. Juin, I. Louveau, and B. Lebret. 2006. Low birth weight is associated with enlarged muscle fiber area and impaired meat tenderness of the longissimus muscle in pigs. J. Anim. Sci. 84:93-103.

Gondret, F., L. Lefaucher, L. Louveau, B. Lebret, X. Pichodo, and Y. Le Cozler. 2005. Influence of piglet birth weight on postnatal growth performance, tissue lipogenic capacity and muscle histological traits at market weight. Livestock Prod. Sci. 93:137-146.

Halevy, O., A. Geyra, M. Barak, Z. Uni, and D. Sklan. 2000. Early posthatch starvation decreases satellite cell proliferation and skeletal muscle growth in chicks. J. Nutr. 130:858–864.

Halevy, O., Y. Nadel, M. Barak, I. Rozenboim, and D. Sklan. 2003. Early posthatch feeding stimulates satellite cell proliferation and skeletal muscle growth in turkey poults. J. Nutr. 133:1376–1382.

Halevy, O., S. Yahav, and I. Rozenbaum. 2006. Enhancement of meat production by environmental manipulations in embryo and young broilers. World's Poult. Sci. J. 62:485-497.

Handel, S. E., and N. C. Stickland. 1987. Muscle cellularity and birth weight. Anim. Prod. 44:311-317.

Harford, I. D., Pavlidis, H. O., and Anthony, N. B. 2014. Divergent selection for muscle color in broilers. Poult. Sci. *93*:1059-1066.

Hartley, R. S., E. Bandman, and Z. Yablonka-Reuveni. 1992. Skeletal muscle satellite cells appear during late chicken embryogenesis. Dev. Biol. 153:206-216.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and B. T. Larson. 1994. Growth, livability, and feed conversion of 1957 vs 1991 broilers when fed "typical" 1957 and 1991 broiler diets. Poult. Sci. 73:1785:1794.

Julian, R. J. 2005. Production and growth related disorders and other metabolic diseases of poultry–a review. Vet. J. 16:350-369.

Katanbaf, M. N., E. A. Dunnington, and P. B. Siegel. 1988. Allomorphic relationships from hatching to 56 days in parental lines and F_1 crosses of chickens selected 27 generations for high or low body weight. Growth Dev. Aging. 52:11-21.

Kuhlers, D. L., and G. R., McDaniel. 1996. Estimates of heritabilities and genetic correlations between tibial dyschondroplasia expression and body weight at two ages in broilers. Poult. Sci. 75:959-961.

Larzul, C., Lefaucheur, L., Ecolan, P., Gogue, J., Talmant, A., Sellier, P., and G. Monin. 1997. Phenotypic and genetic parameters for longissimus muscle fiber characteristics in relation to growth, carcass, and meat quality traits in large white pigs. J. Anim. Sci. 75:3126-3137.

Le Bihan-Duval, E., N. Millet, and H. Remignon. 1999. Broiler meat quality: effect of selection for increased carcass quality and estimates of genetic parameters. Poult. Sci. 78:822-826.

Le Bihan-Duval, C. Berri, E. Baeza, N. Millet and C. Beaumont. 2001. Estimation of the genetic parameters of meat characteristics and of their genetic correlations with growth and body composition in an experimental broiler line, Poult. Sci. 80:839-843.

Lilburn, M. S. 1994. Skeletal growth of commercial poultry. Poult. Sci. 73:897-903.

Luiting, P. 1990. Genetic variation of energy partitioning in laying hens: causes of variation in residual feed consumption. World's Poult. Sci. J. 46:133-152.

Madsen, P., and J. Jensen. 2000. A user's guide to DMU. A package for analysing multivariate mixed models. Version 6, release 4, Tjele, Denmark.

Mahon, M. 1999. Muscle abnormalities-morphological aspects in: Richardson, R.I. and Mead, G.C. (Eds) Poult. Meat Sci., Poult. Sci. Symposium Series, pp 19-64. Wallingford, CAB INTL.

Moss, F. P., R. A. Simmonds, and H. W. McNary. 1964. The growth and composition of skeletal muscle in the chicken. Poult. Sci. 43:1086–1091.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington DC.

Nestor, K. E., W. L. Bacon, Y. M. Salf, and P. A. Renner. 1985. The influence of genetic increases in shank width on body weight, walking ability and reproduction of turkey. Poult. Sci. 64:2248-2255.

Pavlidis, H. O., J. M. Balog, L. K. Stamps, J. D. Hughes, W. E. Huff, and N. B. Anthony. 2007. Divergent selection for ascites incidence in chickens. Poult. Sci. 86:2517-2529.

Rauw, W. M., E. Kanis, E. N. Noordhuizen-Stassen, and F. J. Grommers. 1998. Undesirable side effects of selection for high production efficiency in farm animals: a review. Livestock Prod. Sci. 56:15-33.

Rehfeldt, C., I. Fiedler, G. Dietl, and K. Ender. 2000. Myogenesis and postnatal skeletal muscle cellgrowth as influenced by selection. Livestock Prod. Sci. 66:177-188.

Rehfeldt, C., I. Fiedler, and N. C. Stickland. 2004. "Number and size of muscle fibers in relation to meat production" *Muscle Development of Livestock Animals*. Eds. M.F.W. tePas, M.E. Everts and H.P. Haagsman. CAB International.

Rehfeldt, C., and G. Kuhn. 2006. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J. Anim. Sci. 84S:E113-E123.

SAS Institute Inc. 2001. SAS User's Guide. SAS Inst. Inc., Cary, NC.

Sefton, A. E.,and Siegel, P. B. 1974. Inheritance of body weight in Japanese quail. Poult. Sci. 53:1597-1603.

Siegel, P. B. 2002. Body weight, obesity, and reproduction in meat-type chickens. Avian and Poult. Biol. Rev., 13:209-213.

Siegel, P. B., C. F., Honaker, and W. M., Rauw. 2009. Selection for high production in poultry. In: W.M. Rauw (Ed.), "*Resource allocation theory applied to farm animal production.*" CAB Intl. Wallingford, UK, pp. 230-236.

Smith, J. H. 1963. Relation of body size to muscle cell size and number in the chicken. Poult. Sci. 42:283-290.

Tierce, J. F., and A. W. Nordskog, 1985. Performance of layer-type chickens as related to body conformation and composition. 1. A static analysis of shank length and body weight at 20 weeks of age. Poult. Sci. 64:605-609.

Velleman, S. G., K. E. Nestor, C. S. Coy, I. Harford, and N. B. Anthony. 2010. Effect of posthatch feed restriction on broiler breast muscle development and muscle transcriptional regulatory factor gene and heparin sulfate proteoglycan expression. Int. J. Poult. Sci. 9:417-425.

Wigmore, P. M. C., and N. C. Stickland. 1983. Muscle development in large and small pig fetuses. J. Anat. 137:235-245.

Yalcin, S., S. Özkan, E. Coskuner, G. Bilgen, Y. Delen, Y. Kurtulmus, and T. Tanyalcin, 2001. Effects of strain, maternal age and sex on morphological characteristics and composition of tibial bone in broilers. Brit. Poult. Sci. 42:184-190.

APPENDIX

UNIVERSITYOF

ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Nicholas Anthony
FROM: Craig N. Coon, Chairman Institutional Animal Care And Use Comittee

DATE: May 23, 2012
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: April 6, 2015

The Institutional Animal Care and Use Committee (IACUC) has APPROVED Protocol #12038- "GENERAL REARING OF SELECTED CHICKEN AND QUAIL POPULATIONS ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] prior to initiating the changes. If the study period is expected to extend beyond 04-06-2015, you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

ene/car

cc: Animal Welfare Veterinarian

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3. Growout of broilers divergently selected for relative breast yield at four-days of age and correlated responses.

Abstract

Genetic selections relying on muscle fiber growth have vastly improved production related traits in the modern broiler. Today's broiler reaches a greater market weight at an earlier age than ever before. Regrettably, the improved performance has come at the expense of broiler fitness with the modern broiler experiencing an increase in physiological disorders. In addition meat quality issues are becoming more prevalent as traditional selection methods have been shown to correlate with reduced meat quality. To combat these issues while still promoting growth divergent selection for four-day relative breast yield (BY4) were undergone.

After 4 generations of divergent selection for BY4 in broilers, growth rate and other correlated responses were examined. Included in this study are the two divergently selected BY4 lines (HBY4 and LBY4) and the random bred control (RAN) progenitor line. Divergent selection led to improvements in early growth rate allowing the HBY4 to outperform the LBY4 in both body weight and relative breast yield from 4 to 28 days posthatch. While the impact of divergent selection diminished prior to processing age, the HBY4 exhibited a greater percentage breast than the LBY4 at 42 and 56 days posthatch. The improvements in body composition came without a sacrifice in meat quality with no difference in meat quality being shown between the HBY4, LBY4 and RAN lines.

3.1 Introduction

Rapid genetic progress has been made in meat-type broilers via intensive selections for production traits such as, body weight, growth rate and breast yield (Deeb and Lamont, 2002). The rate of progress intensified as poultry breeders and producers moved from a dual-purpose chicken, selected for both yield and egg production, to the stocks seen today, selected either for egg production or meat yield (Warren, 1996). The broiler today, as a product of selection, has market age body weight approximately three times its predecessor from 50 years ago (Havenstein et al., 1994). Furthermore, market age has continued to decrease roughly one day per generation, thus drastically reducing the days to market (Pollock, 1999)

Improvements in average daily gain and yield are primarily attributable to changes in broiler muscle mass (Hooper et al., 1973). Muscle growth relies not on one, but two major components, hypertrophy and hyperplasia (Stickland and Dwyer, 1996). These gains were made possible by hypertrophic muscle growth (Velleman and McFarland, 2014). Muscle hypertrophy is the expansion of muscle by gains in muscle fiber size (Velleman and McFarland, 2014). Muscle hypertrophy occurs posthatch and relies on the uptake of satellite cell nuclei (Moss and Leblond, 1971). Satellite cells are used to meet the additional demand for nuclei associated with increased fiber size (Goldspink, 2004). While hypertrophy has led to significant gains in growth, increases in growth rate and yield have resulted in reduced fitness (Katanbaf et al., 1988; Dunnington and Siegel, 1996), increased incidence of physiological disorders (Julian, 2005), and reduced meat quality.

Hyperplasia is muscle growth resulting from an increase in muscle fiber number (Rehfeldt et al., 2004). Hyperplasia occurs almost entirely before birth, and is a major determinant of animal performance (Stickland and Handel, 1986; Rehfeldt et al., 1987, 2000). Increased muscle fiber has been suggested as a primary factor in improving muscle growth and meat quality (Stickland and Dwyer, 1996). Inversely, in swine it was shown that piglets with low birth weights had a lower muscle fiber number (Rehfeldt and Kuhn, 2006) and that fiber size increases rapidly when fiber number is low (Rehfeldt et al., 2000). Rapid fiber growth may lead to extreme fiber size and abnormal giant fiber formation, which are known to contribute to poor meat quality in swine and poultry (Klosowska et al., 1979; Fiedler et al., 1999).

Selections for four-day relative breast yield (BY4) are thought to promote both growth and meat quality. Increased muscle fiber number is a trait positively related to muscle growth and quality (Lengerken et al., 1994; Ender et al., 2000). Furthermore, this selection targets a point near maximum muscle cell number per gram of muscle tissue and when satellite cell activity is high (Halevy et al., 2006). Increasing satellite numbers may also promote muscle hypertrophy by increasing the supply of nuclei needed to make gains in fiber size. This combination may prove beneficial to both meat quality and muscle growth.

Divergent selection for BY4, allows for the examination of genetic relationships between muscle growth, meat quality and broiler fitness and other correlated responses that may occur. The two primary

goals of this research were: (1) the effects of selection on muscle growth, and broiler fitness, and (2) determine how broiler meat quality has been impacted by divergent BY4 selection.

3.2 Materials and Methods

Broiler Populations

The populations used for this study were three research broiler lines currently maintained at the University of Arkansas. These lines were divergently selected for BY4 from a random bred control population initiated in 1997 (Harford et al., 2014). In 2012, divergent selection for BY4 was initiated using a sibling selection protocol previously described in Chapter 2. Broilers utilized in the current study originate from selected breeders from the third generation of BY4 selections. These lines are identified as the high breast yield (HBY4), selected for increased percentage breast yield at four-days of age, low breast yield (LBY4), selected for decreased percentage breast yield at four-days of age and the unselected random line (RAN).

Growth Characteristics

To assess how divergent BY4 selection has impacted broiler performance during growout, three one week hatches were obtained from the selected breeders from generation three HBY4, LBY4, and RAN lines. Since we were sampling from a subset of each line it was necessary to generate three hatches to increase sample size and add replication to the study. Data was collected on hatch weight, supply and support systems, as described by Katanbaf and coworkers (1988). Incubation and hatching were performed under standard conditions.

Hatching and Placement

Upon hatch, chicks were pedigreed; wing-banded and weighed. Birds from hatch one and hatch three were reared straight run, and all lines were reared together with roughly 100 birds per pen. Birds from hatch two were grown in cages in a controlled environment. All broilers were provided feed and water *ad libitum* with a starter and finisher ration formulated to meet or exceed NRC (1994) minimum standards. Birds chosen for use in feed conversion ratio (FCR) study were placed in 36 cages, 12 cages

per line. These birds were weighed weekly beginning at eight days of age and continuing up to 42 days of age. In addition, feed consumption was recorded weekly from Day 8 to processing to calculate FCR.

Sample

Birds were sampled at 4, 7, 14, 28, 42, and 56 days of age posthatch. Birds sampled (N ≈ 12 per line per sampling) were taken equally from hatch one and two and each pen at each processing age. Birds from hatch three were used to increase the sample size of the early processings and were sampled at 4, 7, and 14 days of age.

Processing

Prior to euthanasia feed was withdrawn, broilers sampled prior to Day 42 were euthanized via CO2 asphyxiation. Crop contents were surgically removed to minimize the confounding effects of crop contents on body weight. Sampled broilers were then weighed and placed on trays and allowed to refrigerate at 1º C for a minimum of three hours. This was done to promote the onset of rigor mortis and facilitate easy removal of muscle.

Necropsies were performed after the three-hour chill. An incision was made perpendicular to the base of the keel and the skin retracted to reveal the chest cavity. Two cuts were then made along the rib cage toward the clavicle. Cuts were then made along the left and right clavicle to allow for the removal of the bone in breast. Gender was assessed/confirmed for all sampled birds by gonadal examination. Weights were obtained for the gastrointestinal tract, heart, liver, spleen and abdominal fat. The breast and tenders were removed from the bone in breast, then individually weighed and recorded. The skin was removed from the legs of birds sampled prior to Day 42. After removal of the skin, shanks were weighed and shank length recorded (mm). The remaining leg portions were cut into thigh and drum, weighed and recorded. The muscle was separated from the tibia and femur, the tibia and muscle were weighed and recorded. The tibia length, width and height (mm) were then recorded. Data collected on parts yield are expressed as absolute values and as part weight relative to live weight.

Samplings at 42 and 56 days posthatch were conducted at the University of Arkansas processing plant. Processing involved feed removal 12 hours prior to slaughter. Birds were then transported to the

processing plant one hour prior to slaughter. Birds were recorded, weighed and hung on shackles. The shackles then proceeded through the plant, where birds were electrically stunned, bled out via the left carotid artery and jugular vein, scalded, and mechanically defeathered. Birds were then eviscerated by hand to facilitate weighing of the gastro-intestinal tract and abdominal fat. After evisceration, the WOGs were allowed to chill for four hours. WOGs were then re-weighed and recorded, cut into parts, and weights obtained for breast, tenders, keel, wing, drum, and thigh.

Muscle Color, PH, and Drip Loss

For samplings on Day 42 and 56, the deboned breasts were individually bagged and refrigerated at 1ºC. Twenty-four hours post-slaughter breasts were removed from cold storage and blotted dry. The right lobe of the pectoralis major was used for color measurements using a Minolta CR-300 Colorimeter (Minolta Chroma Meter CR-300, Minolta Italia S.P.A., Milano, Italy) to obtain L* value, color measurements were done at the top, center and base of the lobe. PH measurements were taken from the right lobe using a Testo 205 pH probe.

Drip loss measurements were obtained via the EZ Driploss method (Rasmussen and Andersson, 1996). A drill was used to obtain circular cores from the right lobe of the pectoralis major. Samples from the right lobe were then placed individually in driploss tubes, and stored an additional 24 hours at 1º C. At 48 hours post slaughter, the test tubes were weighed to obtain the weight of the combined sample-tube weight. The sample was removed from the test tube and the tube reweighed to obtain the combined weight of the liquid and tube. EZ driploss (Correa et al., 2007), was then calculated as follows:

% Driploss = ((Tube with liquid WT) / (Tube with meat and liquid WT - Tube WT)) * 100

Tenderness

The left lobe of the pectoralis major was used to assess tenderness. Left lobes were cooked in an oven at 190º C until reaching an internal temperature of 74º C. Breasts were then allowed to cool to room temperature prior to further testing. Six 1.27-cm diameter cores were then removed from each breast parallel to the muscle fiber orientation. These cores were sheared through the center with a Warner-

Bratzler shear apparatus attached to an Instron 4466 (Instron Corp., Canton, MA) with a 55-kg load cell with a crosshead speed of 250 mm/min (Burke et al., 2003).

Data Analysis

Data from the growout phase was analyzed by processing age with line as the main effect, to assess body weight, carcass composition, and carcass characteristics. Trait means separation was performed via the GLM procedure along with Duncan's multiple range test using SAS 9.3 (SAS Institute Inc., 2001, Cary, NC). An alpha level of .05 was determined to assign significance.

3.3 Results

BY4 Line Growth from Days 4 to 28.

Body weights from birds sampled at 4, 7, 14 and 28 days posthatch showed no difference between the HBY4 and RAN lines (Table 3.1). The HBY4 consistently had a greater body weight than the LBY4 line. The RAN line had a greater body weight than the LBY4 at 7 days, but the lines did not differ in body weight at days 4, 14 and 28 (Table 3.1). The HBY4 line had a greater breast weight than the RAN line up to Day 7, at which point it failed to differ (Table 3.1). The HBY4 outperformed the LBY4 in breast weight through Day 7 to 28. The RAN line was intermediate for breast weight, showing no difference in breast weight between it and either the HBY4 or LBY4 lines past Day 7 (Table 3.1). Tender weight was greater in the HBY4 line than the LBY4 from days 4 to 28, but not different than the RAN from 4 to 28 (Table 3.1). The RAN line outperformed the LBY4 in tender weight at 4, 7, and 28 days of age (Table 3.1). Total breast weight was lower in the LBY4 than the HBY4 from 4 to 28 days (Table 3.1). Keel weight was greater at Day 4 in the HBY4 compared to the RAN and LBY4 lines and greater than the LBY4 at Day 14 (Table 3.1). Keel weight did not differ between the LBY4 and RAN from 4 to 28 days (Table 3.1) The HBY4 had greater total leg weight than the LBY4 line from Day 4 to 14; it also had a greater leg weight than the RAN line on Day 14 (Table 3.1). No differences were found for leg weight between lines at 28 days posthatch (Table 3.1).

Total supply organ weight, as well as heart, was greater in the HBY4 line when compared to the LBY4 line at four days of age with heart weight continuing to be greater till 14 days posthatch (Table 3.2).

Differences in supply organ weight were found at 4 and 7 days, but no trend was apparent and by Day 14, there were no differences in supply organ weight (Table 3.2).

Percentage breast was greater in the HBY4 line than the LBY4 line from Day 4 to 28 and greater than the RAN line from Day 4 to 14 (Table 3.3). Breast percentage was higher in the RAN than the LBY4 at 4 days of age, at which point it failed to differ (Table 3.3). Total breast percentage followed a similar trend with the HBY4 outperforming the LBY4 at all ages and the RAN at 7 and 14 days (Table 3.3). The RAN had a greater total breast percentage than the LBY4 at days 4 and 7 (Table 3.3). Keel weight relative to live weight differed at 4 days of age with the HBY4 outperforming the RAN and LBY4 (Table 3.3).Leg percentage was greater in the HBY than both the RAN and LBY lines from days 4 to 14 (Table 3.3). Leg percentage was highest in the HBY4 line at 7 and 14 days, but no difference in leg percentage was found between the lines at 28 days (Table 3.3). Leg percentage did not differ between the RAN and LBY4 from 4 to 28 days (Table 3.3).

Despite a difference in heart weight at 4 days of age, heart percentage relative to live weight only differed at Day 28, with the LBY4 having a greater heart percentage than the HBY4 (Table 3.3). The RAN line had a greater supply organ percentage than the HBY4 line at 7 days of age (Table 3.3). However, no other difference in supply organ percentage was found at 14 or 28 days (Table 3.3). No difference was found in abdominal fat weight or percentage at either 14 or 28 days posthatch (data not presented).

Processing Age Characteristics of BY4 Lines

No difference in lifetime FCR was found between the lines with the HBY4, LBY4 and RAN having FCR values of 1.94, 1.99, and 1.95, respectively. Body weight did not differ between the three lines at both 42 and 56 days posthatch (Table 3.4). WOG weight also did not differ between the lines at days 42 and 56 (Table 3.4). WOG post-chill was greater in the RAN line the LBY4 at 42 days, but did not differ from the HBY4 (Table 3.4). Water uptake weight was greater in the RAN, than either the other two lines at 42 days (Table 3.4). Rack weight was not different at 42 or 56 days posthatch (Table 3.4). Leg weights were not different between lines at days 42 or 56 (Table 3.4). Abdominal fat was greater in the RAN line than the HBY4 line at 42 days of age, but no differences were seen at 56 days posthatch (Table 3.4).

The RAN line also had the greatest supply organ weight at 56 days posthatch (Table 3.4). Breast weight was greater in the RAN line at 42 days of age than the LBY4 line, but not different than the HBY4 line (Table 3.4). Total breast weight was greater in the RAN and HBY4 than the LBY4 at 42 days (Table 3.4). Any difference in breast and total breast weight diminished by 56 days posthatch with no differences being shown for either trait (Table 3.4).

When considering the live weight percentages of various traits, numerous differences were found. No difference was found in supply organ percentage at 42 days of age, though at 56 days the RAN line had a greater supply organ percentage than either the HBY4 or LBY4 lines (Table 3.5). Additionally the RAN line had a greater abdominal fat percentage than the HBY4 line at both 42 and 56 days posthatch (Table 3.5). Leg percentage showed no differences between lines at either 42 or 56 days posthatch (Table 3.5). Rack percentage showed no difference at 42 days of age, but the HBY4 line had a greater rack percentage than the LBY4 line at 56 days of age (Table 3.5). Rack percentage failed to differ between the RAN and HBY4 at either processing age (Table 3.5). The HBY4 had a greater breast and total breast percentage than the LBY4 at both processing ages, but did not differ from the RAN at the same ages (Table 3.5).

It is important to consider how divergent selection for BY4 impacted the meat quality of the BY4 lines. Differences were found at 42 days of age, but no differences were found at 56 days posthatch. At 42 days of age the RAN line had a greater muscle color lightness value (L*) than the HBY4 line, but not the LBY4 line (Table 3.6). No difference in shear force was found between the lines at 42 or 56 days (Table 3.6). The RAN line had a greater drip loss percentage at 42 days of age than the HBY4 line but did not differ from the LBY4 (Table 3.6). No differences were shown in drip loss percentage at 56 days of age (Table 3.6). No differences in pH were shown between the lines at either 42 or 56 days (Table 3.6).

3.4 Discussion

Development of BY4 Lines from Days 4 to 28.

Results indicate that selection for increased BY4 promoted both breast and leg percentage at early ages, and body weight. The HBY4 line having a greater body weight and breast percentage than the LBY4 line from ages 4 to 28 and a greater leg percentage from 4 to 14 days posthatch shows this growth

difference. Furthermore, the HBY4 line had greater breast and total breast percentage than the RAN line up to Day 14 and a greater leg percentage at 7 and 14 days posthatch. The growth impact appeared to diminish as the bird aged. At Day 28, the HBY4 line no longer had a greater percentage breast than the RAN line and did not outperform either of the other two BY4 lines in leg percentage.

The change in growth may be an artifact of growth posthatch being based entirely on hypertrophy (Moss and Leblond, 1971), with maximum growth being obtained through a balance between muscle fiber number and size. As only four generations of divergent selection for BY4 have occurred it will be of interest to see how these selections impact satellite cell and muscle fiber number and the resulting impact on the growth of future generations. Theoretically if the HBY4 line continues to make gains in early BY4, it will increase both the muscle fiber number and satellite cell activity leading to further divergence between the HBY4 and LBY4 lines and growth differences between the HBY4 and Ran lines. Increased growth being made possible by providing a better starting point for growth by improving muscle fiber number and available satellite cells needed for increased gain via hypertrophy. While the LBY4 line may continue to decline in percentage breast and body weight, resulting in further divergence from the RAN line.

Research has shown that faster-growing animal strains have higher numbers of muscle fibers than slower growing strains of the same species (Ezekwe and Martin, 1975; Miller et al., 1975). These animals also exhibit improved FCRs when compared to slower-growing strains (Campbell and Taverner, 1988). Contrary to these results FCR over the growout period was equivalent amongst the three BY4 lines. Short-term divergent selection BY4 may not yet have resulted in enough divergence to see the effect on FCR at this point. Alternatively, hypertrophic growth may have obscured potential differences in FCR. Further selection and testing will be needed to assess the effect of BY4 selection on FCR values.

Processing Age Characteristics of BY4 Lines

Results indicate that while the effects of short-term divergent selection for BY4 had diminished by 42 days posthatch, they were present. The increased breast and total breast percentage of the HBY4 line in comparison to the LBY4 line evidence this at 42 and 56 days. Theoretically if selection for BY4 promotes muscle fiber number and satellite cell activity, leg yield percentage should have improved, as

well as breast yield percentage. These gains are contrary to traditional gains in breast yield, which do not result in proportional gains in leg percentage (Nestor et al., 1985; Lilburn, 1994; Yalcin et al., 2001). It may be argued that this increase did, in fact, occur. As evidenced by the HBY4 line having a greater breast yield percentage than the LBY4 line at 42 and 56 days of age while maintaining a leg percentage equal to that of the LBY4 line. Unfortunately, this growth boost may have occurred at the cost of the supply systems of the HBY4 broiler with a lesser supply organ percentage when compared to the RAN at 56 days. This imbalance between supply and demand systems is consistent with research indicating that increased growth comes at the cost of the broiler's supply system (Deeb and Lamont, 2002). The lack of difference in supply organ percentage between the HBY4 and LBY4 may be indicative of divergent selection negatively impacting the supply systems of the LBY4.

Meat quality was neither promoted nor negatively impacted by short-term divergent selection for BY4. While muscle color lightness values (L*) differed between the HBY4 and RAN lines at 42 days of age, no difference was shown between lines at 56 days posthatch. The values collected were consistent with research showing mean L* value for poultry meat not affected by PSE or DFD ranging from 49.70 in broilers processed at 42 days (Harford et al., 2014) to 50.16 also processed at 42 days of age (Zhang and Barbut, 2005). PH measurements at both processing ages were equivalent between the BY4 lines and were consistent with mean pH values from unaffected poultry meat, from 5.88 (Harford et al., 2014) to 5.91 (Zhang and Barbut, 2005). Shear force values obtained were higher than shear force values obtained from broilers processed at between either 42-45 days and 49-52 days of age, 1.82 – 2.19 kg/cm^2 (Lyon et al., 2004), but lower than those obtained from broilers processed at 7 weeks of age and chilled between 2.5 and 3.5 hours ranging from 6.22 to 7.34 kg/cm^2 (Cavitt et al., 2004). Indicating that shear force values for all lines were consistent with values typically seen in poultry. Drip loss percentage was higher than values calculated from broilers processed between 35 and 42 days and measured at 3 days post slaughter, 0.82 - 0.90 percent drip loss and at 6 days post slaughter, 1.59 to 1.74 percent (Berri et al., 2001). The drip loss measurements were also higher than values calculated in three research broiler lines processed at 46 days of age and stored for 24 hours postmortem, 0.88 – 2.27 percent (Harford et al., 2014). Differences in drip loss are believed to be due to differing storage times and the novel method of obtaining drip loss.

Selection did result in less fat percentage in the HBY4 line, having a lower fat percentage than the RAN line at 42 days and the LBY4 line at 56 days of age. While it did not differ from the LBY4 at 42 days and RAN at 56, it had a tendency to be lower than the values obtained for both of these lines. These results are consistent with results of simulated selection for muscle fiber number in swine, which showed selection for fiber number would promote growth (Scheuermann et al., 2004), while minimizing potential negative effects on meat quality (Fiedler et al., 2004).

In summary, divergent selection for BY4 had a significant impact on broiler muscle growth, and minimal impact on broiler fitness and muscle quality. Broilers from the HBY4 line exhibited greater body weights than the LBY4 line from Day 4 up to Day 28. The HBY4 also exhibited greater breast percentage than the LBY4 from 4 to 28 days posthatch, without experiencing declines in leg and supply organ percentage when compared to the LBY4 line, suggesting selection was effective in promoting growth via the creation of additional resources needed to promote growth. However, these gains were temporary, and no difference in body weight was shown between lines at processing age. This may be an effect of posthatch growth relying on hypertrophy (Velleman and McFarland, 2014) and masking differences attributable to changes in muscle fiber number.

Selection was successful in impacting the breast percentage of processing age broilers with the HBY4 outperforming the LBY4. Furthermore, breast yield did not come at the expense of leg percentage. Growth may have impacted supply organ percentage, as the RAN had a greater supply organ percentage than the HBY4 at 56 days, but the HYB4 was not different from the LBY4. Additional growth failed to negatively impact meat quality, as shown by the similarity in meat quality characteristics of the BY4 lines. Consistent with research showing that muscle fiber number is positively correlated with improved meat quality and growth (Fiedler et al., 1993, 2003; Lengerken et al., 1994; Karlsson et al., 1999; Suzuki et al., 2003).

To improve comprehension of how early growth selections and muscle fiber number impacts broiler growth, the divergent lines were formed. The maintenance of these populations at the University of Arkansas will allow for continued research to determine the underlying causes of changes in BY4 and provide a valuable resource population for research into muscle growth.

3.5 Figures and Tables

Table 3.1 Body and breast related trait weight measures (g) (means ± SEM) collected up to 28 days posthatch from the BY4[1] lines after 4 generations of divergent selection. (N ≥ 24 per line)

		Line Designation[2]		
Trait	AGE	HBY4	RAN	LBY4
BWT	4	71.97 ± 2.13a	67.97 ± 1.9ab	63.27 ± 1.95b
	7	107.12 ± 2.14a	107.05 ± 2.65a	100.46 ± 2.14b
	14	272.94 ± 7.43a	262.48 ± 5.84ab	248.28 ± 7.21b
	28	917.04 ± 23.23a	889.66 ± 22.43ab	832.60 ± 28.94b
Breast	4	2.63 ± 0.17a	2.20 ± 0.15b	1.64 ± 0.13c
	7	6.72 ± 0.23a	5.85 ± 0.31b	5.04 ± 0.23c
	14	24.63 ± 0.93a	22.26 ± 0.76ab	20.89 ± 0.91b
	28	108.41 ± 3.86a	101.39 ± 3.71ab	91.61 ± 5.05b
Tenders	4	0.65 ± 0.05a	0.60 ± 0.05a	0.45 ± 0.03b
	7	1.87 ± 0.09a	1.69 ± 0.11a	1.40 ± 0.06b
	14	6.44 ± 0.23a	5.82 ± 0.19ab	5.29 ± 0.31b
	28	28.17 ± 0.94a	26.23 ± 1.03a	22.77 ± 1.13b
TBRST[3]	4	3.28 ± 0.22a	2.80 ± 0.19a	2.09 ± 0.16b
	7	8.59 ± 0.28a	7.54 ± 0.34b	6.44 ± 0.28c
	14	31.08 ± 1.15a	28.07 ± 0.94ab	26.18 ± 1.16b
	28	136.58 ± 4.68a	127.62 ± 4.57ab	114.37 ± 6.11b
Keel[4]	4	1.91 ± 0.09a	1.60 ± 0.08b	1.42 ± 0.06b
	7	3.05 ± 0.12	3.11 ± 0.16	2.92 ± 0.12
	14	10.75 ± 0.54a	9.48 ± 0.35ab	9.27 ± 0.47b
	28	39.29 ± 1.92	38.46 ± 1.54	34.61 ± 1.59
Leg	4	10.70 ± 0.42a	9.88 ± 0.37ab	9.10 ± 0.42b
	7	15.66 ± 0.43a	14.96 ± 0.52ab	14.03 ± 0.40b
	14	45.60 ± 1.53a	41.68 ± 1.15b	39.54 ± 1.44b
	28	169.60 ± 5.65	161.38 ± 5.13	153.46 ± 6.33

[a-c] Means for a trait within an age but across lines are different (P < .05).

[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 lines are selected for high and low % breast at four days of age, respectively. RAN = unselected random bred control.

[3] TBRST = Total Breast (Breast + Tenders)

[4] Keel = Skeletal support of the breast post breast removal

Table 3.2 Supply related trait weight measures (g) (mean ± SEM) collected up to 28 days posthatch from the BY4[1] lines after4 generations of divergent selection. (N ≥ 24)

		Line Designation[2]		
Trait	AGE	HBY4	RAN	LBY4
Heart	4	0.65 ± 0.03a	0.57 ± 0.02b	0.56 ± 0.02b
	7	0.92 ± 0.02a	0.90 ± 0.03ab	0.84 ± 0.03b
	14	2.30 ± 0.08a	2.07 ± 0.09b	2.05 ± 0.08b
	28	6.13 ± 0.25	6.07 ± 0.22	6.04 ± 0.29
Supply Organs	4	21.00 ± 0.69a	20.07 ± 0.76ab	19.00 ± 0.50b
	7	30.82 ± 0.81ab	32.51 ± 0.87a	30.03 ± 0.78b
	14	60.45 ± 1.93	59.74 ± 1.67	56.49 ± 1.83
	28	131.73 ± 3.36	127.55 ± 3.70	124.46 ± 4.35

[a-b] Means within an age but across lines are different (P < .05).

[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 lines are selected for high and low % breast at four days of age, respectively. RAN = unselected random bred control.

Table 3.3 Relative weights (%) (mean ± SEM) of breast, leg and organ weights collected up to 28 days posthatch from the BY4[1] lines after 4 generations of divergent selection. (N ≥ 24)

		Line Designation[2]		
Trait	AGE	HBY4	RAN	LBY4
Breast	4	3.58 ± 0.15a	3.16 ± 0.15b	2.51 ± 0.13c
	7	6.24 ± 0.14a	5.35 ± 0.21b	4.95 ± 0.15b
	14	8.95 ± 0.17a	8.41 ± 0.15b	8.30 ± 0.18b
	28	11.79 ± 0.24a	11.37 ± 0.27ab	10.85 ± 0.30b
TBRST[3]	4	4.47 ± 0.19a	4.01 ± 0.20a	3.21 ± 0.16b
	7	7.99 ± 0.18a	6.96 ± 0.21b	6.33 ± 0.18c
	14	11.30 ± 0.20a	10.62 ± 0.20b	10.41 ± 0.24b
	28	14.87 ± 0.29a	14.31 ± 0.31ab	13.57 ± 0.36b
Keel[4]	4	2.65 ± 0.10a	2.34 ± 0.09b	2.25 ± 0.07b
	7	2.83 ± 0.08	2.88 ± 0.12	2.89 ± 0.10
	14	3.89 ± 0.14	3.61 ± 0.11	3.71 ± 0.13
	28	4.31 ± 0.20	4.33 ± 0.14	4.22 ± 0.20
Leg	4	14.80 ± 0.26	14.47 ± 0.26	14.16 ± 0.28
	7	14.60 ± 0.25a	13.89 ± 0.24b	13.93 ± 0.21b
	14	16.63 ± 0.21a	15.83 ± 0.18b	15.81 ± 0.19b
	28	18.43 ± 0.24	18.13 ± 0.33	18.37 ± 0.29
Heart	4	0.91 ± 0.02	0.84 ± 0.02	0.90 ± 0.03
	7	0.86 ± 0.02	0.84 ± 0.02	0.84 ± 0.02
	14	0.84 ± 0.02	0.79 ± 0.03	0.83 ± 0.03
	28	0.67 ± 0.02b	0.69 ± 0.02ab	0.73 ± 0.02a
Supply Organs	4	29.42 ± 0.81	29.53 ± 0.73	30.27 ± 0.64
	7	28.75 ± 0.48b	30.44 ± 0.46a	29.96 ± 0.57ab
	14	22.33 ± 0.56	23.03 ± 0.69	23.06 ± 0.67
	28	14.49 ± 0.39	14.43 ± 0.38	15.13 ± 0.47

[a-c] Means for a trait within an age but across lines are different (P < .05).

[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 lines are selected for high and low % breast at four days of age, respectively. RAN = unselected random bred control.

[3] TBRST = Total Breast (Breast + Tenders)

[4] Keel = Skeletal support system of the breast post breast removal

Table 3.4 Processing trait weight measures (g) (mean ± SEM) of BY4[1] broilers processed at 42 and 56 days posthatch after 4 generations of divergent selection. (N ≥ 55)

		Line Designation[2]		
Trait	AGE	HBY4	RAN	LBY4
BWT	42	1825 ± 26	1866 ± 27	1806 ± 26
	56	2424 ± 45	2474 ± 45	2446 ± 38
WOG[3]	42	1234 ± 19	1262 ± 20	1209 ± 19
	56	1718 ± 34	1735 ± 34	1699 ± 29
WOGPC[4]	42	1282 ± 19ab	1318 ± 20a	1259 ± 19b
	56	1778 ± 34	1804 ± 34	1767 ± 29
Uptake[5]	42	46 ± 1b	55 ± 1a	48 ± 1b
	56	64 ± 1	69 ± 2	67 ± 2
Rack[6]	42	409.51 ± 6.44	424.27 ± 5.92	410.61 ± 6.36
	56	565.06 ± 12.14	571.13 ± 11.52	553.06 ± 8.59
Breast	42	226.60 ± 4.23ab	231.80 ± 4.73a	216.30 ± 4.23b
	56	309.78 ± 6.60	307.64 ± 6.68	301.40 ± 6.15
TBRST[7]	42	297.03 ± 5.28a	302.29 ± 5.85a	281.50 ± 5.36b
	56	410.82 ± 8.64	408.07 ± 8.53	396.35 ± 7.90
Leg	42	421.51 ± 6.88	433.45 ± 7.99	414.73 ± 7.02
	56	576.49 ± 12.27	593.36 ± 13.52	591.65 ± 12.58
AB Fat	42	42.09 ± 1.87b	50.02 ± 1.72a	45.74 ± 1.82ab
	56	72.43 ± 3.22	79.35 ± 2.42	81.06 ± 2.94
Supply Organs	42	214.63 ± 6.16	218.70 ± 6.26	214.58 ± 6.04
	56	267.31 ± 6.96b	298.56 ± 9.10a	274.73 ± 6.61b

[a-b] Means for a trait within an age but across lines are different (P < .05).

[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 lines are selected for high and low % breast at four days of age, respectively. RAN = unselected random bred control.

[3] WOG = Without Giblets.

[4] WOGPC = Without Giblets Post Chill.

[5] Uptake = Water absorbed during chill.

[6] Rack = Skeletal system of the bird post part removal.

[7] TBRST = Total Breast (Breast + Tenders)

Table 3.5 Relative weight measures (%) (mean ± SEM) of processing traits of BY4[1] broilers processed at 42 and 56 days posthatch after 4 generations of divergent selection. (N ≥ 55)

		Line Designation[2]		
Trait	AGE	HBY4	RAN	LBY4
Rack[3]	42	22.42 ± 0.12	22.76 ± 0.12	22.73 ± 0.12
	56	23.27 ± 0.16a	23.07 ± 0.17ab	22.64 ± 0.15b
Breast	42	12.39 ± 0.12a	12.40 ± 0.14a	11.94 ± 0.11b
	56	12.77 ± 0.12a	12.43 ± 0.13ab	12.30 ± 0.13b
TBRST[4]	42	16.25 ± 0.14a	16.27 ± 0.16a	15.54 ± 0.13b
	56	16.94 ± 0.18a	16.50 ± 0.18ab	16.18 ± 0.15b
Leg	42	23.07 ± 0.12	23.09 ± 0.15	22.93 ± 0.14
	56	23.73 ± 0.14	23.91 ± 0.19	24.11 ± 0.21
AB Fat	42	2.30 ± 0.1b	2.69 ± 0.09a	2.53 ± 0.09ab
	56	2.96 ± 0.11b	3.21 ± 0.08ab	3.34 ± 0.12a
Supply Organs	42	11.74 ± 0.28	11.73 ± 0.30	11.85 ± 0.27
	56	11.08 ± 0.29b	12.20 ± 0.37a	11.30 ± 0.27b

[a-b] Means for a trait within an age but across lines are different (P < .05).
[1] BY4 (Four-Day Relative Breast Yield)
[2] HBY4 and LBY4 lines are selected for high and low % breast at four days of age, respectively. RAN = unselected random bred control.
[3] Rack = Skeletal system of the bird post parts removal.
[4] TBRST = Total Breast (Breast + Tenders)

Table 3.6 Meat quality trait measures (mean ± SEM) of BY4[1] broilers processed at 42 and 56 days posthatch after 4 generations of divergent selection. (N ≥ 55)

		Line Designation[2]		
Trait	AGE	HBY4	RAN	LBY4
[3]L*	42	48.86 ± 0.31b	49.84 ± 0.36a	49.60 ± 0.23ab
	56	50.53 ± 0.36	51.14 ± 0.41	51.43 ± 0.31
[4]Shear Force (kg/cm^2)	42	2.71 ± 0.22	2.59 ± 0.18	2.50 ± 0.18
	56	2.75 ± 0.20	2.92 ± 0.26	2.94 ± 0.24
[5]Drip Loss %	42	3.31 ± 0.33b	4.40 ± 0.38a	4.23 ± 0.37ab
	56	4.00 ± 0.41	3.34 ± 0.36	3.88 ± 0.35
[6]PH	42	5.84 ± 0.03	5.76 ± 0.03	5.81 ± 0.03
	56	5.68 ± 0.03	5.65 ± 0.03	5.73 ± 0.03

[a-b] Means for a trait within an age but across lines are different (P < .05).

[1] BY4 (Four-Day Relative Breast Yield)

[2] HBY4 and LBY4 lines are selected for high and low % breast at four days of age, respectively. RAN = unselected random bred control.

[3] L* = Muscle Color Lightness Value measured using a Minolta CR-300 Colorimeter.

[4] Shear force as measured using WB Method previously described by Burke et al., 2003.

[5] Drip loss as measured using EZ-Driploss Method as described by Correa et al., 2007.

[6] PH measured using Testo 205 pH probe.

3.6 Works Cited

Berri, C., N. Wacrenier, N. Millet, and E. Le Bihan-Duval. 2001. Effect of selection for improved body composition on muscle and meat characteristics of broilers from experimental and commercial lines. Poult. Sci. 80:833-838.

Burke, J. M., J. K. Apple, W. J. Roberts, C. B. Boger, and E. B. Kegley. 2003. Effect of breed-type on performance and carcass traits of intensively managed hair sheep. J. Meat Sci. 63:309-315.

Campbell, R. G., and M. R. Taverner. 1988. Genotype and sex effects on the relationship between energy intake and protein deposition in growing pigs. J. Anim. Sci. 66:676-686.

Cavitt, L. C., J. F. C. Meullenet, R. Xiong, and C. M. Owens. 2004. The relationship of Razor Blade shear, Allo-Kramer shear, Warner-Bratzler shear and sensory tests to changes in tenderness of broiler breast fillets. J. Muscle Foods 14:11-23.

Correa, J. A., S. Methot, and L. Faucitano. 2007. A modified meat juice container (EZ-Driploss) procedure for a more reliable assessment of drip loss and related quality changes in pork meat. J. Muscle Foods 18:67-77.

Deeb, N., and S. J. Lamont. 2002. Genetic architecture of growth and body composition in unique chicken populations. J. Heredity 93:107-188.

Dunnington, E. A., and P. B. Siegel. 1996. Long-term divergent selection for eight-week body weight in white Plymouth. Poult. Sci. 76:1121-1125.

Ender, K., M. Wicke, I. Fiedler, J. Wegner and K. Nürnberg. 2000. Biological bases of muscle meat quality. Daily report "Quality of feed and animal primary products," 17-18. Nov, Halle (Saale), pp. 2-9.

Ezekwe, M. O., and R. J. Martin. 1975. Cellular characteristics of skeletal muscles in selected strains of pigs and mice and the unselected controls. Growth 39:95-106.

Fiedler, I., G. Dietl, C. Rehfeldt, J. Wegner, and K. Ender. 2004. Muscle fibre traits as additional selection criteria for muscle growth and meat quality in pigs – results of a simulated selection. J. Anim. Breed. Genet. 121:331-344.

Fiedler, I., K. Ender, M. Wicke, and G. V. Lengerken. 1993. Zusammenhänge zwischen der mikrostruktur des muskelgewebes bei schweinen der landrasse und ihrer stressempfindlichkeit. Arch. Tierz. 36:525-538.

Fiedler, I., K. Ender, M. Wicke, S. Maak, and G. V. Lengerken. 1999. Structural and functional characteristics of muscle fibres in pigs with different malignant hyperthermia susceptibility (MHS) and different meat quality. Meat Sci. 53:9-15.

Fiedler, I., K. Nürnberg, T. Harge, G. Nürnberg, and K. Ender. 2003. Phenotypic variations of muscle fiber and intramuscular traits in Longissimus muscle of F2 population Duroc x Berlin Miniature Pig and relationships to meat quality. Meat Sci. 63:131-139.

Goldspink, G. 2004. “Local and systemic regulation of muscle growth” *Muscle Development of Livestock Animals*. Eds. M.F.W. tePas, M.E. Everts and H.P. Haagsman. CAB International.

Halevy, O., S. Yahav, and I. Rozenbaum. 2006. Enhancement of meat production by environmental manipulations in embryo and young broilers. World’s Poult. Sci. J. 62:485-497.

Harford, I. D., Pavlidis, H. O., and Anthony, N. B. 2014. Divergent selection for muscle color in broilers. Poult. Sci. *93*:1059-1066.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and D. V. Rives. 1994. Carcass composition and yield of 1991 vs 1957 meat-type chickens when fed “typical” 1957 and 1991 broiler diets. Poult. Sci. 73:1795-1804.

Hooper, A. C., I. Bryne, and J. C. McCarthy. 1973. The effects of selection for body weight on muscle structure in mice. J. Anat. 115:146.

Julian, R. J. 2005. Production and growth related disorders and other metabolic diseases of poultry–a review. Vet. J. 16:350-369.

Karlsson, A. H., R. E. Klont, and X. Fernandez. 1999. Skeletal muscle fibres as factors for pork quality. Livestock Prod. Sci. 60:255-270.

Katanbaf, M. N., E. A. Dunnington, and P. B. Siegel. 1988. Allomorphic relationships from hatching to 56 days in parental lines and F_1 crosses of chickens selected 27 generations for high or low body weight. Growth Dev. Aging. 52:11-21.

Klosowska, D., A. Niewarowicz, B. Klosowski, and M. Trojan, 1979. Histochemische und histologische Untersuchungen adm M. pectoralis superficialis mit beschleunigter, normaler und verzögerter Glykolyserate in Broilern. Fleischwirtschaft. 7:1004-1008.

Lengerken, G. V., S. Maak, M. Wicke, I. Fiedler, and K. Ender. 1994. Suitability of structural and functional traits of skeletal muscle for genetic improvement of meat quality in pigs. Arch. Tierz. 37:133-143.

Lilburn, M. S. 1994. Skeletal growth of commercial poultry. Poult. Sci. 73:897-903.

Lyon, B. G., D. P. Smith, C. E. Lyon, and E. M. Savage. 2004. Effects of diet and feed withdrawal on the sensory descriptive and instrumental profiles of broiler breast fillets. Poult. Sci. 83:275-281.

Miller, L. R., V. A. Garwood, and M. D. Judge. 1975. Factors affecting porcine muscle fiber type, diameter and number. J. Anim. Sci. 41:66-77.

Moss, F. P., and C. P. Leblond. 1971. Satellite cells as the source of nuclei in muscles of growing rats. Anat. Rec. 170:421-436.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington DC.

Nestor, K. E., W. L. Bacon, Y. M. Salf, and P. A. Renner. 1985. The influence of genetic increases in shank width on body weight, walking ability and reproduction of turkey. Poult. Sci. 64:2248-2255.

Pollock, D. L. 1999. A geneticist's perspective from within a primary breeder company. Poult. Sci. 78:414-418.

Rasmussen, A., and M. Andersson. 1996. New method for determination of drip loss in pork muscles. In: Hildrum, K.I. (ed.) *Meat for the consumer. Poster Proceedings of the 42nd International Congress of Meat Science and Technology*. Lillehammer, Norway, pp. 286-287.

Rehfeldt, C., I. Fiedler, G. Dietl, and K. Ender. 2000. Myogenesis and postnatal skeletal muscle cellgrowth as influenced by selection. Livestock Prod. Sci. 66:177-188.

Rehfeldt, C., I. Fiedler, and N. C. Stickland. 2004. "Number and size of muscle fibers in relation to meat production" *Muscle Development of Livestock Animals*. Eds. M.F.W. tePas, M.E. Everts and H.P. Haagsman. CAB International.

Rehfeldt, C., and G. Kuhn. 2006. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J. Anim. Sci. 84S:E113-E123.

Rehfeldt, C., I. Fiedler, and J. Wegner. 1987. Veränderungen der mikrostruktur des muskelgewebes bei Labormäusen, Rindern und Schweinen während des Wachstums. Z. Mikrosk. Anat. Forsch. 101:669-680.

SAS Institute Inc. 2001. SAS User's Guide. SAS Inst. Inc., Cary, NC.

Scheuermann, G. N., S. F. Bilgili, S. Tuzun, and D. R. Mulvaney. 2004. Comparison of chicken genotypes: myofiber number in pectoralis muscle and myostatin ontogeny. Poult. Sci. 83:1404-1412.

Stickland, N. C., and C. M. Dwyer. 1996. The pre-natal influence on post-natal growth. Mol. Physiol. Growth. 60:135-150.

Stickland, N. C., and S. E. Handel. 1986. The numbers and types of muscle fibers in large and small breeds of pigs. J. Anat. 174:181-189.

Suzuki, K., T. Shibata, H. Kadowaki, and T. Toyoshima. 2003. Meat quality comparison of Berkshire, Duroc and crossbred pigs sired by Berkshire and Duroc. Meat Sci. 64:35-42.

Velleman, S. G., and McFarland, D.C. 2014. Skeletal muscle. In: C. G. Scanes, (Ed). *Sturkie's Avian Physiology* (Sixth Edition). pp. 379-402. Academic Press, San Diego.

Warren, J. J. 1996. Breeding and genetics. In: J. L. Skinner, (Ed). *American Poultry History: Volume II*. pp. 12-24. Watt Publ. Co., Mt. Morris, IL.

Yalcin, S., S. Özkan, E. Coskuner, G. Bilgen, Y. Delen, Y. Kurtulmus, and T. Tanyalcin, 2001. Effects of strain, maternal age and sex on morphological characteristics and composition of tibial bone in broilers. Brit. Poult. Sci. 42:184-190.

Zhang, L., and S. Barbut. 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. Brit. Poult. Sci. 46:687-693.

APPENDIX

UNIVERSITYOF

ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Nicholas Anthony
FROM: Craig N. Coon, Chairman Institutional Animal Care And Use Comittee

DATE: May 23, 2012
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: April 6, 2015

The Institutional Animal Care and Use Committee (IACUC) has APPROVED Protocol #12038- "GENERAL REARING OF SELECTED CHICKEN AND QUAIL POPULATIONS ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] prior to initiating the changes. If the study period is expected to extend beyond 04-06-2015, you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

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4. Thermal manipulation of commercial lines selected for different market ages and the impact on breast yield and meat quality characteristics.

Abstract

Thermal manipulation (TM) from embryonic days 16 to 18 (E16 to E18) for 6 hours daily has been shown to promote growth. Previous TM research focused on the effect of TM on embryos from standard yield broiler strains. Of interest to research was the effect of TM on high-yield broiler strain growth and body composition. TMs were performed on embryos from Hubbard x Cobb 500 (HxC) and R708 broiler crosses. TM embryos showed improved body weights up to 35 days of age. Furthermore, the TM HxC showed an improved FCR compared to the control HxC. TM had no effect on leg percentage, but did improve breast percentage in the TM HxC at 46 and 60 days and the TM R708 at 60 days. Evaluations of meat quality parameters showed no difference between control and TM lines.

4.1 Introduction

Muscle mass is primarily made up of two components: muscle fiber number and fiber size. Currently, growth selection practiced by the industry promotes increased body weight and growth through muscle fiber hypertrophy, i.e. increased muscle fiber size. Such selection practices coupled with selection for yield and feed conversion have resulted in vast genetic improvements in the modern broiler (Havenstein et al., 2003). Unfortunately, it has been suggested that selection utilizing hypertrophy as the means of increasing muscle growth and yield may be limited in the future as fiber size reaches a physiological limit in growth (Mahon, 1999).

Therefore, it is important for the poultry industry to continue to explore avenues that promote growth. One of the possible techniques being examined is TM during incubation. As the time needed to reach market weight in poultry has decreased, embryogenesis has increased as a proportion of the broiler's lifespan (Halevy et al., 2006). This prenatal period is of particular importance, as the number of muscle fibers formed during this stage will be a determining factor in the rate of hypertrophy during postnatal growth. To understand the mechanics behind this, it is important to have a working knowledge of myogenesis.

During embryogenesis, muscle precursor cells arise from the dermomytome, which is part of the mesodermal layer. These muscle precursor cells undergo myogenic determination and are then referred

to as myoblasts. These myoblasts proliferate and differentiate to form muscle fibers. In avian species, there are three unique myoblast populations: (1) embryonic myoblasts, (2) fetal myoblasts, and (3) adult myoblasts. Of particular interest to this research are adult myoblasts, also referred to as satellite cells (Mauro, 1961). As muscle cells are incapable of proliferation, satellite cells represent the only source of new nuclei needed for additional muscle growth.

Myogenesis relies on a complex, multi-step process to signal muscle precursor cells to proliferate, differentiate, and fuse into myofibrils. Three families of transcription factor networks are responsible for controlling myogenic development (Perry and Rudnick, 2000). These networks are the paired-box transcription factors (Pax), basic helix-loop-helix (bHLH) superfamily and MADS (MCM1, agamous, deficiens, serum response factor). Pax genes regulate stem cell determination and proliferation (Montarras et al., 2005). BHLH genes are needed for the activation of muscle genes and MADS work to regulate myogenesis.

TM was originally devised as a method for improving thermotolerance by affecting the development of the hypothalamus-hypophysis-thyroid axis (HHTA), and the hypothalamus-hypophysis-adrenal axis (HHAA) (Uni and Yahav, 2010). Temperature was chosen as the incubation factor to be altered as it is considered to be a major controlling factor in embryo development (Meijerhof, 2000). Multiple studies have been conducted to determine the optimal embryonic age, and incubation temperature needed to promote improved thermotolerance (Yahav et al., 2004a; b). Improvements in thermotolerance made using TM have been demonstrated by lower body temperatures prior to slaughter (Piestun et al., 2008a; 2009a).

Postnatal muscle growth is dependent on the prenatal formation of muscle fibers and the postnatal addition of satellite cell nuclei to increase muscle protein buildup (Halevy et al., 2006). TM applied from E16 to E18 is thought to promote satellite cell proliferation as it targets a key period of satellite cell population expansion (Hartley et al., 1992). An increased satellite cell population provides more genetic material to increase growth via hypertrophy. Increased hypertrophy in broilers that underwent embryonic TM suggests TM results in increased satellite cell proliferation (Piestun et al., 2013).

It was also thought that the decrease in core body temperature resulting from TM may impact growth through a reduction in maintenance energy requirements. This reduction leaves more resources available for lean growth. TM embryos exhibit increased breast yield as well as an improved FCR (Piestun et al., 2013). Increased breast yield coupled with improved FCR would be of significant benefit to the poultry industry.

Meat Quality

Meat quality relies on several factors such as water holding capacity (WHC), texture, tenderness, color, and pH. Meat quality has recently become a more prevalent issue in the poultry industry, as several of the factors that contribute negatively to meat quality, also negatively impact the production of value added products. As the poultry industry now relies much more on value added products, as opposed to whole birds, maintaining/improving meat quality is a major industry concern.

PSE and DVD

Specifically two primary conditions affect meat quality in the poultry industry: Pale, Soft, Exudative (PSE) and Dry, Firm, Dark (DFD). PSE is characterized by pale, soft almost gel-like meat, low pH with a poor WHC. Both meat classifications result from lactic-acid production prior to slaughter. This lactic-acid production is a byproduct of glycolsis and results in the lowering of pH (Lawrie, 1998). PSE meat is associated with pre-slaughter stress that increases glycolysis and lactic production. The increased lactic acid contributes to increased contraction of muscle fiber during rigor mortis, thus leaving less space in between muscle cells for water absorption. DFD, in contrast, is dark in color, firm with increased pH (Allen et al., 1997, 1998; Wulf et al., 2002). DFD results from stress hours prior to slaughter exhausting glycogen reserves and giving time for lactic-acid metabolism (Hedrick et al., 1989; Gregory, 1994; Lawrie, 1998). This results in meat that doesn't reach typical pH levels, hence the relatively high pH for DFD meat. This meat is more prone to spoilage and is not favored by consumers, as the dark color is off-putting.

Industry Impact

Meat quality becomes an issue as poor quality meat has a negative impact on the profitability of poultry producers. Consumers rely on characteristics of visual appeal to judge the quality and freshness of poultry products (Froning, 1995). A package of breasts with inconsistent color or excess water will often be passed over for a more uniform ideal product. Furthermore, DFD meat's increased pH provides an ideal environment for bacterial growth (Newton and Gill, 1981; Allen et al., 1998). Increased bacteria growth increases the chance the product will not sell prior to spoilage and also displeases the consumer as it may spoil prior to consumer use. Finally, PSE meat negatively impacts value added products as it has issues with emulsification, binding, and water retention (Barbut, 1996). This limits the use of PSE meat in value-added products due to a reduction in protein functionality resulting in estimated losses upwards of $30 million per year in the US turkey industry (Strasburg and Linz, 1997).

Evaluating Meat Quality

Of interest to the poultry industry is how to adequately evaluate properties of meat that affect its quality. As mentioned previously, some of the more common characteristics used in the evaluation of meat quality are muscle color, pH, tenderness and WHC.

Muscle Color

The method for evaluating muscle color via a colorimeter has been previously described (Barbut, 1993, 1996; McCurdy et al., 1996). This measure produces three variables, lightness (*L), redness (a*) and yellowness (b*). L* value is a measurement commonly used for analysis. L* values for poultry meat unaffected by PSE or DFD range from 48.0 to 53.0 (Qiao et al., 2001; Zhang and Barbut, 2005; Harford et al., 2014), while PSE meat expresses L* values greater than 53 and DFD meat L* values are typically less than 46 (Qiao et al., 2001).

Muscle pH

The spear probe method described by (Dosler et al., 2007) is being used in this experiment. The spear probe method was chosen as it is cost efficient, quick and repeatable. PH measurement done using this method involves the insertion of a pH probe into breast tissue and the recording of the resulting

pH measurement. Normal poultry meat has a pH between 5.7 and 6.1, with PSE meat pH values less than 5.7 and DFD pH values greater than 6.1 (Barbut, 1997; Woelfel et al., 1998; Zhang and Barbut, 2005).

Water Holding Capacity

WHC is measured by cook loss, drip loss or expressible product moisture. Cook loss is measured by weighing the breast before and after cooking. Cook loss is an effective measurement, but some weight loss may also be attributed to fat loss in addition to moisture loss. Drip loss is measured by weighing breasts then storing the breast for a predetermined time period, then reweighing the breast. Expressible moisture is moisture forced out of the breast or other product when put under pressure for a given period of time. Of use in this research is the EZ-Driploss method (Correa et al., 2007), which is being used to measure drip loss in poultry for the first time in this study as well as in chapter 3.

Tenderness

Tenderness is a measure of the total force or force/mm required to tear or shear a product. There are three common methods of assessing the tenderness of products: Allo-Kramer (AK), Warner-Bratzler (WB), and Razor Blade (RB) method (Bratzler, 1932; Cavitt et al., 2004; 2005). Tenderness in this study is being evaluated using the WB method as previously described by Burke et al. (2003).

4.2 Materials and Methods

Broiler Populations

Two broiler crosses were sourced for this study based on their differing growth rates. A commercial broiler cross (Hubbard Male x Cobb 500) (HxC) was chosen to represent a standard yield commercial broiler commonly used in the whole bird/parts market. A high yield bird (Ross x Ross 708) (R708) was also added to represent higher yielding birds produced for the debone market. This dissimilarity in growth was ideal for the study in order to compare the effect of thermal manipulations in lines exhibiting different growth rates.

Data Collection

Fertile hatching eggs (N = 720 per cross) from HxC and R708 crosses were evenly divided across two thermal treatments. Eggs were randomly distributed throughout 8 trays in two identical incubators (4 trays per incubator) in order to minimize potential variation attributable to differences in incubator microenvironment. Incubation conditions for the control HxC and R708 populations were 37.8°C with a 56% relative humidity (RH) for the duration of the incubation. Incubation conditions were identical for the test HxC, and R708 populations up to 384 hours at which time thermal manipulations were implemented. Thermal manipulations involved the test HxC, and R708 incubator conditions being set at 39.5°C with a 65% RH for 6 hours beginning at 384 and repeated every 24 hours (384, 408, and 432) until 438 hours. At 438 hours eggs were transferred into 8 hatching trays by cross and treatment combination (2 hatching trays per cross-treatment combination).

Hatch Window

Beginning 484 hours into incubation, and repeated every 4 hours until 512 hours of incubation the hatcher was opened and hatched chicks were collected, individually weighed, wing banded and recorded according to cross-treatment combination. Chicks were then returned to hatching trays by cross-treatment combination and removed at the end of the hatch window study period. Chicks were re-weighed prior to placement and placed according to cross-treatment combination. Birds were reared straight run in pens containing birds from an individual cross-treatment combination. Stocking density was approximately 40 birds per pen, but all pens were equalized to 24 birds at 7 days. All birds were provided feed and water *ad libitum* with a starter and finisher ration formulated to meet or exceed National Research Council (NRC) (1994) minimum standards. Beginning with Day 7, birds were weighed weekly, and feed consumption recorded, to assess feed conversion ratio (FCR).

Sample

Birds were sampled at 4, 7, 46, and 60 days of age posthatch. At each sampling period birds (N≈48 per cross-treatment) were randomly selected regardless of sex to be necropsied. Birds sampled

were taken equally (N≈8 per pen) each processing age and all remaining birds were processed at the end of the trial.

Processing

Body weight, tender, breast, yolk, keel, drum, thigh weight and part weight relative to live weight were assessed beginning at four days posthatch, hereby referred to as Day 4. In addition, tbe broiler weight without giblets (WOG), WOG post chill, and wing weight were obtained at 46 and 60 days of age. Body weight measurements were taken on all un-sampled birds at each sampling date, as well as 14, 21, 28, 35, 42, 49 and 56 days of age posthatch.

Broilers sampled prior to Day 46 were euthanized via CO2 asphyxiation. Feed was withdrawn 12 hours prior to euthanasia and the contents of the crop removed to avoid artificial body weight contributed by crop contents. Sampled broilers were individually weighed, recorded and placed on trays and allowed to refrigerate at 1ºC for a minimum of three hours to promote the onset of rigor mortis.

Muscle Color, pH and Drip Loss

For birds processed on Day 46 and 60, the deboned pectoralis major was individually bagged and refrigerated at 1º C. Twenty-four hours post slaughter breasts were removed from cold storage, and blotted dry. The right lobe of the pectoralis major was used for color measurements using a Minolta CR-300 Colorimeter (Minolta Chroma Meter CR-300, Minolta Italia S.P.A., Milano, Italy) to obtain L* value, color measurements were done at the top, center and base of the lobe. PH measurements were taken on the right lobe using a Testo 205 pH probe.

Drip loss measurements were obtained via the EZ Driploss method (Rasmussen and Andersson, 1996). A drill was used to obtain circular cores from the right lobe. Samples were then placed individually in driploss tubes, and stored for an additional 24 hours at 1º C. At 48 hours post slaughter, the test tubes were weighed to obtain the weight of the combined sample-tube weight. The sample was then removed from the test tube and the tube reweighed to obtain the combined weight of the liquid and tube. EZ driploss (Correa et al., 2007) was then calculated as follows:

% Driploss = ((Tube with liquid WT) / (Tube with meat and liquid WT - Tube WT)) * 100

Tenderness

The left lobe of the pectoralis major was used to assess tenderness. Left lobes were cooked in an oven at 190º C until they reached an internal temperature of 74º C. Breast lobes were then allowed to cool to room temperature prior to further testing. Six 1.27-cm diameter cores were removed from each breast parallel to the muscle fiber orientation. These cores were sheared through the center with a Warner-Bratzler shear apparatus attached to an Instron 4466 (Instron Corp., Canton, MA, USA) with a 55-kg load cell with a crosshead speed of 250mm/min (Burke et al., 2003).

Data Analysis

Data from the four processing dates (d 4, 7, 46 and 60) were run in a 3-way full-factorial ANOVA with two crosses (HxC and R708), two incubation profiles (CTRL and TM), two sexes and the resulting interactions. No significant interaction between sex and other factors was found, and sex was removed from the model. Cross by incubation was significant for many traits, so cross means by incubation were analyzed for presentation. The GLM procedure of SAS along with Duncan's multiple range test using SAS 9.3 (SAS Institute Inc., 2001, Cary, NC) was used to compare cross means within incubation treatments and to compare the incubation treatments within cross. An alpha level of .05 was used to determine significance.

4.3 Results

Thermal manipulation led to differences in hatch time between treatment groups. The HxC CTRL had a longer hatching time than the HxC TM and the R708 CTRL had a longer hatching time than the R708 TM (Table 4.1). There were no differences in hatching time between the CTRL crosses, but the R708 TM had a longer hatching time than the HxC TM (Table 4.1). The distribution of hatching times for the four line-treatment combinations are shown in Figure 4.1. TM resulted in differences in hatchability in the R708 cross; the R708 TM had a greater hatchability than the R708 CTRL (Table 4.1). Within treatment, the HxC CTRL had a greater hatchability than the R708 CTRL, and the HxC TM had a greater hatchability than the R708 TM (Table 4.1). TM did not affect hatching weight, with no differences in

hatching weight being shown between the HxC CTRL and TM groups, or the R708 CTRL and TM groups (Table 4.1). Within treatment, there were differences in hatching weight. Within the CTRL treatment, the HxC CTRL had a greater hatching weight than the R708 CTRL (Table 4.1). This difference was mirrored in the TM groups with the HxC TM having a greater hatching weight than the R708 TM (Table 4.1).

Body Weight and FCR

At 7, 14, 21, 28, and 35 days posthatch TM resulted in a greater body weight in the R708 TM than the R708 CTRL (Table 4.2). At Day 21 the HxC CTRL had a greater body weight than the HxC TM, this was the only weekly weigh in which a difference in body weight was shown between the HxC CTRL and HxC TM (Table 4.2). The HxC CTRL had a greater body weight than the R708 CTRL from Day 7 to Day 56 (Table 4.2). The HxC TM exhibited a greater body weight than the R708 TM at days 21, 28 and 35, then again had a greater body weight at 49 and 56 days posthatch (Table 4.2).

TM resulted in an improved FCR in the HxC TM compared to the HxC CTRL at 14 and 21 days posthatch (Table 4.2). Differences in FCR would again emerge at 56 days with the HxC CTRL having a greater FCR than the HxC TM (Table 4.2). TM showed a difference in the R708 cross at Day 35 with the R708 CTRL having a greater FCR than the R708 TM (Table 4.2). No differences were found within the control treatment groups, but differences in FCR were shown at days 14 and 21, with the R708 TM having a greater FCR than the HxC TM (Table 4.2).

Processing Data

At days 4 and 7 posthatch, the HxC broiler strain had a greater body weight than the R708 for both the CTRL and TM groups (Table 4.3). The TM strains did however express a greater four-day weight than their respective strains in the CTRL treatment group (Table 4.3). Keel weight was greater in the HxC TM, than the R708 TM at 4 days of age (Table 4.3). Keel weight was also greater at 7 days of age in the HxC CTRL than the R708 CTRL (Table 4.3). No differences in keel weight were found at either 4 or 7 days between treatments within line (Table 4.3). Differences in leg weight were found in both treatment groups at 4 and 7 days, with the HxC CTRL outperforming the HxC CTRL at both 4 and 7 days, while the HxC TM had a greater leg weight than the R708 TM at 4 days (Table 4.4). The TM strains did

however express a greater leg weight than their CTRL counterparts at 4 days of age (Table 4.4). No differences in breast, tender or total breast weight were found between strain within treatments at either 4 or 7 days (Table 4.4). However, the TM strains had greater breast, tender, and total breast weights than the CTRL strain counterparts at 4 days (Table 4.4). The TM strains also had greater tender weights than their respective CTRL strains at 7 days of age (Table 4.4). Furthermore, the R708 TM exhibited greater breast and total breast weight at 7 days, than the R708 CTRL (Table 4.4).

The differences seen in absolute leg weight were not shown in leg weight relative to live weight, with the only difference shown being the HxC CTRL having a greater leg percentage than the HxC TM at Day 7 (Table 4.5). Differences in breast weight relative to live weight and total breast weight relative to live weight were found at 4 days with the TM strains having a greater breast and total breast percentage than their corresponding CTRL strains (Table 4.5). This trend continued in the R708 strain, with the R708 TM having a greater breast and total breast percentage than the R708 CTRL at 7 days (Table 4.5). Differences between strains within treatment were shown at 7 days, with the R708 CTRL and TM strains outperforming the HxC strains in breast and total breast percentage (Table 4.5).

No difference in body weight was shown within strain between treatments at 46 days of age (Table 4.3.) The HxC CTRL did express greater body weight than the R708 CTRL at 46 days (Table 4.3). At 60 days of age, the HxC strain had a greater body weight than the R708 in both the CTRL and TM treatment groups (Table 4.3). Furthermore, the HxC CTRL had a greater body weight than the HxC TM at 60 days (Table 4.3). The HxC CTRL WOG and WOGPC weights were greater than R708 CTRL at 46 and 60 days (Table 4.3). No differences in WOG or WOGPC were found between the HxC CTRL and HxC TM (Table 4.3). The HxC TM and R708 TM were equivalent in WOG and WOGPC weight at 46 days (Table 4.3). Differences between the HxC TM and R708 TM were found for both traits at 60 days (Table 4.3). No differences were found for WOG or WOGPC weight within strain between treatments (Table 4.3). The HxC CTRL had a greater rack weight than the R708 CTRL at 46 and 60 days, but did not differ from the HxC TM (Table 4.3). The HxC TM displayed a greater rack weight than the R708 TM at Day 60 (Table 4.3). No differences in rack weight were shown within strain between treatments (Table 4.3).

The HxC CTRL had a greater leg weight than the R708 CTRL at 46 and 60 days (Table 4.4). The HxC CTRL also had a greater leg weight than the HxC TM at 60 days (Table 4.4). The drum and thigh weights for the HxC CTRL showed similar differences from the R708 CTRL, with the HxC CTRL having greater thigh and drum weights at 46 and 60 days (Table 4.4). Furthermore, the HxC CTRL had a greater thigh weight than the HxC TM (Table 4.4). The HxC TM had a greater drum weight at 46 days and a greater leg, thigh and drum weight at 60 days than the R708 TM at 60 days (Table 4.4). No differences in these traits were found between treatments within the R708 strain at 46 or 60 days (Table 4.4). Breast, tender, and total breast weight showed no differences within strain between treatment at days 46 and 60 (Table 4.4). Furthermore, no differences were found within treatment between strains for either breast or total breast at 46 or 60 days (Table 4.4). Differences in tender weight between strains within treatment were found with the HxC CTRL having a greater tender weight than the R708 CTRL at 60 days and the HxC TM having a greater tender weight than the R708 TM at 46 and 60 days (Table 4.4).

Leg weight relative to live weight was not impacted by TM, with neither the R708 nor the HxC expressing differences in leg percentage between treatments (Table 4.5). Within the CTRL treatment, the HxC expressed a greater leg percentage than the R708 at both 46 and 60 days (Table 4.5). Within the TM treatment, the HxC had a greater leg percentage than the R708 at 60 days (Table 4.5). Thigh percentage was also not impacted by TM, with no differences being found between treatments within strain (Table 4.5). The HxC CTRL had a greater percentage thigh than the R708 CTRL at 46 and 60 days (Table 4.5). The HxC TM had a greater thigh percentage than the R708 TM at Day 60 (Table 4.5). Drum percentage was impacted by treatment with the R708 CTRL having a greater percentage drum than the R708 TM, at 46 and 60 days (Table 4.5). Drum percentage was greater in the HxC CTRL than R708 CTRL at Day 60 (Table 4.5). The HxC TM had a greater drum percentage than the R708 TM at 46 and 60 days (Table 4.5). No differences in breast percentage between treatments within strain were found at 46 days (Table 4.5). However, the HxC TM and R708 TM both displayed a greater breast percentage than their CTRL counterparts at 60 days (Table 4.5). Differences in breast percentage were found between strains within treatment at 46 and 60 days with the R708 CTRL and TM outperforming their respective HxC counterparts (Table 4.5). The R708 exhibited a difference in total breast percentage between treatments within strain with the R708 TM having a greater total breast percentage than the

R708 CTRL (Table 4.5). At 46 and 60 days, the R708 CTRL and TM displayed a greater total breast percentage than their corresponding HxC strains (Table 4.5).

Meat Quality

Within strains and between treatments only drip loss percentage showed a difference with the R708 CTRL having a greater drip loss percentage than the R708 TM at 46 days (Table 4.6). Between strains and within treatment several differences in meat quality parameters were found. The HxC CTRL and TM had a greater muscle color lightness value (L*) than their R708 counterparts at 46 and 60 days (Table 4.6). The HxC CTRL also had a greater shear force mean than the R708 CTRL at 60 days (Table 4.6). No differences were found for drip loss percentage between strains within treatment at either 46 or 60 days (Table 4.6). No difference in pH was found between strains within treatment at 46 days (Table 4.6). The R708 CTRL did exhibit a greater pH than the HxC CTRL at 60 days (Table 4.6). No difference in pH was shown between the HxC TM and R708 TM strains (Table 4.6).

4.4 Discussion

The purpose of this study was to evaluate how thermal manipulation affects lines selected for different processing ages. Previous studies have utilized a standard yield strain exclusively (Piestun et al., 2008a, b, 2009a, 2011). It was of interest to researchers to determine how differing genetics may impact the effect of thermal manipulation. The decrease in hatch time in TM groups is an artifact of the increase in hatch temperature. There was no difference in hatchability between the HxC treatment groups, and the R708 TM had a greater hatchability than the R708 CTRL. The lack of a negative impact on hatchability is consistent with research using TM from E16 to E18 for 3 hours per day (Piestun et al., 2009b). Research utilizing longer TM duration 12 hours per day from E7 to E16, showed similar results with no impact on hatchability found by Piestun et al. (2008a); and a slight negative impact found by Piestun et al. (2013) using the same extended TM procedure. The lack of a negative impact by TM on hatchability is of particular importance because if thermal manipulation is to be regularly used to boost performance, it will need to show minimal to no impact on hatchability.

Growth performance was temporarily improved through the use of thermal manipulation with increases in body weight being shown in the TM groups of both lines at 4 days of age and with the

increase in body weight continuing in the R708 up to 35 days of age. At typical broiler processing ages (46 and 60 days) there was no impact on live weight within the R708 between treatments, but the HxC TM was negatively impacted exhibiting a 5.3% lower body weight than HxC CTRL. The negative impact on body weight was not shown in research using TM for 12 hours/day from E 7 to E16 reared in cages to 35 days (Piestun et al., 2008a) but was shown in studies done using the same TM procedure with broilers being reared on wood shavings to 35 days (Loyau et al., 2013; Piestun et al., 2013). No difference in FCR was shown between strains within treatment at either 46 or 60 days. However, TM did result in a decreased FCR for the HxC TM when compared to the HxC CTRL consistent with previous research (Piestun et al., 2013) but not to the same extent as previously shown. Given that feed costs represent a significant portion of total production costs, producers may be willing to accept a slight decrease in growth in return for an improved FCR.

Light meat (breast and tenders) and dark meat (drum, thigh) were affected differently by TM. No differences in leg or thigh percentage were shown between treatments within strain at 46 or 60 days and only drum percentage showed a difference with the R708 CTRL having a greater percentage drum than the R708 TM at 46 and 60 days. Given there appears to be no trend in any of the leg traits, TM may not be useful in impacting leg muscle development. Differences in how TM affect light and dark meat development may relate to muscle fiber type, as light meat consists primarily of glycolytic fibers, while dark meat primarily consists of oxidative muscle fibers (Brandenbourg, 2013). TM having a greater effect in glycolytic fibers may relate to glycolytic fibers having a greater growth potential than other fibers (Henckel, 1992). Furthermore, oxidative fibers relying on oxidative metabolism may be limited in size as a smaller diameter is more conducive to oxygen metabolism (Macrae et al., 2006). A larger diameter therefore would negatively impact oxygen diffusion, and create a limit to oxidative fiber size. Further research will be needed to expound on differences in TM effect relating to fiber type. However, breast percentage was impacted by TM showing improvement at four days in both TM strains compared to the CTRL strains and again at 60 days. Total breast weight relative to live weight was also improved in the HxC TM compared to the HxC CTRL at 60 days. Improvement in body composition in TM strains is consistent with previous TM research (Halevy et al., 2006; Collin et al., 2007; Piestun et al., 2011; 2013). Improvements in breast yield are attributable to increased muscle fiber hypertrophy in TM broilers

(Piestun et al., 2009b; 2011) made possible by TM targeting a period of satellite cell population expansion (Hartley et al., 1992) leading to increased satellite cell proliferation and subsequent muscle fiber hypertrophic growth.

TM appeared to have no effect on the muscle quality of TM embryos at broiler age. Drip loss at 46 days was the only trait to exhibit a treatment effect and the drip loss values were consistent with control and TM treated Cobb 500 broilers processed at 35 days ranging from 1.99 - 3.67% (Loyau et al., 2013). This study also found no difference in drip loss percentage between control and TM broilers (Loyau et al., 2013). Muscle color values (L*) failed to differ between TM and CTRL strains and were consistent with research showing mean L* value for poultry meat unaffected by PSE and DFD ranging from 48.0 to 53.0 (Qiao et al., 2001; Zhang and Barbut, 2005; Harford et al., 2014). The L* values calculated are consistent with research showing TM had no impact on muscle color (Loyau et al., 2013). Shear force values were not affected by TM and were consistent with shear force values in broilers processed between 42 and 52 days of age (Cavitt et al., 2004, Lyon et al., 2004). PH was also unaffected in TM embryos at broiler age with results being consistent with pH calculated from broilers not affected by PSE or DFD (Zhang and Barbut, 2005; Harford et al., 2014) and lower than pH values calculated from DFD meat (Zhang and Barbut, 2005).

In summary, TM of embryos of lines expressing different growth rates was effective in eliciting responses in body composition at broiler age and broiler growth up to 35 days. These responses are evidenced by the increased breast weight relative to live weight percentage exhibited by both TM strains when compared to their control counterparts, as well as the improvements in body weights shown in the by the R708 up to Day 35. These responses occurred with little negative impact on hatchability and no negative impact on meat quality. FCR improved at broiler age in the HxC TM embryos, but no impact was on FCR was shown in the R708. The lack of impact on hatchability plus increased relative breast yield at processing age suggests TM of embryos may be a viable method to improve body composition in broilers. However, as it appears the HxC strain showed greater benefit from TM than the R708 at processing age, further research and testing is necessary to implement TM effectively in broilers expressing differing growth rates and selected for different market ages.

4.5 Figures and Tables

Figure 4.1 Distribution of hatching time of two broiler strains (HxC, R708), incubated under control or thermally manipulated conditions.

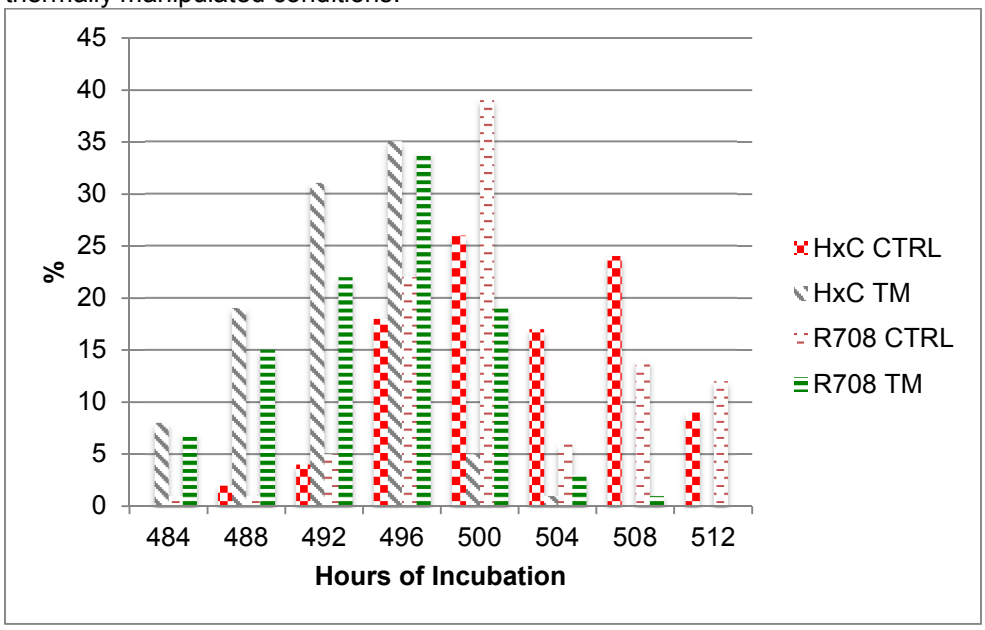

Table 4.1 Hatch of fertile, hatch weight and hatch time measures (mean ± SEM) of chicks from two broiler strains (HxC, R708), incubated under control or thermally manipulated (TM) conditions.

	Control		TM	
Variable	HxC	R708	HxC	R708
Hatchability	89.98 ± 0.05a	83.20 ± 0.07b,y	89.98 ± 0.05a	86.85 ± 0.05b,x
Hatch Weight	51.41 ± 0.27a	49.88 ± 0.27b	50.85 ± 0.27a	49.51 ± 0.29b
Hatch Time	501.96 ± 0.41x	501.53 ± 0.48x	492.32 ± 0.35b,y	493.78 ± 0.37a,y

[a-b] Means for a variable within an incubation treatment are different (P < .05).

[x-y] Means for a variable within a genetic group are different (P < .05).

Table 4.2 Body Weight (BWT) and Feed Conversion Ratio (FCR) measures (means ± SEM) of two broiler strains (HxC, R708), incubated under control or thermally manipulated conditions over time. (N = 6)

		Control		TM	
Trait	Age	HxC	R708	HxC	R708
BWT (g)	7	184 ± 2a	164 ± 5b,y	185 ± 3	179 ± 3x
	14	501 ± 6a	429 ± 7b,y	486 ± 6	471 ± 7x
	21	1036 ± 6a,x	891 ± 14b,y	1006 ± 11a,y	950 ± 4b,x
	28	1726 ± 56a	1475 ± 25b,y	1652 ± 28a	1573 ± 11b,x
	35	2447 ± 15a	2148 ± 36b,y	2375 ± 18a	2244 ± 12b,x
	42	3154 ± 29a	2795 ± 69b	3087 ± 37	2935 ± 57
	49	3731 ± 37a	3375 ± 71b	3610 ± 73a	3426 ± 21b
	56	4613 ± 49a	4162 ± 62b	4501 ± 37a	4088 ± 119b
FCR	14	1.16 ± 0.02x	1.10 ± 0.10	1.06 ± 0.01b,y	1.14 ± 0.02a
	21	1.28 ± 0.01x	1.29 ± 0.05	1.23 ± 0.01b,y	1.27 ± 0.01a
	28	1.37 ± 0.01	1.37 ± 0.03	1.34 ± 0.01	1.35 ± 0.01
	35	1.62 ± 0.05	1.71 ± 0.01x	1.65 ± 0.03	1.64 ± 0.01y
	42	1.75 ± 0.01	1.79 ± 0.01	1.71 ± 0.01	1.72 ± 0.04
	49	1.85 ± 0.03	1.85 ± 0.01	1.83 ± 0.03	1.83 ± 0.01
	56	1.89 ± 0.03x	1.87 ± 0.01	1.82 ± 0.04y	1.87 ± 0.04

[a-b] Means for a trait within an incubation treatment at a given age are different (P < .05).

[x-y] Means for a trait within a genetic group at a given age are different (P < .05).

Table 4.3 Processing measures (g) (means ± SEM) of two broiler strains (HxC, R708), incubated under control or thermally manipulated (TM) conditions at four processing ages (4, 7, 46, 60) (N ≥ 42).

		Control		TM	
Trait	Age	HxC	R708	HxC	R708
BWT	4	100 ± 1a,y	90 ± 2b,y	108 ± 1a,x	101 ± 1b,x
	7	181 ± 2a	169 ± 3b	186 ± 2a	177 ± 3b
	46	3491 ± 62a	3179 ± 65b	3321 ± 60	3197 ± 60
	60	4979 ± 88a,x	4259 ± 100b	4713 ± 83a,y	4378 ± 102b
Keel[1]	4	2.30 ± 0.08	2.14 ± 0.08	2.45 ± 0.08a	2.26 ± 0.06b
	7	4.86 ± 0.13a	4.51 ± 0.15b	5.07 ± 0.14	4.92 ± 0.14
Rack[2]	46	739 ± 15a	646 ± 14b	705 ± 17	662 ± 16
	60	1089 ± 28a	906 ± 24b	1044 ± 22a	922 ± 27b
WOG[3]	46	2534 ± 46a	2308 ± 83b	2426 ± 47	2343 ± 77
	60	3769 ± 47a	3264 ± 68b	3638 ± 44a	3358 ± 80b
WOGPC[4]	46	2619 ± 47a	2379 ± 78b	2497 ± 48	2412 ± 81
	60	3880 ± 47a	3348 ± 71b	3714 ± 44a	3413 ± 79b

[a-b] Means for a trait within an incubation treatment at a given age are different ($P < .05$).

[x-y] Means for a trait within genetic group at a given age are different ($P < .05$).

[1] Keel = Skeletal support of the breast post breast removal.

[2] Rack = Skeletal system of the bird post part removal.

[3] WOG = Without Giblets.

[4] WOGPC = Without Giblets Post Chill.

Table 4.4 Breast and leg weight measures (g) (means ± SEM) of two broiler strains (HxC, R708), incubated under control or thermally manipulated (TM) conditions at four processing ages (4, 7, 46, 60) (N ≥ 42).

		Control		TM	
Trait	Age	HxC	R708	HxC	R708
Breast	4	3.99 ± 0.17y	3.56 ± 0.17y	4.85 ± 0.11x	4.79 ± 0.16x
	7	13.91 ± 0.37	14.32 ± 0.46y	14.66 ± 0.37	15.84 ± 0.47x
	46	659 ± 18	674 ± 18	639 ± 14	676 ± 13
	60	1044 ± 23	996 ± 28	1035 ± 24	1044 ± 23
Tenders	4	0.87 ± 0.04y	0.79 ± 0.04y	1.08 ± 0.03x	1.07 ± 0.04x
	7	3.26 ± 0.09y	3.34 ± 0.13y	3.60 ± 0.09x	3.78 ± 0.12x
	46	137 ± 3	132 ± 3	141 ± 2a	132 ± 2b
	60	207 ± 5a	185 ± 4b	202 ± 4a	176 ± 5b
TBRST[1]	4	4.85 ± 0.21a,y	4.35 ± 0.21b,y	5.93 ± 0.13x	5.86 ± 0.20x
	7	17.17 ± 0.45	17.66 ± 0.57y	18.25 ± 0.45	19.62 ± 0.57x
	46	805 ± 19	807 ± 21	780 ± 17	809 ± 14
	60	1252 ± 27	1179 ± 31	1237 ± 27	1220 ± 26
Leg	4	14.57 ± 0.36a,y	13.51 ± 0.38b,y	15.56 ± 0.25a,x	14.72 ± 0.25b,x
	7	28.37 ± 0.57a	25.93 ± 0.19b	28.40 ± 0.57	27.25 ± 0.64
	46	766 ± 17a	683 ± 13b	722 ± 17	681 ± 16
	60	1125 ± 30a,x	933 ± 26b	1042 ± 24a	932 ± 26b
Thigh	46	452 ± 10a	398 ± 8b	425 ± 9	404 ± 10
	60	668 ± 18a,x	556 ± 16b	617 ± 13a,y	557 ± 15b
Drum	46	314 ± 6a	283 ± 6b	297 ± 7a	276 ± 7b
	60	457 ± 12a	380 ± 10b	424 ± 11a	375 ± 11b

[a-b] Means for a trait within an incubation treatment at a given age are different ($P < .05$).

[x-y] Means for a trait within genetic group at a given age are different ($P < .05$).

[1] TBRST = Total Breast (Breast + Tenders).

Table 4.5 Breast and leg weight relative to live weight measures (%) (means ± SEM) of two broiler strains (HxC, R708), incubated under control or thermally manipulated conditions at four processing ages (4, 7, 46, 60) (N ≥ 42).

		Control		TM	
Trait	Age	HxC	R708	HxC	R708
Breast	4	3.92 ± 0.11y	3.81 ± 0.13y	4.43 ± 0.07x	4.64 ± 0.10x
	7	7.62 ± 0.12b	8.46 ± 0.12a,y	7.84 ± 0.12b	8.88 ± 0.14a,x
	46	19.02 ± 0.24b	20.99 ± 0.29a	19.35 ± 0.26b	21.25 ± 0.33a
	60	21.29 ± 0.22b,y	23.16 ± 0.28a,y	22.02 ± 0.29b,x	23.97 ± 0.27a,x
TBRST[1]	4	4.85 ± 0.15y	4.64 ± 0.16y	5.47 ± 0.09x	5.68 ± 0.13x
	7	9.40 ± 0.14b	10.43 ± 0.15a,y	9.75 ± 0.14b	11.00 ± 0.16a,x
	46	23.15 ± 0.30b	25.16 ± 0.35a	23.65 ± 0.30b	25.43 ± 0.37a
	60	25.53 ± 0.24b,y	27.43 ± 0.32a	26.36 ± 0.32b,x	28.06 ± 0.35a
Leg	4	14.69 ± 0.18	14.76 ± 0.19	14.38 ± 0.16	14.48 ± 0.13
	7	15.67 ± 0.14x	15.24 ± 0.19	15.08 ± 0.17y	15.36 ± 0.18
	46	22.04 ± 0.19a	21.45 ± 0.17b	21.78 ± 0.19	21.25 ± 0.20
	60	22.65 ± 0.21a	21.73 ± 0.18b	22.14 ± 0.19a	21.24 ± 0.19b
Thigh	46	13.00 ± 0.15a	12.57 ± 0.13b	12.81 ± 0.15	12.62 ± 0.14
	60	13.44 ± 0.15a	12.87 ± 0.15b	13.08 ± 0.13a	12.70 ± 0.13b
Drum	46	9.04 ± 0.08	8.88 ± 0.08x	8.97 ± 0.09a	8.63 ± 0.09b,y
	60	9.21 ± 0.10a	8.83 ± 0.08b,x	9.07 ± 0.11a	8.53 ± 0.10b,y

[a-b] Means for a trait within an incubation treatment at a given age are different ($P < .05$).

[x-y] Means for a trait within genetic group at a given age are different ($P < .05$).

[1] TBRST = Total Breast (Breast + Tenders)

Table 4.6 Meat quality means ± SEM of two broiler strains (HxC, R708), incubated under control or thermally manipulated (TM) conditions collected at broiler processing ages (46,60).

		Control		TM	
Trait	Age	HxC	R708	HxC	R708
[1]L*	46	51.40 ± 0.36a	49.82 ± 0.40b	51.05 ± 0.38a	49.69 ± 0.30b
	60	53.56 ± 0.36a	52.22 ± 0.42b	53.31 ± 0.36a	52.12 ± 0.40b
[2]Shear Force	46	4.00 ± 0.69	3.72 ± 0.39	3.43 ± 0.30	3.21 ± 0.40
(kg/cm^2)	60	3.43 ± 0.37a	2.41 ± 0.16b	2.73 ± 0.25	3.02 ± 0.31
[3]Drip Loss %	46	3.38 ± 0.25	3.96 ± 0.27x	3.00 ± 0.29	2.83 ± 0.29y
	60	3.34 ± 0.30	3.62 ± 0.31	4.23 ± 0.41	3.59 ± 0.36
[4]PH	46	6.00 ± 0.05	6.15 ± 0.07	6.13 ± 0.07	6.16 ± 0.05
	60	5.88 ± 0.04b	6.01 ± 0.04a	5.92 ± 0.03	5.91 ± 0.04

[a-b] Means for a trait within an incubation treatment at a given age are different (P < .05).
[x-y] Means for a trait within genetic group at a given age are different (P < .05).
[1] L* = Muscle Color Lightness Value measured using a Minolta CR-300 Colorimeter.
[2] Shear force as measured using WB Method previously described by Burke et al., 2003.
[3] Drip Loss as measured using EZ-Driploss Method as described by Correa et al., 2007.
[4] PH measured using Testo 205 pH probe.

4.6 Works Cited

Allen, C. D., S. M. Russell, and D. L. Fletcher. 1997. The relationship of broiler breast meat color and pH to shelf-life and odor development. Poult. Sci. 76:1042-1046.

Allen, C. D., D. L. Fletcher, J. K. Northcutt, and S. M. Russell. 1998. The relationship of broiler breast color to meat quality and shelf life. Poult. Sci. 77:361-366.

Barbut, S. 1993. Color measurements for evaluating the pale soft exudative (pse) occurrence in turkey meat. Food Res. Int. 26:39-43.

Barbut, S. 1996. Estimates and detection of the PSE problem in young turkey breast meat. Can. J. Anim. Sci. 76:455-457.

Barbut, S. 1997. Problem of pale soft exudative meat in broiler chickens. Brit. Poult. Sci. 38:355-358.

Brandenbourg, T. 2013. Growth of muscle from myoblast to whole muscle. In: Kerth, C. R. (ed.) *The Science of Meat Quality*. John Wiley and Sons, Inc., Oxford, pp. 1-27.

Bratzler, L. J. 1932. Measuring tenderness of meat by use of the Warner-Bratzler method. MSc Thesis, Kansas State College, Manhattan, KS.

Burke, J. M., J. K. Apple, W. J. Roberts, C. B. Boger, and E. B. Kegley. 2003. Effect of breed-type on performance and carcass traits of intensively managed hair sheep. J. Meat Sci. 63:309-315.

Cavitt, L. C., J. F. C. Meullenet, R. K. Gandhapuneni, G. W. Youm, and C. M. Owens. 2005. Rigor development and meat quality of large and small broilers and the use of Allo-Kramer shear, needle puncture, and razor blade shear to measure texture. Poult. Sci. 84:113-118.

Cavitt, L. C., J. F. C. Meullenet, R. Xiong, and C. M. Owens. 2004. The relationship of Razor Blade shear, Allo-Kramer shear, Warner-Bratzler shear and sensory tests to changes in tenderness of broiler breast fillets. J. Muscle Foods 14:11-23.

Collin, A., C. Berri, S. Tesseraud, F. Requena, S. Cassy, S. Crochet, M. J. Duclos, N. Rideau, K. Tona, J. Buyse, V. Bruggerman, E. Decuypere, M. Picard, and S. Yahav. 2007. Effects of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. Poult. Sci. 86:795-800.

Correa, J. A., S. Methot, and L. Faucitano. 2007. A modified meat juice container (EZ-Driploss) procedure for a more reliable assessment of drip loss and related quality changes in pork meat. J. Muscle Foods 18:67-77.

Dosler, D., T. Polak, B. Zlender, and L. Gasperlin. 2007. Relation of myofibril fragmentation to textural and chemical parameters of aged pork Longissimus dorsi. Acta agric. Slovenica 90:5-16.

Froning, G. W. 1995. Color of poultry meat. Poult. Avian Biol. Rev. 6(1): 83-93.

Gregory, N. G. 1994. Preslaughter handling, stunning and slaughter. Meat Sci. 36:45-56.

Halevy, O., S. Yahav, and I. Rozenbaum. 2006. Enhancement of meat production by environmental manipulations in embryo and young broilers. World's Poult. Sci. J. 62:485-497.

Harford, I. D., Pavlidis, H. O., and Anthony, N. B. 2014. Divergent selection for muscle color in broilers. Poult. Sci. *93*:1059-1066.

Hartley, R. S., E. Bandman, and Z. Yablonka-Reuveni. 1992. Skeletal muscle satellite cells appear during late chicken embryogenesis. Dev. Biol. 153:206-216.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1509-1518.

Hedrick, H. B., E. D. Aberle, J. C. Forrest, M. D. Judge, and R. A. Merkel. 1989. Principles of Meat Science. 3rd ed. Kendall/Hunt Publishing Co., Dubuque, IA.

Henckel, P. 1992. Properties of muscle fibre types as a source of variation in meat quality. *Proceedings of the 19th World's Poultry Congress*. Amsterdam, The Netherlands, pp. 87-92.

Lawrie, R. A. 1998. Lawrie's Meat Science. 6th ed. Woodhead Publishing, Cambridge, UK.

Loyau, T., C. Berri, L. Bedrani, S. Métayer-Coustard, C. Praud, M. J. Duclos, S. Tesseruad, N. Rideau, N. Everaert, S. Yahav, S. Mignon-Grasteua and A. Collin. 2013. Thermal manipulations of the embryo modifies the physiology and body composition of broiler chickens reared in floor pens without affecting breast meat processing quality. J. Anim. Sci. 91:3674-3685.

Lyon, B. G., D. P. Smith, C. E. Lyon, and E. M. Savage. 2004. Effects of diet and feed withdrawal on the sensory descriptive and instrumental profiles of broiler breast fillets. Poult. Sci. 83:275-281.

Macrae, V. E., M. Mahon, S. Gilpin, D. A. Sandercock, and M. A. Mitchell. 2006. Skeletal muscle fibre growth and growth associated myopathy in the domestic chicken (*Gallus domesticus*). Brit. Poult. Sci. 47:264-272.

Mahon, M. 1999. Muscle abnormalities-morphological aspects in: Richardson, R.I. and Mead, G.C. (Eds) Poult. Meat Sci., Poult. Sci. Symposium Series, pp 19-64. Wallingford, CAB INTL.

Mauro, A. 1961. Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Cytol. 9:493-495.

McCurdy, R. D., S. Barbut, and M. Quinton. 1996. Seasonal effect on pale soft excudative (PSE) occurrence in young turkey breast meat. Food Res. Int. 29:363-366.

Meijerhof, R. 2000. Embryo temperature as a tool in the incubation process. Incubation and Fertility Research Group [WPSA Working Group 6 (Reproduction)], St. Edmand's Hall, Oxford, UK.

Montarras, D., J. Morgan, C. Collins, F. Relaix, S. Zaffran, A. Cumano, T. Partridge, and M. Buckingham. 2005. Direct isolation of satellite cells for skeletal muscle regeneration. Science 5743:2064-2067.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington DC.

Newton, K. G., and C. O. Gill. 1981. The microbiology of DFD fresh meats: A review. Meat Sci. 5:223-232.

Perry, R. L., and M. A. Rudnick, 2000. Molecular mechanisms regulating myogenic determination and differentiation. Front Biosci. 5:D750-767.

Piestun, Y., S. Druyan, J. Brake, and S. Yahav. 2013. Thermal manipulations during broiler incubation alter performance of broilers to 70 days of age. Poult. Sci. 92:1155-1163.

Piestun, Y., O. Halevy, D. Shinder, M. Ruzal, S. Druyan, and S. Yahav. 2011. Thermal manipulations during broiler embryogenesis improves post hatch performance under hot conditions. Therm. Biol. 36:469-474.

Piestun, Y., O. Halevy, and S. Yahav. 2009a. Thermal manipulations of broiler embryos - The effect on thermoregulation and development during embryogenesis. Poult. Sci. 88:2677-2688.

Piestun, Y., M. Harel, M. Barak, S. Yahav and O. Halevy. 2009b. Thermal manipulations in late-term chick embryos have immediate and longer term effects on myoblast proliferation and skeletal muscle hypertrophy. J. Appl. Physiol. 106:233-240.

Piestun, Y., D. Shinder, M. Ruzal, O. Halevy, J. Brake, and S. Yahav. 2008a. Thermal manipulations during broiler embryogenesis: Effect on the acquisition of thermotolerance. Poult. Sci. 87:1516-1525.

Piestun, Y., D. Shinder, M. Ruzal, O. Halevy, and S. Yahav. 2008b. The effect of thermal manipulations during the development of the thyroid and adrenal axes on in-hatch and post-hatch thermoregulation. J. Therm. Biol 33:413-418.

Qiao, M, D. L. Fletcher, D. P. Smith, and J. K. Northcutt. 2001. The effect of broiler breast meat color on pH, moisture, water-holding capacity, and emulsification capacity. Poult. Sci. 80:676–680.

Rasmussen, A., and M. Andersson. 1996. New method for determination of drip loss in pork muscles. In: Hildrum, K.I. (ed.) *Meat for the consumer. Poster Proceedings of the 42nd International Congress of Meat Science and Technology*. Lillehammer, Norway, pp. 286-287.

SAS Institute Inc. 2001. SAS User's Guide. SAS Inst. Inc., Cary, NC.

Strasburg, G. M., and J. E. Linz. 1997. "Biochemical characterization of pale, soft exudative turkey." 2USDA NRICGP Abstracts of Funded Research. FY 1997.

Uni, Z., and Yahav, S. 2010. Managing prenatal development of broiler chickens to improve productivity and thermotolerance. In: P. L. Greenwood, (Ed). *Managing the Prenatal Environment to Enhance Livestock Productivity*. pp. 71-90. Springer, Netherlands.

Woelfel, R. L., C. M. Owens, E. M. Hirschiler, and S. R. Sams. 1998. The incidence and characterization of pale, soft and exudative chicken meat in commercial plant. Poult. Sci. 77(Suppl. 1):62.

Wulf, D. M., R. S. Emnett, J. M. Leheska, and S. J. Moeller. 2002. Relationships among glycolytic potential, dark cutting beef, and cooked beef palatability. J. Anim. Sci. 80:1895-1903.

Yahav, S., Collin, A., Shinder, D., & Picard, M. 2004a. Thermal manipulations during broiler chick embryogenesis: Effects of timing and temperature. Poult. Sci. 83:1959-1963.

Yahav, S., Rath, R. S., & Shinder, D. 2004b. The effect of thermal manipulations during embryogenesis of broiler chicks (*Gallus domesticus*) on hatchability, body weight and thermoregulation after hatch. J. Therm. Biol. *29*:245-250.

Zhang, L., and S. Barbut. 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. Brit. Poult. Sci. 46:687-693.

CONCLUSION

Selection for increased broiler growth and yield has made the poultry industry the number one source of protein. However, it is clear that growth selection has negatively impacted broiler physiology and fitness. The continued use of modern selection techniques has resulted in increases in traditional meat quality issues, as well as the introduction of new muscle myopathies such as white striping and wooden breast. To combat these issues development of novel methods of growth selection are required. To that end divergent selection for BY4 was undergone to utilize increases in muscle fiber number, as opposed to traditional growth selections utilizing the increase in muscle fiber size. Furthermore, TM an embryonic technique used to promote postnatal broiler growth and thermotolerance was evaluated to assess its impact on broiler lines selected for different growth rates and the subsequent impact on meat quality. The purpose of this dissertation was to develop a new selection technique for promoting growth and test a promising new technique, as well as evaluating the impact of these techniques on broiler growth, fitness and meat quality.

Short term divergent selection for BY4 has shown that selection for muscle fiber number is possible and provides a heritability that lends itself to effective use in selection. Additionally, divergent BY4 selection posthatch was capable of manipulating embryonic development, suggesting that selection was effective in targeting the prenatal period in which changes in muscle fiber number occur. Further experimentation and research will be needed to evaluate the nature of these changes and provide a selection technique that can be adopted by the poultry industry.

Short term divergent selection for BY4 was also shown to promote broiler muscle growth, while having minimal impact on broiler fitness and muscle quality. This was demonstrated by increases in broiler growth up to Day 28, increased breast yield at processing age and no differences in meat quality. It is believed that further selection will result in continued divergence in body weight and breast percentage. Future research will focus on the impact of long term divergent BY4 selection and histological examination of muscle tissue from the BY4 lines.

TM was shown to be effective in altering the body composition of broiler strains expressing different growth rates. The improvements in breast percentage expressed by TM strains compared to

their control counterparts demonstrate this change. Contrary to traditional growth selection these improvements in body composition occurred without concomitant decreases in meat quality. While both strains showed improved body composition, the degree to which performance improved differed. Further research will be needed to tailor TM use based upon growth rates and differences in genetics. Overall results of this research suggest that promising new techniques exist to promote broiler growth, fitness and muscle quality, given further development these techniques may one day be of benefit to the poultry industry.

APPENDIX

UNIVERSITYOF

ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Nicholas Anthony
FROM: Craig N. Coon, Chairman Institutional Animal Care And Use Comittee

DATE: May 23, 2012
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: April 6, 2015

The Institutional Animal Care and Use Committee (IACUC) has APPROVED Protocol #12038- "GENERAL REARING OF SELECTED CHICKEN AND QUAIL POPULATIONS ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] prior to initiating the changes. If the study period is expected to extend beyond 04-06-2015, you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

ene/car

cc: Animal Welfare Veterinarian

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